Gap junction modulation and its implications for heart function

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GAP JUNCTION COMMUNICATION IN HEALTH AND DISEASE

Gap junction communication (GJC) describes the electrical and metabolic coupling of cells through specialized cell contacts called gap junctions. In vertebrates, gap junctions are present in most tissues having important roles in development, growth regulation, tissue homeostasis, and communication. They assemble from homo- or hetero-hexameric connexin hemichannels encoded by 20 (rodents) or 21 (human) different genes (Söhl et al., 2005). GJC has been studied in great detail for the last 50 years. These studies emphasized important molecular, biophysical properties, and physiological roles of connexin channels. Other studies revealed connexin structures down to atomic resolution (Maeda et al., 2009; Groscly and Sorgen, 2013) and a multitude of regulatory mechanisms controlling the entire life cycle of these channels from transcription, post-translational modification, to removal of gap junctions and degradation (Laird, 2006; Johnstone et al., 2012a; Su et al., 2012; Thévenin et al., 2013). More recent work demonstrated connexin hemichannel functions under physiological conditions (Bruzzone et al., 2001; Anselmi et al., 2008; Garré et al., 2010) and evidence for channel independent function, e.g., in cell growth and death (Vinken et al., 2012) or migration (Kameritsch et al., 2012). Mutations in connexins were discovered in inherited human diseases like oculodentodigital dysplasia (ODDD, Cx43, GJA1; Huang et al., 2013), X-linked Charcot-Marie-Tooth disease (Cx32, GJB1; Scherer and Kleopa, 2012), Pelizaeus-Merzbacher-like disease or a milder spastic paraplegia (Cx47; Kleopa et al., 2010), Vohwinkel syndrome as well as Keratitis-Icthyosis-Deafness (KID) syndrome (Cx26, GJB2; Lee and White, 2009; Xu and Nicholson, 2013), Erythrokeratodermia variabilis (Cx31, GJB3; Cx30.3, GJB4), Clouston syndrome (Cx30, GJB6) or secondary lymphedema following breast cancer treatment (Cx47, GJC2; Finegold et al., 2012). Furthermore, transcriptional and post-transcriptional alterations and dysfunctional degradation by autophagy (Lichtenstein et al., 2011; Fong et al., 2012) may represent indirect mechanisms causing impaired GJC. Today, a causal relationship, e.g., in the context of seizures (Li et al., 2001; Gajda et al., 2005; Samoilova et al., 2008), cerebral ischemia (Contreras et al., 2004; Talhouk et al., 2008; Orellana et al., 2010), autism (Fatemi et al., 2008), schizophrenia (Meyer et al., 2002; Aleksic et al., 2007), and after trauma (Frantsëva et al., 2002) seems plausible. Thus, understanding the exact roles of GJC in health and disease is a highly relevant and timely objective in biomedical and preclinical research. Transcriptome studies have started to provide valuable insight into the consequences of altered connexin expression in animal models (Spray and Iacobas, 2007; Iacobas et al., 2012, 2007a), exploring the use of coordination analysis of gene expression as a strategy to identify connexin related gene networks. The huge amount of transcriptome data available in public databases, together with more sophisticated data processing tools, suggest that investigating transcriptional changes within a physiologically relevant “gap junction network” (GJN) will have wide application potential.

MODULATION OF GAP JUNCTION COMMUNICATION

The major cardiac connexin proteins are Cx40 (GJA5), Cx43 (GJA1), and Cx45 (GJC1), having distinct expression patterns and essential roles in propagation of action potentials, metabolic coupling, tissue homeostasis and heart development (Lo, 2000; Nishii et al., 2001; Rohr, 2004; Bernstein and Morley, 2006; Zacchigna et al., 2009; Jansen et al., 2010). Given these important functions,
it is not surprising that GJC has been associated with various heart diseases (Jongsma and Wilders, 2000; Severs, 2001; Severs et al., 2004, 2008; Tribulová et al., 2008; Rodríguez-Sinovas et al., 2012, 2013). Here, we will focus on interacting and modulating proteins, clustered in functional groups, forming the basis for a draft GJN (Figure 1). A complete list of proteins, isoforms, and putative interactions in the GJN can be found in Table 1, a list of proven interactions in Table 2, while functional evidence is presented below. We will not discuss the structurally related, non-gap junction forming pannexins, or LRRC8 (Abascal and Zardoya, 2012), although it is interesting to note that pannexins release cardioprotectants during ischemic events in the heart (Wang et al., 2009; Vessey et al., 2010, 2011; Rodríguez-Sinovas et al., 2012).

### CELL-CELL JUNCTIONAL AND SCAFFOLDING PROTEINS

A shared communality among connexins is the binding to junctional, scaffolding and cytoskeletal/transport proteins. Interactions between connexins and the tight junction proteins ZO-1, ZO-2, and ZO-3 (TJP1, TJP2, TJP3) vary regarding different connexin and ZO proteins (Giepmans and Moolenaar, 1998; Toyofuku et al., 1998; Kausalya et al., 2001), regulating connexon to gap junction transition (Rhett et al., 2011) and, as shown for ZO-1, can be regulated by c-Src in cardiac myocytes (Toyofuku et al., 2001). Increased interaction of ZO-1 with Cx43 plays a role in Cx43 down-regulation and reduced Cx43 gap junction size in congestive heart failure (Bruce et al., 2008). Cell adhesion proteins like E-cadherin (CDH1) and α-catenin are co-localized in newly formed gap junctions (Fujimoto et al., 1997), and E-cadherin mediated cell–cell contacts were shown to increase GJC (Jongen et al., 1991). p120ctn (CTNND1) (Xu et al., 2001) and β-catenin (CTNNB1) (Ai et al., 2000) also co-localize with Cx43, and Cx43 was further found to immunoprecipitate with β-catenin (Li et al., 2009). N-cadherin (CDH2)/connexin interactions were also reported (Li et al., 2009). CDH2 antibodies inhibit gap junction formation (Meyer et al., 1992), and cardiac specific CDH2 knockout in mice causes reduced GJC and sudden death (Li et al., 2005). Vinculin (VCL) interacts with connexins (Iacobas et al., 2007b), and cardiac myocyte specific VCL knockout caused Cx43 dislocation, dilated cardiomyopathy, and sudden death (Zemljic-Harpf et al., 2007). VCL also binds directly to ZO-1, stabilizing gap junctions in the heart (Zemljic-Harpf et al., 2014). The tight junction protein occludin (OCLN) was shown to interact with Cx32 (Kojima et al., 1999) and ZO-1 as well as ZO-2 (Furuse, 1994; Itoh et al., 1999).

AGS8 (FNDC1) forms a scaffold for Gβγ subunits and Cx43 and elicits phosphorylation and subsequent internalization, an effect involved in hypoxia-induced apoptosis in cardiomyocytes (Sato et al., 2009). In the brain, the scaffolding proteins MUPP1 (MPDZ) and AF6 (MLLT4) interact with Cx36 (Li et al., 2012). Membrane targeting, cellular migration and wound healing are modulated by Cx43 and interaction with the multidomain scaffolding protein CASK (Márquez-Rosado et al., 2012). Further, all three known human caveolins (CAV), a group of proteins found in lipid rafts and the membrane, interact with Cx43 (Langlois et al., 2008; Liu et al., 2010), increasing GJC (shown for CAV1 and CAV2). Drebrin (DBN1) interacts with Cx43 maintaining Cx43-containing gap junctions in their functional state (Butkevich et al., 2004), likely involving further interactions with the cytoskeleton.

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**FIGURE 1 | Simplified summary of the gap junction network.** This cartoon summarizes important signaling pathways, modulators, and interacting proteins of connexins, which converge exemplarily on a (green) connexin gap junction channel. The major functional groups outlined in the main text have been color-coded and relations between groups indicated by arrows. Further, phosphorylation (P) and dephosphorylation (-P) is highlighted. Note that the depicted pathways/interactions will vary for individual connexins. The gap junction network includes G proteins (light blue), cyclases (dark blue), kinases (violet), MAPK/ERK related signaling pathways (orange), receptors (red), scaffolding and junctional proteins (pink), cytoskeleton (dark pink), and cell cycle associated proteins (yellow).
plakophilin-2 (PKP2) (Li et al., 2009; Sato et al., 2011), a GFAP, cofilin-1) were differentially regulated when Cx43 expression changes and contribution to heart diseases (Liu et al., 2009; Joshi et al., 2012). Mammals inherit two PRKGs, cGKI (cGK, PKG, PRKG) was also reported to phosphorylate connexins and modulate their expression (Kwak et al., 1995; Patel et al., 2006; Joshi et al., 2012). Mammals inherit two PRKGs, cGKI (PRKG1) and cGKII (PRKG2), where PRKG1 is the main PRK in the heart. PRKG1 has well-known functions in the cardiovascular system, including excitation-contraction coupling, contractility, CM hypertrophic remodeling and more, where elevated cGMP levels protect against adverse ventricular remodeling (Balligand and Hammond, 2013; Frantz et al., 2013). In the failing human heart, PKA, as well as PKC and PKG, can phosphorylate cardiac ryanodine receptors, resulting in defective channel function due to increased sensitivity (Takasago et al., 1991; Marx et al., 2000). Ca2+/calmodulin-dependent protein kinase II (CaMKII) can phosphorylate Cx43, and its activation and/or increased expression occurs in cardiac disease states like infarction, hypertrophy, and myocardial ischemia (see Erickson and Anderson, 2008; Huang et al., 2011 and references within) and is therefore considered a drug target in heart failure (Bers, 2010). The δ(CAMK2D) subunit is the highest expressed CaMKII in the heart, besides the γ (CaMK2G) subunit being expressed at lower levels (Schwerer et al., 1993; Edman and Schulman, 2013). Calmodulin (CaM) activates CaMKII, and also directly modulates connexin gating properties and mediating Ca2+-induced uncoupling of gap junctions (review: Zou et al., 2014). Connexins can be modulated by casein kinase 1 (CK1) and CK2 (Cheng and Louis, 1999; Yin, 2000). Besides the finding that CK1δ (CSNK1D) regulates Cx43 gap junction assembly (Cooper and Lampe, 2002), little is known about which CKs targets for other connexins. CK2α1 dependent phosphorylation may be involved in the development of cardiac hypertrophy (Eom et al., 2011).

MAP KINASE SIGNALING CASCADES

The mitogen-activated protein kinase (MAPK) cascades are key intracellular signaling pathways regulating diverse cellular functions such as proliferation, differentiation, survival, development, stress response, and apoptosis. Multiple MAPK cascades have been identified, and although often described as linear, they display significant cross talk (Keshet and Segel, 2010). In the heart, H-Ras, K-Ras, and N-Ras are expressed (Potenza et al., 2005). MAPKs have functions in heart development and are also
Table 2 | Summary of connexin interacting proteins.

| Interacting protein | Connexin | Type of detection | References |
|---------------------|----------|------------------|------------|
| α-catenin           | GJ       | co-loc, EM, FRIL  | Fujimoto et al., 1997 |
| β-catenin           | Cx43     | co-loc in cardiac myocytes, β-catenin-IP (N) | Ai et al., 2000; Lee and White, 2009 |
| actin               | Cx43     | co-loc            | Wall et al., 2007; Smyth et al., 2012 |
| AF6                 | Cx36     | AF6-IP (N), Cx36-IP (N), co-loc | Li et al., 2012 |
| AGS8                | Cx43     | IVB, co-loc       | Sato et al., 2009 |
| CASK                | Cx36     | IVB, co-loc, FarWB, CASK-IP (N, RE) | Marquez-Rosado et al., 2012 |
| CAV1                | Cx43     | CAV1-IP (RE), co-loc, IVB | Langlois et al., 2008 |
| CAV2                | Cx43     | CAV2-IP (RE), co-loc, IVB | Langlois et al., 2008 |
| CAV3                | Cx43     | CAV3-IP (N), co-loc, IVB | Liu et al., 2010 |
| CDH1                | GJ       | co-loc, EM, FRIL  | Fujimoto et al., 1997 |
| CDH2                | Cx43     | N-cadherin-IP (N), co-loc | Lee and White, 2009 |
| drebrin             | Cx43     | co-loc, IVB       | Butkevich et al., 2004 |
| MUPP1               | Cx36     | Cx36-IP (N), MUPP1-IP (N), co-loc | Li et al., 2012 |
| occludin            | Cx43     | occludin-IP (RE), co-loc | Kojima et al., 1999 |
| p120ctn             | Cx43     | co-loc            | Xu et al., 2001 |
| PKP2                | Cx43     | PKP2-IP (N)       | Giepmans et al., 2001a,b |
| tubulin             | Cx43     | co-loc, IVB       | Iacobas et al., 2007a,b |
| vinculin            | Cx43     | AB-array, Cx43-IP (N) | Iacobas et al., 2007a,b |
| ZO-1                | Cx43     | co-loc cardiac myocytes, ZO1-IP (RE, N), Cx43-IP (RE, IVB) | Giepmans and Moelenaar, 1998; Toyofuku et al., 1998 |
| ZO-2                | Cx43     | IVB, ZO2-IP (N), Cx43-IP (N), co-loc, FarWB | Singh et al., 2005 |
| ZO-3                | Cx45     | Y2H (PDZ domains) | Kausalya et al., 2001 |
| PKA                 | Cx35     | IV/P              | Ouyang et al., 2005 |
| PKG                 | Cx36     | IV/P              | Urschel et al., 2006 |
| PKG                 | Cx50     | IV/P, in vivo phosphorylation, | Liu et al., 2011a,b |
| PKC                 | Cx43     | PKCa-IP (N), Cx43-IP (N; PKCa, PKCx, PKCs), co-loc (PKCa, PKCx, PKCs), IV/P (PKCs), PKCa-IP | Bowling et al., 2001; Niget et al., 2010 |
| PKG                 | Cx35     | IV/P              | Patel et al., 2006 |
| PKG                 | Cx43     | IV/P              | Kwak et al., 1995; Patel et al., 2006 |
| CaMKII              | Cx43     | IV/P, co-loc      | Hud et al., 2008; Huang et al., 2011 |
| calmodulin          | Cx32     | co-loc            | Peracchia et al., 2000 |
| CKI                 | Cx49     | IV/P              | Cheng and Louis, 1999 |
| CKII                | Cx45.6(a) | IV/P, Cx43-IP (N) | Cooper and Lamp, 2002 |
| MAPK7/ERK5          | Cx43     | IV/P, ERK5-IP (RE), Cx43-IP (RE) | Cameron et al., 2003 |
| c-Src               | Cx43     | Cx43-IP (N, RE)   | Toyofuku et al., 2001; Li et al., 2009 |
| CIP85               | Cx43     | Co-loc, CIP85-IP (RE, N) | Lan et al., 2005 |
| RPTPμ               | Cx43     | RPTPμ-IP (RE), Cx43-IP (N, RE) | Giepmans et al., 2003 |
| AQP0                | Cx45.6(a) | Cx45.6-IP (N), co-loc | Yu and Jiang, 2004; Yu et al., 2005 |
| P2X7                | Cx43     | Cx43-IP (N), P2X7 (N), co-loc, AB-array, | Fortes et al., 2004; Iacobas et al., 2007a,b |
| RPTPμ               | Cx43     | RPTPμ-IP (RE), Cx43-IP (N, RE) | Giepmans et al., 2003 |
| AQP0                | Cx45.6(a) | Cx45.6-IP (N), co-loc | Yu and Jiang, 2004; Yu et al., 2005 |
| P2X7                | Cx43     | Cx43-IP (N), P2X7 (N), co-loc, AB-array, | Fortes et al., 2004; Iacobas et al., 2007a,b |

Summary of connexin interacting proteins. This table summarizes documented interactions described in the text and the detection methods used. It does not include indirect interactions with regulatory pathways. Abbreviations in alphabetic order: AB-array, antibody array; av, avian connexin; co-loc, co-localization in cells or tissues; IVB, in vitro binding, binding of peptides or functional domains; FarWB, Far western blot; IVP, in vitro phosphorylation; N, native, non-transfected tissues, cells, or cell lines; RE, one or both IP partners were expressed in recombinant cells; Y2H, yeast two hybrid assay.
involved in heart disease formation (Rose et al., 2010). MAPK phosphorylation of connexins is well-documented (reviews: Giepmans, 2004; Solan and Lampe, 2005), e.g., MAPK7/ERK5 was reported to phosphorylate and associate with Cx43, regulating gap junction uncoupling (Cameron et al., 2003). The non-receptor protein tyrosine kinase protein c-Src inhibits the interaction of Cx43 and ZO-1 in cardiac myocytes (Toyofuku et al., 2001). Further, c-Src activation was shown to inhibit gap junctional coupling and remodeling in ischemic heart disease (review: Giepmans, 2004; Rutledge et al., 2012). A Rab-GAP-like protein, CIP85, interacts with Cx43 and induce its internalization and degradation (Lan et al., 2005; Cochrane et al., 2013).

**HETERTRIMERIC G-PROTEINS**

G proteins can interact with GJC by their activation/inhibition of different signaling cascades, e.g., via adenyl cyclase or phospholipase C (see below). General consent is that GNAI2 is the main Gα in the heart, GNAI3 is expressed in lower amounts and GNAI1 is not expressed (Eschenhagen et al., 1992). However, there are few studies investigating expression of GNAI1 in detail. One newer study reports some cardiac GNAI1 expression (Dizayee et al., 2011) in the heart, alongside the knowledge of its expression in erythrocytes (Olearczyk et al., 2004) and thrombocytes (Patel et al., 2003). GNAI2 is thought to be up-regulated in various heart diseases, but maybe not in ischemic heart disease (ICM) (Feldman et al., 1988; Neumann et al., 1988; Böhm et al., 1990; Eschenhagen et al., 1992). Lack of Gαq leads to tachycardia and defects in short-term heart rate dynamics (Zuberi et al., 2008). Gαq and G11 may be involved in gap junction assembly, as pertussis toxin (PTX) sensitive G proteins were linked to Cx43 trafficking (Lampe et al., 2001). Overexpression of GαqGNAS) causes many features of dilated cardiomyopathy (DCM) (Iwase et al., 1997), and haplotypes causing different expression levels of Gαq have been found in humans (Frey et al., 2009), providing a putative link to heart disease risk. Gαq (GNAQ) overexpression leads to heart hypertrophy and contractile failure in transgenic mice (D’Angelo et al., 1997; Fan et al., 2005), and knockout prevents ventricular hypertrophy in response to pressure-overload (Wettchureck et al., 2001). Gα13 regulates the expression of hypertrophic and fibrotic genes in cardiomyocytes, and inactivation prevents cardiac decompensation (Finn, 1999; Takefuji et al., 2012).

**CYCLASES AND PHOSPHOLIPASE C**

Modulators of the soluble guanylate cyclase (sGC, GUCY) are promising new drugs for heart failure treatment (Mitrovic et al., 2011). sGC is a heterodimer composed of one α (GUCYA), and one heme-binding β domain (GUCYB), of which sGCα1β1 is the principal heteromer in the heart (see Mitrovic et al., 2011 and references within). Adenyl cyclase type III (ACDy) is considered a therapeutic target for heart diseases, where from 10 known ADCYs ADCY5 and ADCY6 are the predominant ones in the heart, expressed in a development-dependent way (e.g., Feldman, 2002 and references within), but several others are also expressed (Ludwig and Seuwen, 2002). Phospholipase C (PLC) cleaves phosphatidylinositol 4,5-bisphosphate (PIP2) into DAG and inositol 1,4,5-trisphosphate (IP3). DAG remains bound to the membrane, and IP3 is released as a soluble structure into the cytosol activating IP3 calcium channels in the smooth endoplasmic reticulum. In addition, calcium and DAG active PKC. A majority of the 15 known PLCs is present in the heart and some were linked to heart dysfunction (Schwertz and Halverson, 1992; Meij et al., 1997; Hwang et al., 2004; Mangat et al., 2006; Ichise et al., 2009; Otaegui et al., 2010). PLCβ3 was reported to colocalize with Cx43, via the scaffolding protein ZO-1 (see below), where localized changes in PIP2 levels dictate channel inhibition (Van Zeijl et al., 2007).
with cyclin E (CCNE1), for example after MAPK phosphorylation, promoting smooth muscle cell proliferation (Johnstone et al., 2012b). Cx43 also competes with cyclin D1 for binding to heat shock protein 70 (HSP70) (Hatakeyama et al., 2013). Further, degradation of connexins was linked to binding to tumor susceptibility gene 101 (TSG101), an ubiquitin–conjugating enzyme associated with the cell cycle, turnover of proteins, and transcriptional regulation (Auth et al., 2009). Cyclin-dependent kinase 2 (CDC2) was shown to phosphorylate Cx43 in a cell-cycle dependent manner (Kanemitsu et al., 1998; Lampe et al., 1998). Connexins also interact with BAX, a member of the Bcl-2 protein family located in the outer mitochondrial membrane, to regulate apoptosis (Sun et al., 2012).

**FUTURE DIRECTIONS: TOWARD META-ANALYSIS OF THE GAP JUNCTION NETWORK?**

Experimental investigation of the GJN is challenging, due to the large number of putative interactions, procedural issues or the huge experimental variations caused by small sample sizes frequently found in studies using human tissues. However, meta-analyses can capitalize from the growing number of multiple microarray and other “–omics” studies publicly available. Technically, different approaches to merge and perform a statistical analysis have been established and various software tools allow users to process microarray data (Saeed et al., 2003; Gentleman et al., 2004; Reich et al., 2006; Tseng et al., 2012; Xia et al., 2013). Unfortunately, cross-comparison of studies is still a major challenge, but the recently developed online platform INMEX (Xia et al., 2013), or a LabVIEW-based software tool called Array Data Extractor (ADE) (Kurtenbach et al., 2013) are efforts toward making microarray data available in a user-friendly way to a large community. This opens the opportunity to test physiologically relevant changes of the proposed GIN in health and disease.

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**REFERENCES**

Abascal, F., and Zardoya, R. (2012). LRRCS8 proteins share a common ancestor with pannexins, and may form hexameric channels involved in cell-cell communication. *Bioessays* 34, 551–560. doi: 10.1002/bies.201100173

Abdelmohsen, K., Gerber, P. A., von Montfort, C., Sies, H., and Klotz, L.-O. (2003). Epidermal growth factor receptor is a common mediator of quinone-induced signaling leading to phosphorylation of connexin-43: role of glutathione and tyrosine phosphatases. *J. Biol. Chem.* 278, 38360–38367. doi: 10.1074/jbc.M306785200

Ai, Z., Fischer, A., Spray, D. C., Brown, A. M., and Fishman, G. I. (2000). Wnt-1 regulation of connexin43 in cardiac myocytes. *J. Clin. Invest.* 105, 161–171. doi: 10.1172/JCI7798

Alekseic, B., Ishihara, R., Takahashi, N., Maeno, N., Ji, X., Saito, S., et al. (2007). Gap junction coding genes and schizophrenia: a genetic association study. *J. Hum. Genet.* 52, 498–501. doi: 10.1007/s10038-007-0142-2

Anselmi, F., Hernandez, V. H., Crispino, G., Seydel, A., Ortolano, S., Roper, S. D., et al. (2008). ATP release through connexin hemichannels and gap junction transfer of second messengers propagate Ca2+ signals across the inner ear. *Proc. Natl. Acad. Sci. U.S.A.* 105, 18770–18775. doi: 10.1073/pnas.0807931015

Auth, T., Schlüter, S., Urschel, S., Kussmann, P., Sonntag, S., Höher, T., et al. (2009). The TSG101 protein binds to connexins and is involved in connexin degradation. *Exp. Cell Res.* 315, 1053–1062. doi: 10.1016/j.yexcr.2008.12.025

Balligand, J.-L., and Hammond, J. (2013). Protein kinase G type I in cardiac myocytes: unmasked at last? *Eur. Heart J.* 34, 1181–1185. doi: 10.1093/eurheartj/ehr145

Barbino, R., Gierschik, P., Jakobs, K. H., Piekars, B., Schnabl, P., Ungerer, M., et al. (1999). Increase of GI alpha in human hearts with dilated but not ischemic cardiomyopathy. *Circulation* 82, 1249–1265.

Boling, N., Huang, X., Sandusky, G. E., Fouts, R. L., Mintze, K., Esteman, M., et al. (2001). Protein kinase C-alpha and -epsilon modulate connexin-43 phosphorylation in human heart. *J. Mol. Cell. Cardiol.* 33, 789–798. doi: 10.1016/j.mcc.2000.1349

Bruce, A. F., Rothery, S., Dupont, E., and Severs, N. J. (2008). Gap junction remodelling in human heart failure is associated with increased interaction of connexin43 with ZO-1. *Cardiac Res.* 77, 757–765. doi: 10.1016/crvm083

Bruzzone, S., Guida, L., Zocchi, E., Franco, L., and De Flora A. (2001). Connexin 43 hemi channels mediate Ca2+–regulated transmembrane NAD+ fluxes in intact cells. *FASEB J.* 15, 10–12. doi: 10.1096/fj.00-05666e

Butkevich, E., Hülsmann, S., Wenzel, D., Shirao, T., Duden, R., and Majouli, I. (2004). Drehrin is a novel connexin-43 binding partner that links gap junctions to the submembrane cytoskeleton. *Carr. Biol.* 14, 650–658. doi: 10.1016/j.cub.2004.03.063

Cameron, S. I., Malik, S., Akaïke, M., Lerner-Marmarosh, N., Yan, C., Lee, J.-D., et al. (2003). Regulation of epidermal growth factor-induced connexin 43 gap junction communication by big mitogen-activated protein kinase 1/ERK5 but not ERK1 kinase activation. *J. Biol. Chem.* 278, 18682–18688. doi: 10.1074/jbc.M213283200

Chansons, M., Kotisias, B. A., Peracchia, C., and Grady, S. M. (2007). Interactions of connexins with other membrane channels and transporters. *Prog. Biophys. Mol. Biol.* 94, 233–244. doi: 10.1016/j.pbiomolbio.2007.03.002

Cheng, H. L., and Louis, C. F. (1999). Endogenous casein kinase I catalyzes the phosphorylation of the lens fiber cell connexin49. *Eur. J. Biochem.* 263, 276–286. doi: 10.1046/j.1432-1327.1999.00502.x

Chintalattu, V., Ai, D., Langley, R. K., Zhang, J., Bankson, J. A., Shih, T. L., et al. (2010). Cardiomyocyte PDGF-beta signaling is an essential component of the mouse cardiac response to load-induced stress. *J. Clin. Invest.* 120, 472–484. doi: 10.1172/JCI49343

Chong, I. J. H., Reinecke, H., Iwata, M., Torok-Storb, B., Stempien-Otero, A., and Murry, C. E. (2013). Progenitor cells identified by PDGF-beta alpha expression in the developing and diseased human heart. *Stem Cells Dev.* 22, 1932–1943. doi: 10.1089/scd.2012.0542

Cochrane, K., Su, V., and Lau, A. F. (2013). The connexin43-interacting protein, CIP35, mediates the internalization of connexin43 from the plasma membrane. *Cell Commun. Adhes.* 20, 53–66. doi: 10.3109/15419061.2013.784745

Contreras, J. E., Sánchez, H. A., Veliz, L. P., Bukauskas, F. F., Bennett, M. V., and Slez, J. C. (2004). Role of connexin-based gap junction channels and hemichannels in ischemia-induced cell death in nervous tissue. *Brain Res. Brain Res. Rev.* 47, 290–303. doi: 10.1016/j.brainresrev.2004.08.002

Cooper, C. D., and Lampe, P. D. (2002). Casein kinase 1 regulates connexin-43 gap junction assembly. *J. Biol. Chem.* 277, 44962–44968. doi: 10.1074/jbc.M209427200

D’Angelo, D. D., Sakata, Y., Lorenz, J. N., Boivin, G. P., Walsh, R. A., Liggett, S. B., et al. (1997). Transgenic Galphai overexpression induces cardiac contractile failure in mice. *Proc. Natl. Acad. Sci. U.S.A.* 94, 8121–8126.

Diez, J. A., Elvira, M., and Villalobo, A. (1998). The epidermal growth factor receptor tyrosine kinase phosphorylates connexin32. *Mol. Cell. Biochem.* 187, 210–213. doi: 10.1016/S0300-9825(97)00272-5

Dizayee, S., Kaestner, S., Kuck, F., Hein, P., Klein, C., Piekorz, R. P., et al. (2011). Guz1- and Guz3-specific regulation of voltage-dependent L-type calcium channels in cardiomyocytes. *PLoS ONE* 6:e24979. doi: 10.1371/journal.pone.0024979
Dubé, E., Dufresne, J., Chan, P. T. K., and Cyr, D. G. (2012). Epidermal growth factor regulates connexin 43 in the human epidermis: role of gap junctions in azoospermia. *Hum. Reprod.* 27, 2285–2296. doi: 10.1093/humrep/des164

Edman, C. F., and Schulman, H. (1994). Identification and characterization of delta B-CaM kinase and delta C-CaM kinase from rat heart, two new multifunctional Ca"++/calmodulin-dependent protein kinase isoforms. *Biochim. Biophys. Acta* 1221, 89–101.

Eom, G. H., Cho, Y. K., Ko, J.-H., Shin, S., Choe, N., Kim, Y., et al. (2011). Casein kinase-2 induces hypertrophic response by phosphorylation of histone deacetylase 2 S394 and its activation in the heart. *Circulation* 123, 2392–2403. doi: 10.1161/CIRCULATIONAHA.110.003665

Erickson, J. R., and Anderson, M. E. (2008). CaMKII and its role in cardiac arrhythmia. *J. Cardiovasc. Electrophysiol.* 19, 1332–1336. doi: 10.1110/jcn.1540-8167.2008.01295x

Eschenhagen, T., Mende, U., Nose, M., Schmitz, W., Haverich, A., et al. (1992). Increased messenger RNA level of the inhibitory G protein alpha subunit Gi alpha-2 in human end-stage heart failure. *Circ. Res.* 70, 688–696. doi: 10.1161/01.RES.70.4.688

Fan, G., Jiang, Y.-P., Lu, Z., Martin, D. W., Kelly, D. J., Zuckerman, J. M., et al. (2005). A transgenic mouse model of heart failure using inducible Galapha q. *J. Biol. Chem.* 280, 40337–40346. doi: 10.1074/jbc.M506812000

Fatemi, S. H., Folsom, T. D., Reitman, T. J., and Lee, S. (2008). Expression of astrocytic markers aquaporin 4 and connexin 43 is altered in brains of subjects with autism. *Synapse* 62, 501–507. doi: 10.1002/syn.20519

Feldman, A. M. (2002). Adenyl cyclase: a new target for heart failure therapeutics. *Circulation* 105, 1876–1878. doi: 10.1161/01.CIR.0000019695.24080.12

Feldman, A. M., Cates, A. E., Vaezy, W. B., Hershberger, R. E., Bristow, M. R., Baughman, K. L., et al. (1988). Increase of the 40,000-mol wt pertussis toxin substrate (G protein) in the failing human heart. *J. Cardiovasc. Electrophysiol.* 19, 893–913. doi: 10.1161/01.RES.70.4.688

Fujimoto, K., Nagafuchi, A., Tsukita, S., Kuraoka, A., Ohokuma, A., and Shibata, H. (2002). Connexin43 functions as a novel interacting partner of heat shock cognate protein 70. *Sci. Rep.* 3, 2719. doi: 10.1038/srep02719

Hossain, M. Z., Ao, P., and Boynton, A. L. (1998a). Platelet-derived growth factor-induced disruption of gap junctional communication and phosphorylation of connexin 43 involves protein kinase C and mitogen-activated protein kinase. *J. Cell. Physiol.* 176, 66–77. doi: 10.1002/(SICI)1097-4652(199808)176:2<66::AID-JCP2>3.0.CO;2-5

Hossain, M. Z., Ao, P., and Boynton, A. L. (1998b). Rapid disruption of gap junctional communication and phosphorylation of connexin43 by platelet-derived growth factor receptor. *J. Cell. Physiol.* 174, 66–77. doi: 10.1002/(SICI)1097-4652(199801)174:1<66::AID-JCP8>3.0.CO;2-E

Hossain, M. Z., Jagdale, A. B., Ao, P., and Boynton, A. L. (1999a). Mitogen-activated protein kinase and phosphorylation of connexin43 are not sufficient for the disruption of gap junctional communication by platelet-derived growth factor and tetradecanoylphorbol acetate. *J. Cell. Physiol.* 179, 87–96. doi: 10.1002/(SICI)1097-4652(199904)179:1<87::AID-JCP11>3.0.CO;2-5

Hossain, M. Z., Jagdale, A. B., Ao, P., Kazlauskas, A., and Boynton, A. L. (1999b). Disruption of gap junctional communication by the platelet-derived growth factor is mediated via multiple signaling pathways. *J. Biol. Chem.* 274, 10489–10496. doi: 10.1074/jbc.M110.10489

Huang, R. Y.-C., Laing, J. G., Kanter, E. M., Berthoud, V. M., Bao, M., Rohrs, H. W., et al. (2011). Identification of CaMKII phosphorylation sites in Connexin43 by high-resolution mass spectrometry. *J. Proteome Res.* 10, 1098–1109. doi: 10.1021/pr1008702

Huang, T., Shao, Q., MacDonald, A., Xin, L., Lorentz, R., Bai, D., et al. (2013). Autosomal recessive GJA1 (Cx43) gene mutations cause oculodenatal dystasia by distinct mechanisms. *J. Cell Sci.* 126, 2857–2866. doi: 10.1242/jcs.123515

Hund, T. J., Decker, K. F., Kanter, E., Mohler, P. J., Boyden, P. A., Schuessler, R. B., et al. (2008). Role of activated CaMKII in abnormal calcium homeostasis and I(Na) remodeling after myocardial infarction: insights from mathematical modeling. *J. Mol. Cell. Cardiol.* 45, 420–428. doi: 10.1016/j.yjmcc.2008.06.007

Iacobas, D. A., Iacobas, S., and Spray, D. C. (2007a). Connexin-dependent transcellular transmyocardial exchanges in mouse brain. *Prog. Biophys. Mol. Biol.* 94, 169–185. doi: 10.1016/j.pbiomolbio.2007.03.015

Iacobas, D. A., Suadicani, S. O., Iacobas, S., Chrismas, C., Cohen, M. A., Spray, D. C., et al. (2007b). Gap junction and purinergic P2 receptor proteins as a functional unit: insights from transcriptions. *J. Membr. Biol.* 217, 85–91. doi: 10.1007/s00232-007-9039-7

Gentleman, R. C., Carey, V. J., Bates, D. M., Bolstad, B., Dettling, M., Dudoit, S., et al. (2004). Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol.* 5, R80. doi: 10.1186/gb-2004-5-10-r80

Giepmans, B. N., and Moolenaar, W. H. (1998). The gap junction protein connexin 43 interacts with the PDZ domain of the zona occludens-1 protein. *Curr. Biol.* 8, 931–934. doi: 10.1016/S0960-9821(07)00357-2

Grosely, R., and Sorgen, P. L. (2013). A history of gap junction structure: hexagonal arrays to atomic resolution. *Cell Commun. Adhes.* 20, 11–20. doi: 10.3109/15419060.2013.773259

Hatakeyama, T., Dai, P., Harada, Y., Hino, H., Tsukahara, F., Maru, Y., et al. (2013). Connexin43 functions as a novel interacting partner of heat shock cognate protein 70. *Sci. Rep.* 3, 2719. doi: 10.1038/srep02719

Huang, R. Y.-C., Laing, J. G., Kanter, E. M., Berthoud, V. M., Bao, M., Rohrs, H. W., et al. (2011). Identification of CaMKII phosphorylation sites in Connexin43 by high-resolution mass spectrometry. *J. Proteome Res.* 10, 1098–1109. doi: 10.1021/pr1008702
Solan, J. L., and Lampre, P. D. (2005). Connexin phosphorylation as a regulatory event linked to gap junction channel assembly. *Biochim. Biophys. Acta* 1711, 154–163. doi: 10.1016/j.bbamem.2004.09.013

Spray, D. C., and Jacobs, D. A. (2007). Organizational principles of the connexin-related brain transcriptome. *J. Membr. Biol.* 218, 39–47. doi: 10.1007/s00232-007-9049-5

Su, V., Cochrane, K., and Lau, A. F. E. (2012). Degradation of connexins through the proteasomal, endolyosomal and phagolysosomal pathways. *J. Membr. Biol.* 245, 389–400. doi: 10.1007/s00232-012-9461-3

Sun, Y., Zhao, X., Yao, Y., Qi, X., Yuan, Y., and Hu, Y. (2012). Connexin 43 interacts with Bax to regulate apoptosis of pancreatic cancer through a gap junction-independent pathway. *Int. J. Oncol.* 41, 941–948. doi: 10.3892/ijo.2012.1524

Takasago, T., Imagawa, T., Furukawa, K., Ogurusu, T., and Shigekawa, M. (1991). Regulation of the cardiac ryanodine receptor by protein kinase-dependent phosphorylation. *J. Biochem.* 109, 163–170.

Takefuji, M., Wirth, A., Lukasova, M., Takefuji, S., Boettger, T., Braun, T., et al. (2012). G13-mediated signaling pathway is required for pressure overload-induced cardiac remodeling and heart failure. *Circulation* 126, 1972–1982. doi: 10.1161/CIRCULATIONAHA.112.109256

Talhouk, R. S., Zeinieh, M. P., Mikati, M. A., and El-Sabban, M. E. (2008). Gap junction modulation heart disease. *Circ. Res.* 103, E28–E34. doi: 10.1161/CIRCRESAHA.107.162356

Vessey, D. A., Li, L., and Kelley, M. (2010). Pannexin-I/P2X7 purinergic receptors mediate the release of cardioprotectants induced by ischemic preconditioning. *Biochim. Biophys. Acta* 1801, 167–178. doi: 10.1016/j.bbamem.2012.06.024

Xu, X., Li, W. E., Huang, G. Y., Meyer, R., Chen, T., Luo, Y., et al. (2001). Modulation of mouse neural crest cell motility by N-cadherin and connexin 43 gap junctions. *J. Cell Biol.* 154, 217–230. doi: 10.1083/jcb.200105047

Yin, X. (2000). Casein Kinase II Phosphorylates Lys Connexin 45.6 and is involved in its degradation. *J. Biol. Chem.* 275, 6850–6856. doi: 10.1074/jbc.275.10.6850

Yu, X. S., and Jiang, J. X. (2004). Interaction of major intrinsic protein (aquaporin-0) with fiber connexins in lens development. *J. Cell Sci.* 117, 871–880. doi: 10.1242/jcs.000945

Yu, X. S., Yin, X., Lafer, E. M., and Jiang, J. X. (2005). Developmental regulation of the direct interaction between the intracellular loop of connexin 45.6 and the C terminus of major intrinsic protein (aquaporin-0). *J. Biol. Chem.* 280, 22081–22090. doi: 10.1074/jbc.M414377200

Zacchigna, S., Oh, H., Wilsch-Bräuning, M., Missol-Kolka, E., Jäszai, I., Jansen, S., et al. (2009). Loss of the cholesterol-binding protein prominin-1/CD133 causes disk morphogenesis and photoreceptor degeneration. *J. Neurosci.* 29, 2297–2308. doi: 10.1523/JNEUROSCI.2043-08.2009

Zemljic-Harpf, A. E., Godoy, J., Platoshyn, O., Asfaw, E. K., Busija, A. R., Domenighetti, A. A., et al. (2014). Vinculin directly binds zonula occludens-1 and is essential for stabilizing connexin 43 containing gap junctions in cardiac Myocytes. *J. Cell Sci.* 127, 14374–14373. [Epub ahead of print].

Zemljic-Harpf, A. E., Miller, J. C., Henderson, S. A., Wright, A. T., Manso, A. M., Elsherif, L., et al. (2007). Cardiac myocyte-specific excision of the vinculin gene disrupts cellular junctions, causing sudden death or dilated cardiomyopathy. *Mol. Cell. Biol.* 27, 7522–7537. doi: 10.1128/MCB.00728-07

Wettchureck, N., Rütten, H., Zywietz, A., Gehring, D., Wilkie, T. M., Chen, J., et al. (2001). Absence of pressure overload induced myocardial hypertrophy after conditional inactivation of Galpha(q)/Galpha11 in cardiomyocytes. *Nat. Med.* 7, 1236–1240. doi: 10.1038/nm1166

Winter, C. R., and Baker, R. C. (1995). L-glutamate-induced changes in intracellular calcium oscillation frequency through non-classical glutamate receptor binding in cultured rat myocardial cells. *Life Sci.* 57, 1925–1934. doi: 10.1016/0024-3205(95)01217-9

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