REVIEW

Determining How Defects in Connexin43 Cause Skeletal Disease

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Summary: Gap junction channels mediate direct cell–cell communication via the exchange of second messengers, ions, and metabolites from one cell to another. Mutations in several human *connexin* (*cx*) genes, the subunits of gap junction channels, disturb the development and function of multiple tissues/organs. In particular, appropriate function of Cx43 is required for skeletal development in all vertebrate model organisms. Importantly, it remains largely unclear how disruption of gap junctional intercellular communication causes developmental defects. Two groups have taken distinct approaches toward defining the tangible molecular changes occurring downstream of Cx43-based gap junctional communication. Here, these strategies for determining how Cx43 modulates downstream events relevant to skeletal morphogenesis were reviewed. genesis 00:1–8, 2012. **© 2012 Wiley Periodicals, Inc.**

Key words: gap junction; Cx43; bone growth; skeletal development; skeletal tissue; limb/wing/appendage tissue; signaling process

Gap junctions are proteinaceous channels that lie in the plasma membranes between adjacent cells, permitting the exchange of small molecules including ions and second messengers (Goodenough *et al*., 1996; Kumar and Gilula, 1996), and playing critical roles in development and homeostasis. Connexins (cx), the subunits of gap junctions, consist of four transmembrane-spanning domains, two extracellular loops, one intracellular loop, and intracellular amino and carboxy ends. Six connexins comprise a connexon, or hemichannel, and a functional gap junction channel is composed of two connexons (one from each cell, see Fig. 1). Connexons may be homomeric (same connexin isotype) or heteromeric (two or more connexin isotypes), increasing the potential for specificity differences in the types of molecules shared between cells. For example, mouse Cx43 and Cx45 can assemble into heteromeric gap junctions that exhibit coupling properties distinct from either homomeric type (Martinez *et al*., 2002). Several studies show that Cx43 is important for the growth and development of the vertebrate skeleton. However, little is known mechanistically regarding how Cx43 regulates events such as cell proliferation and cell differentiation. Thus, the challenge is to identify the tangible events regulated by gap junctional intercellular communication (GJIC). In this review, we will first summarize the important roles of Cx43 in skeletal development. Second, we will discuss two independent experimental strategies aimed at revealing how Cx43 mediates downstream molecular signaling events, in turn altering cell function.

CX43 EXHIBITS CONSERVED FUNCTIONS IN THE VERTEBRATE SKELETON

Defective Cx43 function causes skeletal defects in human, mouse, chick, and zebrafish. Missense mutations in human *CX43* result in an autosomal dominant disorder called oculodentodigital dysplasia (ODDD, Paznekas *et al*., 2003). Patients with ODDD exhibit major skeletal malformations and craniofacial abnormalities such as cranial hyperostosis and broad tubular bones. This disease has additional pleiotropic phenotypes, including eye abnormalities leading to vision loss and dental anomalies (reviewed in Pfenniger *et al*., 2010). The range of phenotypes affecting multiple tissues is

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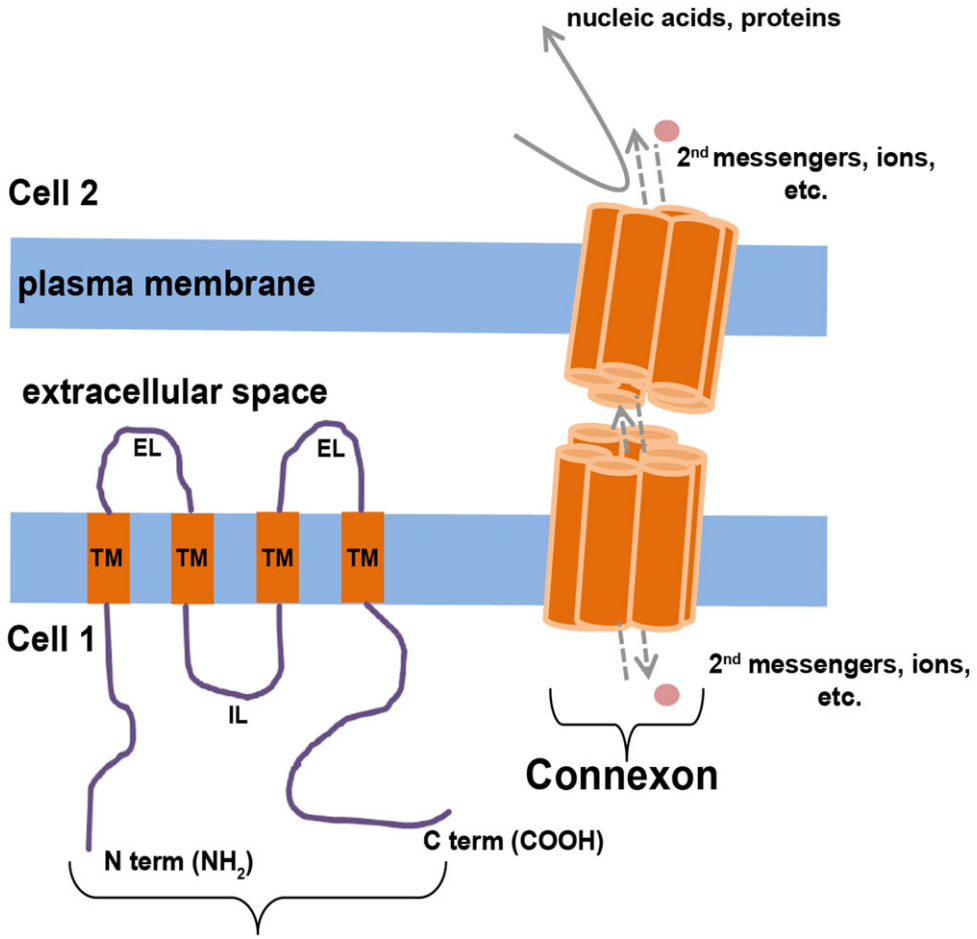

FIG. 1. Gap junction channels connect the cytoplasm of adjacent cells. Each connexin contains four transmembrane-spanning domains, with both the amino and carboxy ends located in the cytoplasm. Six connexins comprise a connexon, or hemichannel. Two connexons, one from each cell, dock together at the plasma membrane to make a single gap junction channel. IL, intracellular loop; EL, extracellular loop; TM, transmembrane-spanning domain.

likely due to the fact that *CX43* is expressed in most cell types. The $Gja1^{jrt/+}$ mouse carries a mutation coding for a dominant missense mutation in the *CX43* gene, and exhibits skeletal and pleiotropic phenotypes similar to those observed in human ODDD (Flenniken *et al.*, 2005). Indeed, recently generated knock-in alleles, where human Cx43-missense mutations replace the endogenous wild-type Cx43 allele in the mouse, also mimic human ODDD (Dobrowolski *et al.*, 2008; Watkins *et al.*, 2011). In contrast, the *CX43* knockout mouse ($CX43^{-/-}$) dies perinatally because of defects in the heart outflow tract and reduced blood flow to tissues (Reaume *et al.*, 1995). Continued investigation revealed that these mice exhibit delayed ossification of both the intramembranous and endochondral skeletons (Lecanda *et al.*, 2000). To further overcome the lethality of $CX43^{-/-}$, conditional *CX43* knockout mouse lines have been generated. Although mice lacking Cx43 activity in osteoblasts are viable, they exhibit reduced bone mineral density throughout their skeletons, consistent with phenotypes observed in the complete knockout (Bivi *et al.*, 2012; Chung *et al.*, 2006; Watkins *et al.*, 2011; Zhang *et al.*, 2011). Targeted gene knockdown of *CX43* in adult chicks and in chick embryos results in facial defects and limb malformation, respectively (Makarenkova and Patel, 1999; McGonnell *et al.*, 2001). Finally, in the zebrafish, homozygous mutations in the *cx43* gene cause the short fin (*sof* ^{b123}) phenotype, which primarily affects the fin skeleton. The *sof* ^{b123} mutant exhibits short bony fin rays because of short bony segments and reduced levels of cell proliferation (Iovine *et al.*, 2005). Targeted gene knockdown of *cx43* causes embryonic heart defects, suggesting *cx43* is essential (Iovine *et al.*, 2005). Collectively, these data demonstrate that Cx43 function is conserved from fish to man and is required for typical skeletal morphogenesis in vertebrates.

There is some evidence that connexins, including Cx43, may exhibit activities independent of, or in addition to, GJIC. For example, as Cx43 in particular has a

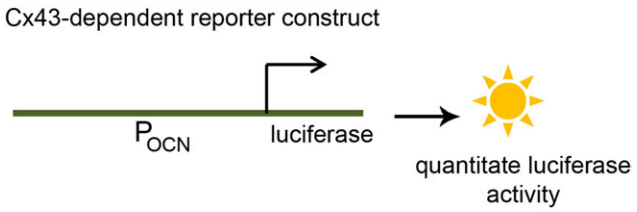

FIG. 2. The in vitro system used to evaluate Cx43-dependent regulation of gene expression. The reporter construct contains the osteocalcin (OCN) promoter followed by the coding sequence for the luciferase gene. The level of luciferase transcription is determined by quantitating the amount of luciferase activity. Osteoblast cell lines transfected with this reporter demonstrate that luciferase activity is dependent on the level of Cx43-based function.

long carboxy tail exposed to the cytoplasm, it may serve as a signaling platform that functions independent of GJIC. Indeed the carboxy tail of Cx43 is known to bind to the components of signaling complexes including β-catenin and src (Giepmans, 2004; Herve *et al.*, 2004; Saez *et al.*, 2003). Alternatively, the signaling complexes may be activated by GJIC, such that channel function regulates signaling events. There is also evidence that Cx43 hemichannels play important functions in skeletal growth and development (reviewed in Plotkin, 2011). Connexin hemichannels are unpaired connexons that may permit the exchange of small molecules between the cytoplasm and the extracellular space. The focus of this review is on current strategies used to define the cellular events occurring downstream of Cx43 function, whether as a gap junction channel, a hemichannel, or in a channel-independent manner. Thus, it should be noted that while GJIC is often the presumed function for Cx43 in the described studies, its precise role(s) has not been exclusively determined.

Cx43 Regulates Gene Expression in Osteoblast Cell Lines

It is well established that Cx43 enhances the expression of osteoblast genes. Recently, considerable effort has gone into defining the molecular mechanisms underlying Cx43-based regulation of gene expression. This work has been completed using a reporter assay in osteoblast cell lines (i.e., ROS17/2.8 cells and/or MC3T3 cells). The reporter construct contains the promoter of the osteoblast-specific gene osteocalcin upstream of the luciferase coding sequence (Fig. 2). Luciferase activity can be precisely calculated and is directly proportional to the level of gene transcription from the osteocalcin promoter. When Cx43 function is high, luciferase activity is high. When Cx43 function is abrogated either by over-expression of Cx45 (which has been shown to reduce Cx43-dependent GJIC by modifying the size and specificity of the heteromeric gap junction channel, Lecanda *et al.*, 1998) or by the addition of pharmacological inhibitors of GJIC (Stains and Civitelli, 2005), luciferase activity is reduced. Using this system, two distinct Cx43-dependent response elements have been identified in the osteocalcin promoter, and a mechanism for Cx43-dependent transcriptional activation has been suggested (described below and shown in Fig. 3).

Using the system described earlier, a minimal Cx43 response element (CxRE) was identified in the osteocalcin promoter (Stains *et al.*, 2003). It was determined that Cx43 function regulated the level of phosphorylation of the transcription factor Sp1, which in turn is bound to the CxRE. Thus, increased phosphorylation of Sp1 favored its recruitment to the CxRE and caused increased gene transcription. In contrast, reduced phosphorylation of Sp1 favored the recruitment of an alternate transcription factor, Sp3, and leads to reduced gene transcription. Continued studies revealed that the phosphorylation of Sp1 occurred through Cx43-dependent activation of the ERK signal transduction cascade (Stains and Civitelli, 2005). Moreover, this Cx43-dependent recruitment of Sp1 was also correlated with recruitment of the osteoblast transcription factor Osx/Sp7 to the osteocalcin promoter (Niger *et al.*, 2011). A second pathway for Cx43-dependent gene transcription occurs via activation of the transcription factor Runx2. Phosphorylated Runx2 interacts with the osteoblast-specific element OSE2, also found in the osteocalcin promoter. Cx43 function leads to activation of PKCδ, which is an intermediate in the FGF2 signaling pathway. Activation of PKCδ leads to the phosphorylation of Runx2 and increased gene transcription (Lima *et al.*, 2009). Alternatively, FGF2 signaling can activate ERK independent of Cx43, also leading to Runx2-phosphorylation (Niger *et al.*, 2012). These data provide evidence that FGF2 and Cx43 synergize to influence Runx2-phosphorylation and therefore increased gene expression of at least a subset of osteoblast-specific genes.

How are the ERK and FGF2 growth factor dependent signaling cascades related to Cx43 function? The Stains group suggests that both primary and secondary responses to growth factor-mediated signal transduction pathways are responsible for coordinated regulation of gene expression among a population of osteoblasts (Fig. 3). The primary response occurs in cells expressing appropriate receptors for externally provided cues, such as growth factors. Growth factor binding to its receptor leads to the intracellular production of second messengers, which in turn can be shared with adjacent cells via gap junctions, thereby promoting the same response in neighboring cells (i.e., even when those cells lack the appropriate receptor). The identification of signal transduction cascades working synergistically with Cx43-based gap junctions suggests that one role of gap junctions may be to amplify typical growth-factor induced responses. Moreover, as the carboxy-tail of connexins may act as a signaling center by binding to com-

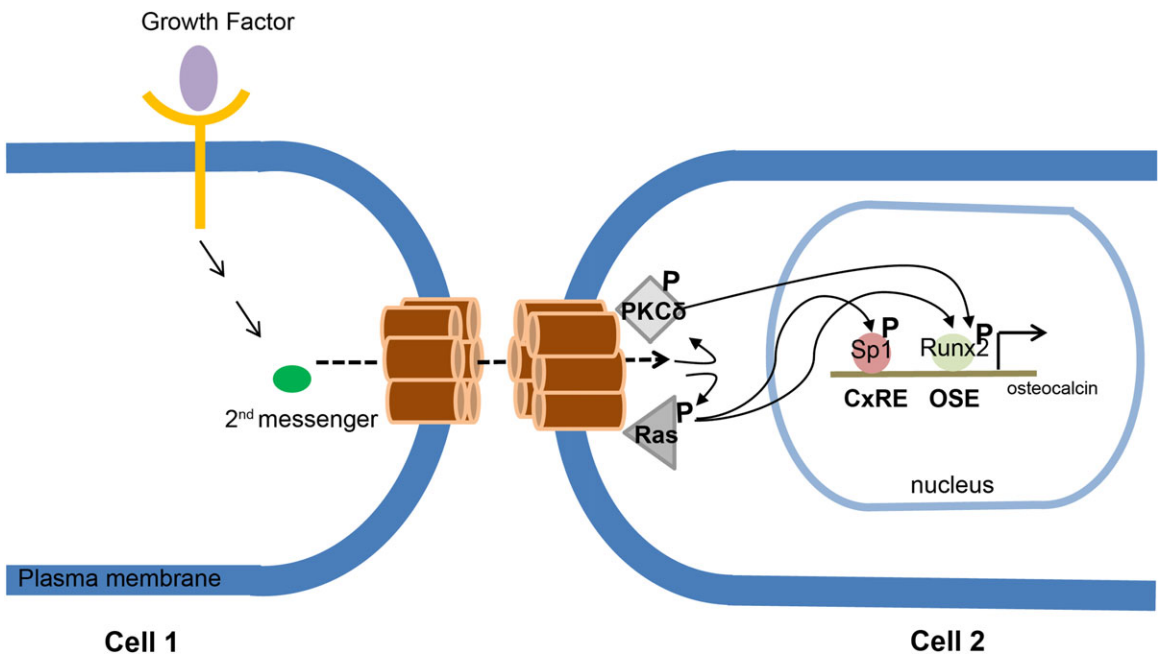

FIG. 3. Model showing how gap junctions may amplify signals originating from growth-factor mediated cascades. A primary signal originates from a growth factor-receptor interaction that can initiate a response/change in gene expression in cell one (not drawn). At the same time, second messengers resulting from the primary signal transverse gap junction channels to initiate a similar change in gene expression in cell two. This is the secondary signal. Described Cx43-dependent changes include Ras/ERK (triangle) mediated phosphorylation of Sp1 (Stains and Civitelli, 2005) and PKCδ (diamond) mediated phosphorylation of Runx2 (Lima *et al*., 2009). PKCδ may also independently activate ERK (Niger *et al*., 2012). Phosphorylated Sp1 and Runx2 favor transcription of certain osteoblast genes.

ponents of various signaling complexes (Bivi *et al*., 2011), the mediators of the second messengers may be closely associated with gap junctions. For example, Ras, a mediator of the ERK pathway, is known to associate on the inner leaflet of the plasma membrane and could associate with gap junctions (Stains and Civitelli, 2005). Moreover, PKCδ, a known mediator of FGF2 signaling, was recently shown to physically associate with the carboxy-tail of Cx43 (Niger *et al*., 2010).

These studies suggested that the Cx43 gap junctions reinforce typical signal transduction cascades by propagating second messengers to neighboring cells that may not have received the primary signal. Such a mechanism allows a signal that is initiated in a small number of cells to be coordinated within a tissue. Furthermore, these studies provide opportunities to begin identifying the relevant second messengers that may permeate Cx43-based gap junction channels (Niger *et al*., 2012).

Cx43 Regulates Gene Expression In Vivo

A second strategy to explore how Cx43 controls gene regulation in a mechanistic pathway is to evaluate Cx43 effects in vivo. Our lab evaluates the fin phenotypes of the *sof* mutant (Fig. 4) to identify Cx43-dependent functions in the skeleton. The fin is composed of multiple bony fin rays and each fin ray consists of multiple bony segments separated by joints. Fin growth occurs at the distal end of the fin ray and can be monitored during typical ontogenetic growth or during regenerative growth following amputation (reviewed in Akimenko *et al*., 2003; Poss *et al*., 2003). The fin ray includes several tissues. The bone matrix of opposed hemirays surrounds the central mesenchymal compartment, whereas the hemirays are surrounded by a multilayered epithelium. The population of dividing cells contributing to new fin growth is located in the distal mesenchyme, and is referred to as the blastema in the regenerating fin. Skeletal precursor cells (the preosteoblasts and prejoint forming cells) are located laterally in the mesenchymal compartment, adjacent to the blastema. During regeneration, the *cx43* mRNA is upregulated in blastemal cells. We have suggested that communication between the blastema and skeletal precursor cells could be mediated by Cx43 function (Brown *et al*., 2009).

The Iovine lab has taken a molecular genetics approach to identify *cx43*-dependent genes contributing to skeletal morphogenesis. The original allele, sof^{b123}, exhibits reduced levels of *cx43* mRNA, short fins, short bony fin ray segments, and reduced levels of cell proliferation (Iovine *et al*., 2005). In addition to the sof^{b123} allele, three alleles coding missense mutations in the *cx43* gene were isolated using a noncomplementation screen. All four *sof* alleles are hypomorphic and exhibit reduced fin length, segment length, and cell proliferation. Further, morpholino-mediated knockdown of *cx43* recapitulates all of the *sof* phenotypes (Hoptak-

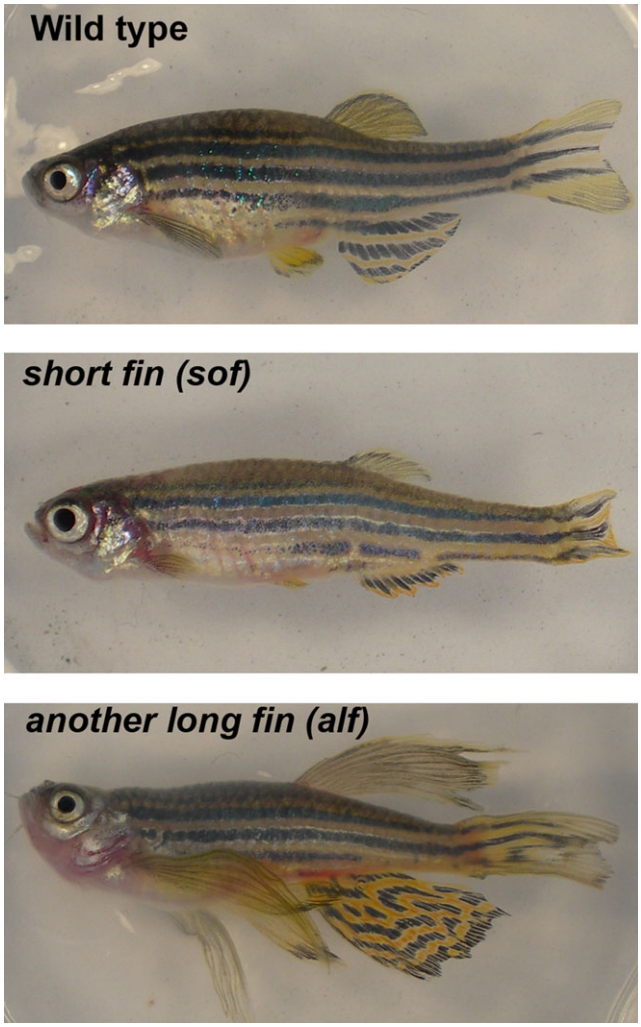

FIG. 4. Fin length mutants exhibit defects in skeletal morphogenesis. Top: wild-type zebrafish. Middle: *sof*^{b123} mutant. Bottom: *alf*^{dty86} mutant.

Solga *et al*., 2008). In contrast to the *sof* mutants, the another long fin (*alf*^{dty86}, van Eeden *et al*., 1996, Fig. 4) mutant exhibits increased levels of *cx43* mRNA, long fins, and stochastic joint failure (i.e., long segments on average). Remarkably, the *alf*^{dty86} phenotypes are rescued by *cx43* knockdown (Sims *et al*., 2009). Because the *alf*^{dty86} mutant exhibits phenotypes opposite to that of *sof*, and as *cx43* knockdown rescues those phenotypes, we consider *alf*^{dty86} to be a mimic of *cx43* over-expression. Based on these and other data, we further hypothesize that the role of Cx43 in the regenerating fin is to coordinate fin growth/cell proliferation with skeletal patterning/joint formation. In other words, Cx43 mediates *two* activities: promoting cell proliferation and suppressing joint formation.

To identify genes whose expression is regulated by Cx43, we completed a microarray analysis. We generated a list of genes that are both downregulated in *sof*^{b123} (when *cx43* is downregulated) and upregulated in *alf*^{dty86} (when *cx43* is upregulated). Notably, we found the *cx43* gene among the top 50 candidates, suggesting that our approach was successful. From this list, we decided to focus first on the *semaphorin3d* (*sema3d*) gene (Ton and Iovine, 2012). The Class 3 semaphorins, such as Sema3d, are secreted signaling molecules known to be involved in activating signaling pathways that influence diverse cellular functions (reviewed in Yazdani and Terman, 2006). Expression of *sema3d* is located in the lateral skeletal precursor cells. As *sema3d* is not expressed in the *cx43*-positive cells, it appears not to be a direct target for Cx43 function. However, as *sema3d* knockdown recapitulates all of the *cx43* knockdown phenotypes (including rescue of the *alf*^{dty86} phenotypes), *sema3d* is a likely candidate for mediating Cx43-dependent functions (Fig. 5a).

Semaphorins utilize Neuropilins (Nrps) and Plexins (Plxns) as typical cell-surface receptors. On the basis of

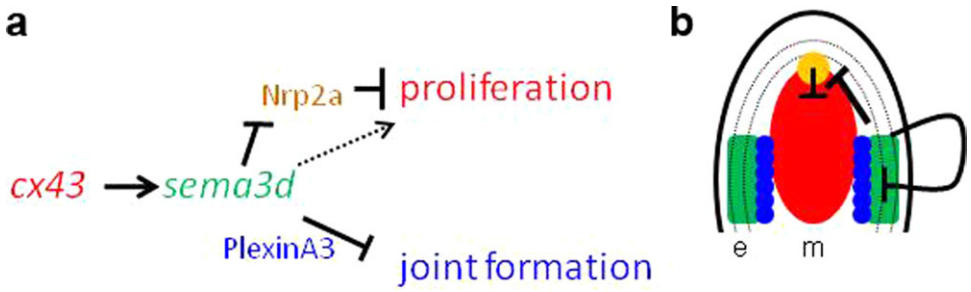

FIG. 5. Cx43 influences cell proliferation and joint formation by regulating the Sema3d pathway. (**a**) Expression of the *sema3d* gene is regulated by *cx43*. The Sema3d protein is secreted and interacts with its putative receptors, Nrp2a and PlxnA3, to regulate cell proliferation and joint formation respectively. (**b**) Compartmental expression of the genes in the Cx43 pathway. The *cx43* mRNA is upregulated in the proliferating cells of the blastema (red), while *sema3d* is upregulated in the lateral skeletal precursor cells and basal layer of the epidermis (green). The *nrp2a* gene is expressed in the distal blastema (yellow), where it may regulate cell proliferation in the blastemal cells. The *plxna3* gene is expressed in the skeletal precursor cells (blue), where it may regulate joint formation. This figure was originally published in Ton and Iovine, 2012, and is reprinted here with permission.

gene expression and gene function of candidate genes encoding the Nrps and Plxns, we found that independent receptors mediate either cell proliferation or joint formation (Ton and Iovine, 2012). For example, the Nrp2a receptor appears to mediate Sema3d-dependent cell proliferation, whereas the PlxnA3 receptor appears to mediate Sema3d-dependent joint formation. Interestingly, knockdown of *nrp2a* causes an increase in cell proliferation, whereas knockdown of *sema3d* causes reduced cell proliferation. We interpret these and other data to suggest that Sema3d inhibits Nrp2a function, thereby promoting cell proliferation by inhibiting a negative signal (Ton and Iovine, 2012). On the basis of these analyses of a single gene identified by our novel microarray strategy, we established the beginning of a molecular pathway dependent on Cx43 function (Fig. 5). Continued insight into Cx43-dependent events may be obtained by placing additional genes identified by the microarray in this pathway. Indeed, of the nine newly validated genes from the microarray, four have established connections to mammalian skeletal diseases (Table 1). We anticipate that continued evaluation of these and other microarray genes will reveal a network of Cx43-dependent events that coordinate tangible cellular functions (such as cell proliferation and joint formation) and thereby control skeletal morphogenesis.

Table 1
Validated Microarray Genes With Known Skeletal Relevance

name	Disease associated with mammalian ortholog	OMIM ID
hsp47	Osteogenesis imperfecta	600943
hapln1a	Dwarfism and craniofacial defects (mouse)	115435
twist1b	Craniosynostosis type I	601622
hoxda13	Brachydactyly; syndactyly	142989

OMIM: Online Mendelian Inheritance in Man is a database that catalogs disease-causing mutations and phenotypes. Mutations in the mammalian orthologs to the four listed zebrafish genes are known to cause skeletal disease. For complete list of identified genes, please see Ton and Iovine, 2012.

The case for Cx43-dependent GJIC during skeletal morphogenesis

Work from both the Stains lab and the Iovine lab provides evidence that Cx43-based GJIC contributes to skeletal development. For example, recall that Cx43 and FGF2 signals synergize to activate the ERK2-mediated phosphorylation of Runx2 and concomitant increased transcription of the luciferase reporter (Niger *et al.*, 2012). When treated cells were examined using flow cytometry it was possible to demonstrate that the increased luciferase activity was due to an increase in the number of cells responding to the Cx43 and FGF2

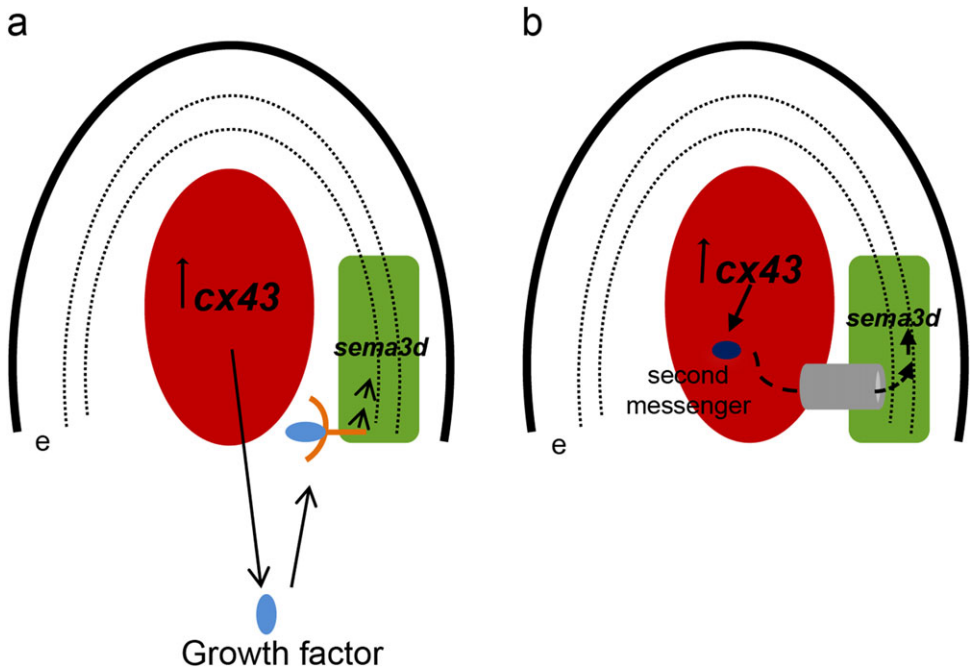

FIG. 6. Examples of how Cx43-dependent GJIC may influence gene expression in the regenerating fin. (**a**) Upregulation of *cx43* in the blastema (red) may lead to the secretion of a growth factor that can interact with its receptor located on adjacent skeletal precursor cells (green), leading to increased *sema3d* expression. (**b**) Heterotypic gap junctions may exist between cells of the blastema and skeletal precursor cells, permitting the direct exchange of secondary messengers. These second messengers may influence *sema3d* expression.

signals, and not due to a more robust response from the same number of cells. This finding is consistent with their proposed model that the primary signal from the FGF2 growth factor is amplified via Cx43-based GJIC. Earlier work from our lab demonstrated that the three missense alleles of *cx43* exhibit reduced levels of GJIC in heterologous assays (these alleles also appear to be capable of trafficking to the plasma membrane, Hoptak-Solga *et al*., 2007). Moreover, the severity of the segment length and cell proliferation phenotypes is correlated with the reduced level of GJIC (Hoptak-Solga *et al*., 2008; Iovine *et al*., 2005), strongly suggesting that reduced Cx43-based GJIC is responsible for the observed skeletal defects.

There are two possibilities to explain how Cx43-dependent GJIC in the blastemal cells influences *sema3d* gene expression in the adjacent skeletal precursor cells. First, Cx43-GJIC causes changes in gene expression in the *cx43*-positive compartment that lead to the secretion of an unidentified growth factor. This growth factor will bind to its receptor on the adjacent cells and cause an increase in *sema3d* expression in the lateral compartment (Fig. 6a). Such a mechanism may be revealed by continued examination of genes identified from the microarray analysis. Alternatively, it remains possible that heterotypic gap junctions (i.e., when each cell contributes a connexon composed of different connexins) exist between cells of the blastema and the skeletal precursor cells, directly influencing gene expression between the two compartments (Fig. 6b). Expression of a *connexin* gene has not been identified in the lateral compartment. However, since the zebrafish genome has at least 37 connexin genes (Eastman *et al*., 2006), one may still be found. This latter model, if true, may represent an in vivo example of second messengers traveling from one cell population to another in order to coordinate changes in gene expression, as elucidated by work from Stains *et al*.

CONCLUSIONS

The goal in defining the relevant Cx43 functions during skeletal morphogenesis is to reveal the initial Cx43-dependent event that regulates changes in cellular function. How do the different connexin functions lead to tangible changes in cell proliferation or cell differentiation? What are the relevant molecules that mediate these functions? Does the channel activity enhance propagation (as suggested in the work of Stains *et al*.), or is the channel-function secondary to the carboxy-tail as a scaffold for signaling molecules? How does hemichannel function fit in? The strategies described here have revealed tangible events downstream of Cx43 function and have provided novel insights into the types of cellular changes that are regulated by Connexins. In the future, we and others hope to work from these downstream events toward the initial Cx43-dependent function.

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LITERATURE CITED

Akimenko MA, Mari-Beffa M, Becerra J, Geraudie J. 2003. Old questions, new tools, and some answers to the mystery of fin regeneration. Dev Dyn 226:190–201.

Bivi N, Lezcano V, Romanello M, Bellido T, Plotkin LI. 2011. Connexin43 interacts with βarrestin: a prerequisite for osteoblast survival induced by parathyroid hormone. J Cell Biochem 112:2920–2930.

Bivi N, Condon KW, Allen MR, Farlow N, Passeri G, Brun LR, Rhee Y, Bellido T, Plotkin LI. 2012. Cell autonomous requirement of connexin 43 for osteocyte survival: consequences for endocortical resorption and periosteal bone formation. J Bone Miner Res 27:374–389.

Brown AM, Fisher S, Iovine MK. 2009. Osteoblast maturation occurs in overlapping proximal-distal compartments during fin regeneration in zebrafish. Dev Dyn 238:2922–2928.

Chung DJ, Castro CH, Watkins M, Stains JP, Chung MY, Szejnfeld VL, Willecke K, Theis M, Civitelli R. 2006. Low peak bone mass and attenuated anabolic response to parathyroid hormone in mice with an osteoblast-specific deletion of connexin43. J Cell Sci 119:4187–4198.

Dobrowolski R, Sasse P, Schrickel JW, Watkins M, Kim JS, Rackauskas M, Troatz C, Ghanem A, Tiemann K, Degen J, Bukauskas FF, Civitelli R, Lewalter T, Fleischmann BK, Willecke K. 2008. The conditional connexin43G138R mouse mutant represents a new model of hereditary oculodentodigital dysplasia in humans. Hum Mol Genet 17:539–554.

Eastman SD, Chen TH, Falk MM, Mendelson TC, Iovine MK. 2006. Phylogenetic analysis of three complete gap junction gene families reveals lineage-specific duplications and highly supported gene classes. Genomics 87:265–274.

Flenniken AM, Osborne LR, Anderson N, Ciliberti N, Fleming C, Gittens JE, Gong XQ, Kelsey LB, Lounsbury C, Moreno L, Nieman BJ, Peterson K, Qu D, Roscoe W, Shao Q, Tong D, Veitch GI, Voronina I, Vukobradovic I, Wood GA, Zhu Y, Zirngibl RA, Aubin JE, Bai D, Bruneau BG, Grynpas M, Henderson JE, Henkelman RM, McKerlie C, Sled JG, Stanford WL, Laird DW, Kidder GM, Adamson SL, Rossant J. 2005. A Gja1 missense mutation in a mouse model of oculodentodigital dysplasia. Development 132:4375–4386.

Giepmans ,BN. 2004. Gap junctions and connexin-interacting proteins. Cardiovasc Res 62:233–245.

Goodenough DA, Goliger JA, Paul DL. 1996. Connexins, connexons, and intercellular communication. Annu Rev Biochem 65:475–502.

Herve JC, Bourmeyster N, Sarrouilhe D. 2004. Diversity in protein-protein interactions of connexins: emerging roles. Biochim Biophys Acta 166:22–41.

Hoptak-Solga AD, Klein KA, Derosa AM, White TW, Iovine MK. 2007. Zebrafish short fin mutations in connexin43 lead to aberrant gap junctional intercellular communication. FEBS Lett 581:3297–3302.

Hoptak-Solga AD, Nielsen S, Jain I, Thummel R, Hyde DR, Iovine MK. 2008. Connexin43 (GJA1) is required in the population of dividing cells during fin regeneration. Dev Biol 317:541–548.

Iovine MK, Higgins EP, Hindes A, Coblitz B, Johnson SL. 2005. Mutations in connexin43 (GJA1) perturb bone growth in zebrafish fins. Dev Biol 278: 208–219.

Kumar NM, Gilula NB. 1996. The gap junction communication channel. Cell 84:381–388.

Lecanda D, Towler DA, Ziambaras K, Cheng SL, Koval M, Steinberg TH, Civitelli R. 1998. Gap junctional communication modulates gene expression in osteoblastic cells. Mol Biol Cell 9:2249–2258.

Lecanda F, Warlow PM, Sheikh S, Furlan F, Steinberg TH, Civitelli R. 2000. Connexin43 deficiency causes delayed ossification, craniofacial abnormalities, and osteoblast dysfunction. J Cell Biol 151:931–944.

Lima F, Niger C, Hebert C, Stains JP. 2009. Connexin43 potentiates osteoblast responsiveness to Fibroblast Growth Factor 2 via a Protein Kinase C-delta/Runx2-dependent mechanism. Mol Biol Cell 20:2697–2708.

Makarenkova H, Patel K. 1999. Gap junction signaling mediated through connexin-43 is required for chick limb development. Dev Biol 207:380–392.

Martinez AD, Hayrapetyan V, Moreno AP, Beyer ,EC. 2002. Connexin43 and connexin45 form heteromeric gap junction channels in which individual components determine permeability and regulation. Circ Res 90:1100–1107.

McGonnell IM, Green CR, Tickle C, Becker DL. 2001. Connexin43 gap junction protein plays an essential role in morphogenesis of the embryonic chick face. Dev Dyn 222:420–438.

Niger C, Hebert C, Stains JP. 2010. Interaction of connexin43 and protein kinase C-delta during FGF2 signaling. BMC Biochem 11:14.

Niger C, Lima F, Yoo DJ, Gupta RR, Buo AM, Hebert C, Stains JP. 2011. The transcriptional activity of osterix requires the recruitment of Sp1 to the osteocalcin proximal promoter. Bone 49:683–692.

Niger C, Buo AM, Hebert C, Duggan BT, Williams MS, Stains JP. 2012. ERK acts in parallel to PKCδ to mediate the connexin43-dependent potentiation of Runx2 activity by FGF2 in MC3T3 osteoblasts. Am J Physiol Cell Physiol 302:C1035–C1044.

Paznekas WA, Boyadjiev SA, Shapiro RE, Daniels O, Wollnik B, Keegan CE, Innis JW, Dinulos MB, Christian C, Hannibal MC, Jabs EW. 2003. Connexin 43 (GJA1) mutations cause the pleiotropic phenotype of oculodentodigital dysplasia. Am J Hum Genet 72: 408–418.

Pfenniger A, Wohlwend A, Kwak BR. 2010. Mutations in connexin genes and disease. Eur J Clin Inv 41: 103–116.

Plotkin ,LI. 2011. Connexin43 and bone: not just a gap junction protein. Actual Osteol 7:79–90.

Poss KD, Keating MT, Nechiporuk A. 2003. Tales of regeneration in zebrafish. Dev Dyn 226:202–210.

Reaume AG, de Sousa PA, Kulkarni S, Langille BL, Zhu D, Davies TC, Juneja SC, Kidder GM, Rossant J. 1995. Cardiac malformation in neonatal mice lacking connexin43. Science 267:1831–1834.

Saez JC, Berthoud VM, Branes MC, Martinez AD, Beyer EC. 2003. Plasma membrane channels formed by connexins: Their regulation and functions. Physiol Rev 83:1359–1400.

Sims K Jr, Eble DM, Iovine MK. 2009. Connexin43 regulates joint location in zebrafish fins. Dev Biol 327: 410–418.

Stains JP, Lecanda F, Screen J, Towler DA, Civitelli R. 2003. Gap junctional communication modulates gene transcription by altering the recruitment of Sp1 and Sp3 to connexin-response elements in osteoblast promoters. J Biol Chem 278:24377–24387.

Stains JP and Civitelli R. 2005. Gap junctions regulate extracellular signal-regulated kinase signaling to affect gene transcription. Mol Biol Cell 16:64–72.

Ton QT and Iovine MK. 2012. Semaphorin3d mediates Cx43-dependent phenotypes during fin regeneration. Dev Biol 366:195–203.

van Eeden FJ, Granato M, Schach U, Brand M, Furutani-Seiki M, Haffter P, Hammerschmidt M, Heisenberg CP, Jiang YJ, Kane DA, Kelsh R., Mullins MC, Odenthal J, Warga RM, Nusslein-Volhard C. 1996. Genetic analysis of fin formation in the zebrafish, Danio rerio. Development 123:255–262.

Watkins M, Grimston SK, Norris JY, Guillotin B, Shaw A, Beniash E, Civitelli R. 2011. Osteoblast connexin43 modulates skeletal architecture by regulating both arms of bone remodeling. Mol Biol Cell 22:1240–1251.

Yazdani U, Terman JR. 2006. The semaphorins. Genome Biol 7:211.

Zhang Y, Paul EM, Sathyendra V, Davison A, Sharkey N, Bronson S, Srinivasan S, Gross TS, Donahue HJ. 2011. Enhanced osteoclastic resorption and responsiveness to mechanical load in gap junction deficient bone. PLoS One 6:e23516.