Structural Changes in the Carboxyl Terminus of the Gap Junction Protein Connexin43 Indicates Signaling between Binding Domains for c-Src and Zonula Ocludens-1*

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Paul L. Sorgen‡§, Heather S. Duffy¶, Prangya Sahoo‡, Wanda Coombs, Mario Delmar**†‡, and David C. Spray‡¶**

From the ‡Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, Nebraska 68198, the ¶Department of Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461, and the §Department of Pharmacology, Upstate Medical University, Syracuse, New York 13210

Regulation of cell-cell communication by the gap junction protein connexin(43) can be modulated by a variety of connexin-associating proteins. In particular, c-Src can disrupt the connexin43 (Cx43)-zonula occludens-1 (ZO-1) interaction, leading to down-regulation of gap junction intercellular communication. The binding sites for ZO-1 and c-Src correspond to widely separated Cx43 domains (∼100 residues apart); however, little is known about the structural modifications that may allow information to be transferred over this distance. Here, we have characterized the structure of the connexin43 carboxyl-terminal domain (Cx43CT) to assess its ability to interact with domains from ZO-1 and c-Src. NMR data indicate that the Cx43CT exists primarily as an elongated random coil, with two regions of α-helical structure. NMR titration experiments determined that the ZO-1 PDZ-2 domain affected the last 19 Cx43CT residues, a region larger than that reported to be required for Cx43CT-ZO-1 binding. The c-Src SH3 domain affected Cx43CT residues Lys-264–Lys-287, Ser-306–Glu-316, His-331–Phe-337, Leu-356–Val-359, and Ala-367–Ser-372. Only region Lys-264–Lys-287 contains the residues previously reported to act as an SH3 binding domain. The specificity of these interactions was verified by peptide competition experiments. Finally, we demonstrated that the SH3 domain could partially displace the Cx43CT-PDZ-2 complex. These studies represent the first structural characterization of a connexin domain when integrated in a multimolecular complex. Furthermore, we demonstrate that the structural characteristics of a disordered Cx43CT are advantageous for signaling between different binding partners that may be important in describing the mechanism of channel closure or internalization in response to pathophysiological stimuli.

Gap junction channels serve to directly interconnect the cytoplasm of neighboring cells, allowing the passage of moderately small ions, metabolites, and signaling molecules. Mammalian gap junction channels are formed by as many as 21 different connexin proteins (1). Of these, connexin43 (Cx43) is the most completely characterized in terms of channel gating properties (2–4), phosphorylation sites (5–7), mechanisms of pH sensitivity (8–11), and overall molecular structure (12). Cx43 is the most abundant gap junction protein in various tissues, including heart and brain. Cx43 null mice have been extensively investigated, with important differences being found as compared with wild types with regard to numerous processes, including cardiac developmental abnormalities, electrical synchrony in the heart, spreading depression in brain, as well as global gene expression changes in heart and astrocytes (13–20).

Connexin molecules are tetraspan membrane proteins, with both amino and carboxyl termini within the cytoplasm. Although the structure of the membrane-spanning portions of Cx43 has been solved to a resolution of about 7.5 Å (in the membrane plane) using electron crystallography (12), a construct truncated near its emergence from the fourth transmembrane domain was used for those studies, and thus, little has been known regarding structures of the cytoplasmic portions of the molecule. We have recently used a combination of NMR and mirror resonance technology to characterize the structure of the second half of the cytoplasmic loop (Cx43L2) and of the carboxyl-terminal domain of Cx43 (Cx43CT (11, 21)). Our data show a direct pH-dependent interaction between these domains and the formation of acidification-induced α-helical structure in the Cx43L2 peptide. Additional studies have shown that solvent acidification induces Cx43CT dimerization (22), suggesting that structural modification of both the cytoplasmic loop and the carboxyl-terminal domains participates in their interaction.

Regulation of Cx43 channels involves not only the intramolecular interactions described above but also intermolecular associations. Cx43 is the first gap junction protein for which non-connexin molecular binding partners were identified. These include zonula occludens-1 (ZO-1) (23, 24), v- and c-Src (25–28), caveolin-1 (29), β-catenin (30), α-catenin (31), and

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The atomic coordinates and structure factors (code 1R5S) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

§ The on-line version of this article (available at http://www.jbc.org) contains two supplementary figures.

To whom correspondence should be addressed. Tel.: 402-559-7557, Fax: 402-559-6650; E-mail: psorgen@unmc.edu.

** Both authors contributed equally to this work.

1 The abbreviations used are: Cx43, connexin43; Cx43CT, connexin43 carboxyl terminus; Cx43CL, connexin43 cytoplasmic loop; CL, cytoplasmic loop; CT, carboxyl terminus; HSQC, heteronuclear single-quantum correlation; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; NOESY, NOE spectroscopy; ZO-1, zonula Occludens-1; SH, Src homology; MAP, mitogen-activated protein; MAPK, MAP kinase.
The role of connexin-binding proteins is presumably in trafficking to sites of gap junction formation between cells, stabilization of the junctional plaque, or perhaps signal transduction (33, 34). Whereas site-directed mutagenesis has been used to infer the sites of protein-protein interactions, no characterization of the structural modifications resulting from these interactions has been reported previously.

Here, we show the solution of the secondary structure of Cx43CT and report the changes that occur upon binding to the SH3 domain of c-Src and to the second PDZ domain of ZO-1. Our experiments identify selected groups of Cx43CT residues, the positions of which in space are altered by the presence of the ligate. The regions of structural reorganization include but span beyond those identified previously by site-directed mutagenesis to be necessary for binding. Our data suggest that the Cx43CT ligand binding platforms may represent elaborate three-dimensional conformations that include amino acid residues in the primary sequence of the protein.

EXPERIMENTAL PROCEDURES

Expression and Purification of Recombinant GST-Cx43CT—Cx43CT (residues 255–382 of Cx43 and 4 additional amino acids appended to the amino terminus; see Ref. 11), [15N,C]Cx43CT, [15N13C]Cx43CT, the SH3 domain of c-Src SH3, and the PDZ domain of ZO-1 were expressed and purified as described previously (11, 28). Short peptides corresponding to selected Cx43CT sequences were synthesized as described in Duffy et al. (11). All proteins were confirmed for purity and analyzed for degradation by SDS-PAGE; synthetic polypeptides and recombinant protein purity were further analyzed by NMR and mass spectroscopy.

Nuclear Magnetic Resonance—NMR data were acquired at 7 °C using a Bruker DRX-300 spectrometer fitted with a broadband probe, a Bruker DRX-600 spectrometer fitted with a triple resonance probe, and a 600 MHz Varian INOVA NMR spectrometer outfitted with a cryo-probe. Experimental data to determine backbone sequential assignments have been described (21). Distance constraints were derived from NOEs observed in 15N-NOESY-HSQC (35) and 13C-NOESY-HSQC spectra, with mixing times of 125 and 150 ms, respectively. NMR spectra were processed using NMRPipe (36) and analyzed using NMR-View (37).

Hydrogen bonds were identified based on temperature dependence studies (38). These studies followed the amide proton chemical shifts from 7 to 37 °C. Any amide proton that shifted less than 4.5 ppb/K was considered likely to be hydrogen-bonded. Each hydrogen bond was imposed as two distance restraints (an H-0 distance of 1.9–2.3 Å and an N-O distance of 2.4–3.0 Å).

Structural Calculation—Model structures were calculated by simulated annealing using torsion angle dynamics as implemented in the program Crystallography and NMR system (CNS) (39). NOE cross-peaks classified as strong, medium, and weak were converted into distance restraints of 1.8–2.5, 1.8–3.5, and 1.8–5.5 Å, respectively. 3JHHH coupling constants (three-bond HNH coupling constants) were measured from a three-dimensional HNHA experiment (40) and used directly as constraints. The 10 best energy minimized structures were evaluated using AQUA and PROCHECK-NMR (41).

RESULTS

Structure Calculation and Description—Our laboratory recently published resonance assignments for the Cx43CT (21). We have now applied NOE spectroscopy to identify the resonance peaks that presented stable secondary structures. The solution structure was obtained in phosphate-buffered saline buffer (pH 5.8) at 7 °C. Structure calculations by torsion angle dynamics followed with refinement by simulated annealing, and energy minimization led to the family of structures shown in Fig. 1. The overall structure of the Cx43CT was mainly random coil; however, two regions of helical structure were present (Fig. 1, A and B). These helical regions were superimposed on the basis of backbone coordinates from Ala-315–Thr-326 and Asp-340–Ala-348 (Fig. 1C). The total root mean square deviations for these alignments were 0.27 and 0.34 Å, respectively. Structural statistics are presented in Table I. The predominantly random coil structure for the Cx43CT is consistent with the narrow chemical shift dispersions in the 15N HSQC (Fig. 1D).

Structural Changes in Cx43CT Resulting from Binding of Cellular Partners—The solution of the structure of Cx43CT allowed for an assessment of the modifications that result from its interaction with other molecules. We focused our attention on two well known binding partners of Cx43 for which we have recently quantified binding affinities to Cx43CT in solution (28): the second PDZ domain of ZO-1 (PDZ-2) and the SH3 domain of c-Src (SH3). Unlabeled PDZ-2 or SH3 fragments were diluted in a phosphate-buffered saline buffer solution (pH 5.8) containing 15N-Cx43CT to a final 1:1 molar ratio, and 15N-HSQC spectra were acquired. The 15N-HSQC is a two-dimensional NMR experiment in which each amino acid (except proline) gives one signal (or chemical shift) that corresponds to the N-H amide group. These chemical shifts are sensitive to the chemical environment, and even small changes in structure and/or dynamics can change the chemical shift of an amino acid.

The addition of the PDZ-2 domain affected the resonance peaks of the last 19 amino acids of the Cx43CT, a region larger than what was expected from the putative “PDZ binding domain” of Cx43 (the final 4 amino acids in the carboxyl terminus (42)). Indeed, as seen in Fig. 2A, the signal from residues Ser-372–Ile-382 completely disappeared (i.e. broadened beyond detection), whereas the amplitude of the peaks corresponding to residues Ser-364–Ala-371 decreased in intensity. These changes can be explained by decreased tumbling rates caused by an increase in molecular weight and/or intermediate chemical exchange rates, both indicative of a structural modification caused by the association of the two molecules.

To verify the specificity of this interaction, resonance peaks were resolved in the presence of excess peptide corresponding to the carboxyl-terminal 20 residues of Cx43 (363–382). As indicated in Fig. 2B, the resonances for each of the residues affected in the presence of ZO-1 PDZ-2 returned to normal signal intensity in the presence of excess peptide. These data are consistent with a specific Cx43CT-PDZ-2 interaction at the carboxy-terminal domain of Cx43.

The addition of the SH3 domain affected the resonance peaks of amino acids Lys-264–Lys-287, Ser-306–Glu-316, His-331–Phe-337, Leu-356–Val-359, and Ala-367–Asp-371 (Fig. 2C). The specificity of these interactions was verified by the use of excess peptides corresponding to the Cx43CT residues affected by the binding of the SH3 domain. The peptide competition experiments indicated that the Cx43CT-SH3 complex was inhibited by peptides 271–287, 312–336, and 346–360 (Fig. 3B and Supplemental Data 2), as indicated by the re-emergence of all resonance peaks affected in Fig. 3A. Control peptides that correspond to the amino-terminal domain (NT) and the first extracellular loop (EL) of Cx43 did not interfere with the Cx43CT-SH3 interaction (see Supplemental Data 2). The results from this experiment indicate that although binding is confined to the SH3 canonical interaction domain of Cx43CT,
conformational changes resulting from this binding extend for
large distances along the Cx43CT.

SH3 Binding Cx43CT Disrupts the Cx43CT-PDZ-2 Complex—
Previous data have indicated that c-Src can disrupt the
Cx43-ZO-1 interaction, leading to down-regulation of gap junc-
tion intercellular communication (28, 42). Duffy et al. (28)
reported a pH-dependent “cross-talk” between Cx43CT inter-
actions in the SH3 binding domain and the PDZ binding do-
main. Here, we determined whether the SH3-induced struc-
tural modifications in Cx43CT affected the conformation of the
region ascribed as the PDZ-2 binding domain (residues Ser-
364–Ile-382; Fig. 2).

15N-Cx43CT (Fig. 4, black) was com-
bined with unlabeled PDZ-2 in phosphate-buffered saline
buffer (pH 5.8) to a final 1:1 molar concentration (Fig. 4,
red); unlabeled SH3 was titrated into the complex (Fig. 4,
green), and 15N-HSQC spectra were acquired. Clearly, the addition of
SH3 led to the reappearance of those Cx43CT resonance peaks
that had disappeared as a result of the Cx43CT-PDZ-2 inter-
action, although reappearance of the resonance peaks was not
complete (black and green intensities not being equal) even
after a 3-fold excess of the SH3 domain. Finally, it is worth
noting that the presence of the PDZ-2 domain did not affect the
changes in Cx43CT resonance peaks caused by the presence of
the SH3 domain. These data, combined with our inability to
detect an SH3-PDZ-2 interaction by chemical cross-linking
(data not shown), is consistent with a hypothesis that Cx43-
ZO-1 dissociation may occur by Cx43CT structural modifi-
cations induced by pH (e.g. dimerization) and Cx43-c-Src inter-
actions rather than via a direct ZO-1-c-Src interaction. We
propose that the Cx43CT domain has the ability to undergo
disorder-to-order transitions in the presence of molecular part-
ners to facilitate signaling between different molecules.

TABLE I
Structural statistics of the 10 lowest energy Cx43CT structures

| NOE distance restraints (total 1177) |  |
|--------------------------------------|---|
| Intra-residue                        | 401 |
| Sequential                           | 471 |
| Medium                               | 304 |
| Long range                           | 1  |
| Backbone hydrogen bonds              | 9  |
| r.m.s.d.\(^a\) of atomic coordinates (residues 315–342, 340–348) |  |
| Backbone (Å)                         | 0.41, 0.38 |
| All non-hydrogens (Å)                | 1.59, 1.41 |
| Ramachandran plot                    |  |
| Residues in most favored regions     | 41.0% |
| Residues in additional allowed regions | 43.1% |
| r.m.s.d.\(^a\) from ideal geometry   |  |
| Bond lengths (Å)                     | 0.004 |
| Bond angles (deg)\(^b\)              | 0.568 |
| Impropers (deg)\(^b\)                | 0.23 |

\(^a\) r.m.s.d., root mean square deviation.
\(^b\) deg, degrees.
FIG. 2. $^{15}$N-HSQC of Cx43CT in the presence of the PDZ-2 domain of ZO-1. This experiment allowed for detection of the regions of Cx43CT that were structurally modified when in the presence of a binding domain from the molecular partner ZO-1. A, Cx43CT was titrated with the PDZ-2 domain of ZO-1 at a 1:1 molar ratio. The control spectra, Cx43CT only (black), have been overlapped with spectra obtained when both molecules were present at a 1:1 molar ratio (red). The amino acid peaks that disappeared have been labeled. B, the Cx43CT-PDZ-2 complex (red) was titrated with a peptide corresponding to residues 363–382 to a final molar concentration of 1:3 (black). The differences in appearance between panels A and B are due to the collection of data on different spectrometers (600 versus 300 MHz), number of scans collected (16 versus 4), and complex points in the $^{15}$N dimension (256 versus 64), respectively.

FIG. 3. $^{15}$N-HSQC of Cx43CT in the presence of the SH3 domain of c-Src. This experiment allowed for detection of the regions of Cx43CT that were structurally modified when in the presence of a binding domain from the molecular partner c-Src. A, Cx43CT was titrated with the SH3 domain of c-Src at a 1:1 molar ratio. The control spectra, Cx43CT only (black), have been overlapped with spectra obtained when both molecules were present at a 1:1 molar ratio (red). The amino acid peaks that disappeared or decreased in intensity (regions Lys-264–Lys-287 (blue) and Glu-306–Glu-316 (red)) have been labeled. B, to verify the specificity of the Cx43CT interaction with the SH3 domain of c-Src, the Cx43CT-SH3 complex (red) from panel A was titrated with a peptide corresponding to residues 271–287 to a final concentration of 1:3 (black). Selected Cx43CT residues from regions Lys-264–Lys-287 and Glu-306–Glu-316 have been labeled.
DISCUSSION

We have characterized the structure of the carboxyl-terminal domain of Cx43 by the use of NMR. Our data indicate that Cx43CT exists primarily as an elongated random coil, with two regions of α-helical structure. We have further characterized the structural modifications brought about by the interaction of Cx43CT with two well known molecular partners and determined that those modifications are flexible, so that binding to one partner can largely displace the interaction with the other. These studies represent the first structural characterization of a connexin domain when integrated in a multimolecular complex and provide insight into the molecular mechanisms involved in the chemical regulation of Cx43.

Analysis of the Cx43CT Structure—Our data show that most of the Cx43CT sequence is configured as a random coil. Analysis of eukaryotic genomes led to the estimation that between 35 and 51% of all proteins contain disordered regions greater than 40 consecutive amino acids in length (46). A protein data base analysis determined that there are four broad functional categories of disordered proteins: molecular recognition, molecular assembly, protein modifications, and entropic chains (46). The major functions of such proteins, which include protein-protein binding, phosphorylation, and flexibility, all apply to the Cx43CT. A disordered Cx43CT may thus offer an ideal protein binding, phosphorylation, and flexibility, all apply to the Cx43CT. A disordered Cx43CT may thus offer an ideal

Dimerization is one of a number of intermolecular interactions described for Cx43CT. All these interactions may act in concert as “molecular switches” changing the properties of the channel (or its ability to be regulated by other factors) in response to the microenvironment. Consistent with this hypothesis is the inhibition of the Cx43CT-SH3 complex by Cx43CT peptides (312 and 346), corresponding to residues involved in the dimer formation of Cx43CT (22). We hypothesize that a coiled-coil structure between a Cx43CT dimer is part of the structural foundation for the “ball-and-chain” model of chemical gating (51), which then enables the direct intramolecular interaction between the Cx43CT and Cx43CL (11) during acidification-induced closure of the channel. Predictions from their sequence indicate that analogous helical structures occur in other connexin CTs (Cx40, Cx46, and Cx50), thus opening the possibility that a pH-dependent coiled-coil structure may be a common feature of α-connexins (52).

FUNCTIONAL SIGNIFICANCE OF THE HELICAL DOMAINS—Numerous binding partners to the carboxyl-terminal domain of Cx43 have been identified; these include tubulin, v- and c-Src, ZO-1, casein kinase 1 (CK1), mitogen-activated protein kinase (MAPK), cGMP-dependent protein kinase, cAMP-dependent protein kinase, and protein kinase C (for a review, see Ref. 33). Interestingly, other than the possible binding of CK1 to Ser-325, all these proteins interact with Cx43 in regions outside the two α-helical domains. We speculate that the α-helical domains: 1) allow the formation of a higher order structure necessary to regulate channel behavior during chemical gating and 2) act as a conduit for a cross-talk between different Cx43 binding partners. In particular, our data show that regions Ser-314–Ile-327 and Gln-342–Ala-348, which form a secondary α-helical structure, also correspond to sites of pH-dependent dimerization of Cx43CT (22). We hypothesize that a coiled-coil structure between a Cx43CT dimer is part of the structural foundation for the “ball-and-chain” model of chemical gating (51), which then enables the direct intramolecular interaction between the Cx43CT and Cx43CL (11) during acidification-induced closure of the channel. Predictions from their sequence indicate that analogous helical structures occur in other connexin CTs (Cx40, Cx46, and Cx50), thus opening the possibility that a pH-dependent coiled-coil structure may be a common feature of α-connexins (52).

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Functional Significance of the ZO-1 Interaction—Recent studies have focused on the relationship between Cx43 and two interacting proteins, ZO-1 and c-Src (28, 42). ZO-1 is important...
for maintaining cell polarity as well as for coupling the extracellular environment to intracellular signaling pathways and the cytoskeleton (53). A Cx43-ZO-1 interaction has been identified at the cardiac intercalated disc and at gap junctions in testis, Rat-1 fibroblasts, lung epithelial cells, and astrocytes (23, 28). ZO-1 binding is not essential for the formation of functional Cx43 channels, as evidenced by channel formation by Cx43 constructs lacking the carboxyl terminus (54, 55); however, data suggest a possible involvement of ZO-1 in the localization of Cx43 to gap junction plaques (24) as well as in the process of internalization and remodeling of Cx43 upon changes in the intracellular environment (28, 56). Another possible role for the Cx43CT-ZO-1 interaction is for ZO-1 to keep in close contact cellular partners that are important in the normal function of Cx43 gap junction channels. An abundant number of proteins involved in cell-cell junctions have been demonstrated to interact with ZO-1 (57–62).

We demonstrated that the PDZ-2 domain of ZO-1 affected residues Ser-364–Ile-382 of Cx43, a region larger than what was expected from the putative PDZ binding domain of Cx43 (42). The different dynamics between residues Ser-364–Ala-371 and Ser-372–Ile-382 indicates a possible tighter association between residues Ser-372–Ile-382, which contains the consensus-binding motif for ZO-1. The looser interactions with residues Ser-364–Ala-371 may play a more regulatory role with other Cx43 molecular partners. It is perhaps noteworthy that this region contains three phosphorylatable residues: protein kinase C phosphorylates Cx43 on Ser-368 and Ser-372 (7, 63), leading to increased junctional conductance (64–66), whereas phosphorylation of Ser-364 by cAMP-dependent protein kinase increases gap junctional communication (67, 68). Whether phosphorylation of this region alters binding affinity to intracellular ligands remains to be determined. Additionally, pH gating of the channel was found to be disrupted by deleting residues Arg-362–Ile-382 or by the point mutation S364P in this same region of Cx43CT (48).

Functional Significance of the Src Interaction and Displacement of the Cx43-ZO-1 Complex—The oncogene v-Src and its cellular homologue, c-Src, are plasma membrane-associated tyrosine kinases (69); Src activation has been correlated with an inhibition of gap junction intercellular communication, due either to Src-mediated phosphorylation on Tyr-265 and possibly Tyr-247 or to indirect activation of MAP kinase (25, 26, 42, 66, 70–74). The mechanism of channel closure has been proposed to occur through a ball-and-chain type of mechanism similar to that proposed for pH gating of Cx43 channels (51, 72, 66, 70–74). The different dynamics between residues Ser-364–Ala-371 is consistent with previous surface plasmon resonance data because under conditions in which the SH3 domain displaces the Cx43CT-PDZ-2 complex, the PDZ-2 domain shows an ~10-fold greater binding affinity for Cx43CT than the SH3 domain (428 nmol/liter versus 4.52 μmol/liter) (28). Additionally, numerous studies have determined a common PXXP motif for recognizing SH3 domains (76–79), and mutation of this motif in Cx43CT (near Pro-280) inhibits the Cx43-Src complex, which suggests that residues Ala-367–Ser-372 are not directly involved in SH3 binding Cx43CT (44). The hypothesis that the SH3 domain can induce indirect long range changes in the Cx43CT structure seems viable, however, requires further experiments.

In summary, the work described here has enabled us to use structural information to define binding domains in Cx43 and describe possible mechanisms for the communication between molecular partners via Cx43CT. This is a step forward over the use of site-directed mutagenesis and primary structure-function analysis and can be extended to the characterization of other protein-protein interactions regulating connexin function. In general, this information may be useful in describing how the structural characteristics of discretized proteins are advantageous for signaling responses to multiple different binding partners.

REFERENCES

1. Sohl, G., and Willecke, K. (2004) Cardiovase. Res. 62, 228–232
2. Moreno, A. P., Chanson, M., Elenes, S., Anunomowo, J., Scerri, I., Gu, H., and Delmar, M. (2002) Circ. Res. 90, 450–457
3. Bukauskas, F. F., Bukauskiene, A., Bennett, M. V., and Verselis, V. K. (2001) Biophys. J. 81, 137–152
4. Bukauskas, F. F., Bukauskiene, A., and Verselis, V. K. (2002) J. Gen. Physiol. 119, 171–185
5. Lampe, P. D., and Lau, A. F. (2000) Arch. Biochem. Biophys. 384, 205–215
6. Saiz, J. C., Nairn, A. C., Czernik, A. J., Fishman, G. I., Spray, D. C., and Hertzberg, E. L. (1997) J. Mol. Cell. Cardiol. 29, 2131–2145
7. Lampe, P. D., TenBroek, E. M., Burt, J. M., Kurata, W. E., Johnson, R. G., and Lau, A. F. (2000) J. Cell Biol. 149, 1503–1512
8. Francis, D., Stergiopoulous, K., Ek-Vitorin, J. F., Cao, P. L., Taffet, S. M., and Delmar, M. (1999) Den. Genet. 24, 123–136
9. Stergiopoulous, K., Alvarado, J. L., Mastroiani, M., Ek-Vitorin, J. F., Taffet, S. M., and Delmar, M. (2001) J. Cardiovasc. Electrophysiol. 2, 939–951
10. Duffy, H. S., Sorgen, P. L., Girvin, M. E., O’Donnell, P., Coombs, W., Taffet, S. M., Delmar, M., and Spray, D. C. (2002) J. Biol. Chem. 277, 36760–36764
11. Unger, V. M., Kumar, N. M., Gillula, N. B., and Veager, M. (1999) Science 283, 1176–1180
12. Siuhansian, R., Beecherberg, J. F., Cechetto, D. F., Hachinski, V. C., and Nau, C. C. (2001) J. Comp. Neurol. 440, 387–394
13. Liao, Y., Day, K. H., Damon, D. N., and Duling, B. R. (2001) Proc. Natl. Acad. Sci. U.S.A. 98, 9989–9994
14. Guerrero, P. A., Schuessler, R. B., Davis, L. M., Beyer, E. C., Johnson, C. M., Yamada, K. A., and Saffitz, J. E. (1997) J. Clin. Investig. 99, 1991–1998
15. Dermietzel, R., Gao, Y., Scemes, E., Vieira, D., Urban, M., Kremer, M., Bennett, M. V., and Spray, D. C. (2000) Brain Res. Brain Res. Rev. 32, 45–56
16. Jacobas, D. A., Urban-Maldonado, M., Jacobs, S., Scemes, E., and Spray, D. C. (2003) Physiol. Genomics 15, 177–190
17. Scemes, E.,and Sorgen, P. L., Girvin, M. E. (2002) J. Cardiovasc. Electrophysiol. 13, 1361–1375
18. Sorgen, P. L., Duffy, H. S., Cahill, S. M., Coombs, W., Spray, D. C., Delmar, M., and Girvin, M. E. (2002) J. Biochem. 25, 245–246
19. Sorgen, P. L., Duffy, H. S., Spray, D. C., and Delmar, M. (2004) Biophys. J. 87,
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Paul L. Sorgen, Heather S. Duffy, Prangya Sahoo, Wanda Coombs, Mario Delmar and David C. Spray

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