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The "Connexin" Between Bone Cells and Skeletal Functions

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ABSTRACT

The processes of bone modeling and remodeling are crucial in the skeleton's functions as a supportive and protective structure, a mineral reservoir, and an endocrine organ. The coordination between bone cell activities (bone formation and bone resorption), necessary to maintain the integrity of the skeleton during these processes, is mediated at least in part by cell–cell and cell–environment interactions across gap junctions and hemichannels. The increasing number of genetically engineered Connexin 43 (Cx43) knockout and missense mouse models have provided insight into the complex and critical roles of Cx43-containing gap junctions and hemichannels in the development and turnover of the skeleton, in differentiation, activity and survival of the bone cell lineages, and in the cellular and molecular mechanisms by which Cx43 functions and assists in mediating cellular responses to stimuli in bone. Cx43 may be an important potential therapeutic target, making it crucial that we continue to gain understanding of the multiple and complex roles of Cx43 in bone. J. Cell. Biochem. 115: 1646–1658, 2014.

KEY WORDS: CONNEXIN 43; BONE DEVELOPMENT; SKELETAL HOMEOSTASIS; OSTEOBLAST AND OSTEOCLAST LINEAGE CELLS; OSTEOPENIA; Cx43 KNOCKOUT AND MISSENSE MOUSE MODELS

Bone is a living and dynamic tissue whose structural and material properties are critical to the skeleton's functions, which include providing structural support to the body, protecting internal organs, assisting in limb mobility, and acting as a reservoir for and participating in calcium and phosphorus homeostasis. Bone not only responds to hormonal signals, but is itself an endocrine organ, with secretion of factors influencing such diverse functions as phosphorus excretion by the kidney and insulin production by pancreatic beta cells, amongst other targets [Fukumoto and Martin, 2009].

Bone tissue consists of a mineralized organic matrix formed and maintained by cells that are continuously engaged in modeling and remodeling to adapt the tissue to the demands (mechanical and physiological) that are put on it. Maintaining the integrity of the adult skeleton requires equilibrium between the amount of bone formed and the amount resorbed, that is, coordinated activity between osteoblasts and osteoclasts. This equilibrium and coordination is mediated at least in part by cell–cell and cell–extracellular environment communication across gap junctions and hemichannels. Understanding the role of gap junctions and hemichannels in bone metabolism has long been an area of pursuit, but has accelerated over the last decade by characterization of novel mutations in humans and genetically engineered mice, in which markedly affected gap junction and hemichannel formation and function are associated with aberrant bone structure and activity.

CONNEXINS: GAP JUNCTIONS AND HEMICHANNELS

Connexins are a multigene family of hemichannel- and gap junction-forming proteins (reviewed in Kumar and Gilula [1996] and Dbouk et al. [2009]). The connexin proteins contain highly conserved transmembrane domains, extracellular domains required for hemichannel or connexon pairing between adjacent cells, and a carboxyl-terminal region that serves as a docking platform for signaling complexes (Fig. 1A). Six connexin proteins form the hemichannel or connexon with a central pore. Intercellular gap

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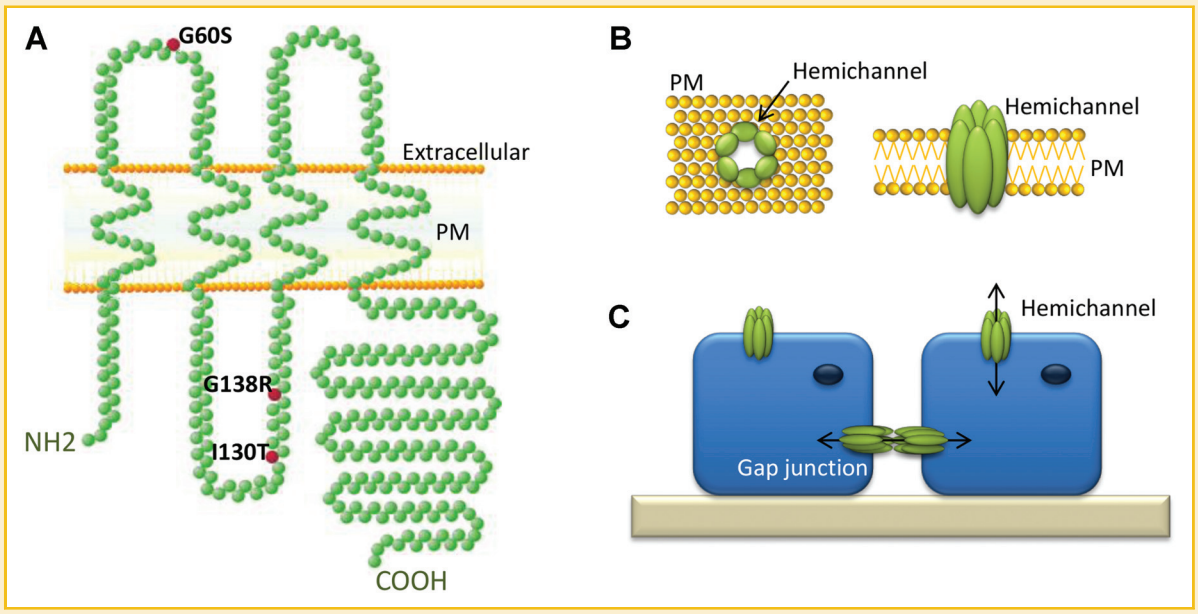

Fig. 1. Illustrations of connexin protein structure, hemichannels, and gap junctions in the plasma membranes of cells. A: Schematic of Cx43 protein structure showing the three missense mutations described in the ODDD mouse models highlighted in red. The mutations are denoted by the correct amino acid followed by the number and the substituted amino acid. B: Illustration of six connexin proteins which oligomerize forming a hemichannel or connexon in the plasma membrane. C: Hemichannels and gap junctions allow the passage of ions and small molecules to the extracellular environment and between cells, respectively. PM, plasma membrane.

junctions form when hemichannels from adjacent cells dock onto one another (Fig. 1B,C).

Hemichannels and gap junctions both display relatively low substrate specificity and allow the passage of ions and small molecules (molecular weight less than 1 kDa). Hemichannels mediate communication between cells and the extracellular environment, including the bone matrix. They are essential for the transduction of signals and can activate intracellular signaling by mediating transport of signaling molecules such as ATP [Stout et al., 2002; Eltzschig et al., 2006] and PGE2 [Jiang and Cherian, 2003; Cherian et al., 2005]. On the other hand, gap junctions are involved in communication between adjacent cells, via transport of intracellular signaling molecules such as calcium [Saez et al., 1989; Christ et al., 1992], cAMP [Kam et al., 1998; Webb et al., 2002], and inositol triphosphate [Saez et al., 1989; Christ et al., 1992].

Many cells express more than one member of the connexin family, and each member forms channels with different functional properties. A connexin channel's permeability to specific cytoplasmic molecules, voltage, and chemical gating properties are determined by the type of channel formed, for example, hemichannel versus gap junction, and the channel's composition, for example, homomeric (composed of a single connexin member) versus heteromeric (composed of two or more different connexins). These differences are the reason that connexins cannot fully substitute or compensate for one another.

CONNEXIN 43 (Cx43) AND THE SKELETON

Cx43, encoded by the gap junction protein alpha 1 gene (*GJA1* [human], *Gja1* [mouse]), is the most widely expressed and abundant vertebrate gap junction protein. It is expressed in cells of almost all tissues in the body, including the brain [Yamamoto et al., 1990; Simburger et al., 1997], heart [Beyer et al., 1987], ovary [Mayerhofer and Garfield, 1995], tooth [Pinero et al., 1994; Fried et al., 1996], eye [Ruangvoravat and Lo, 1992], and bone [Civitelli et al., 1993; Su et al., 1997].

Cx43 is the major connexin protein expressed in developing and mature skeletal tissues, and is expressed in chondrocytes [Zimmermann, 1984; Donahue et al., 1995a], osteoblasts [Civitelli et al., 1993; Yellowley et al., 2000], osteocytes [Yellowley et al., 2000], osteoclasts [Ilvesaro et al., 2000], and bone marrow stromal cells [Dorshkind et al., 1993]. Cx45 [Civitelli et al., 1993; Steinberg et al., 1994], Cx46 [Koval et al., 1997], and Cx37 [Yamada et al., 2007; Paic et al., 2009] are also expressed in bone tissue, albeit at significantly lower levels than Cx43. In bone, as in other tissues, osteoblasts, osteocytes, osteoclasts, and cells in the bone marrow can communicate with one another via passage of signaling molecules and ions across gap junctions and hemichannels (Fig. 2). This communication is crucial in cellular control of the tightly regulated processes of bone formation and bone turnover.

Cx43 globally and conditionally deleted mouse models have provided much insight into the importance of Cx43 in bone formation and function and osteoblast lineage development. The models discussed in this review include: Cx43 global knockout mice ($Cx43^{-/-}$) [Lecanda et al., 2000], and conditional deletion of Cx43 in early osteochondro progenitors (*DM1Cre;*$Cx43^{-/fl}$ and *DM1Cre;* $Cx43^{+/fl(G138R)}$) [Watkins et al., 2011; Grimston et al., 2012], osteoblasts (*ColCre;*$Cx43^{-/fl}$) [Chung et al., 2006; Grimston et al., 2008; Gonzalez-Nieto et al., 2012], mature osteoblasts/ osteocytes (*OcnCre;*$Cx43^{-/fl}$) [Plotkin et al., 2008; Zhang et al., 2011;

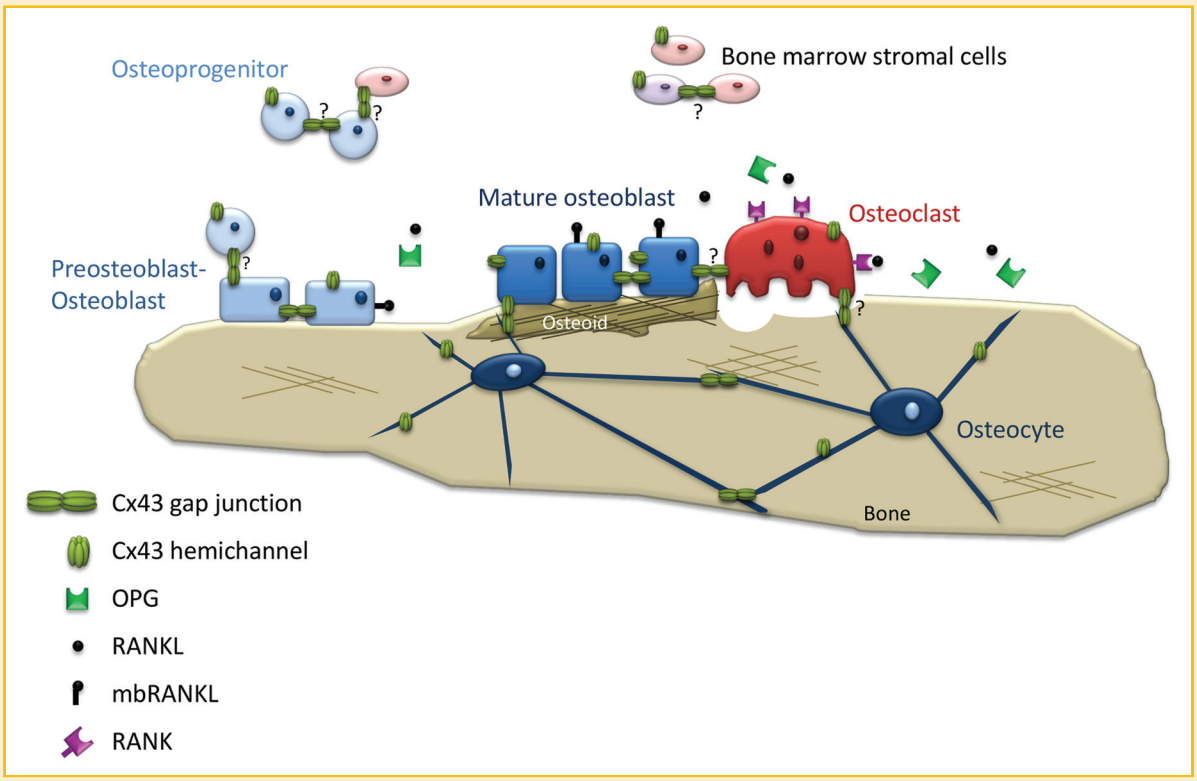

Fig. 2. Connexin 43 in the bone microenvironment. The interconnected network of cells, comprised of cells on/within the bone (osteoblasts, osteocytes, and osteoclasts) and within the bone marrow (osteoprogenitors and other stromal cells) can communicate with one another via Cx43 channels, soluble factors (RANKL–OPG) and other cell–cell interactions. This communication coordinates bone formation and resorption, ensuring that skeletal integrity is maintained. The consequences of aberrant Cx43 channel formation and function are, therefore, complex and multifactorial. Question marks (?) denote instances where direct communication across Cx43 gap junctions has yet to be reported or requires further study to confirm.

Bivi et al., 2012a; Lloyd et al., 2012; Loiselle et al., 2012], and osteocytes (*DMP1Cre;Cx43*$^{fl/fl}$) [Bivi et al., 2012a,b] (Fig. 3).

In addition to the knockout models, three mouse strains with missense point mutations in one allele of the *Gja1* gene have been generated (*Gja1*$^{Jrt(G60S)}$/+ [Flenniken et al., 2005], *Cx43*^{I130T}/+ [Kalcheva et al., 2007], *Cx43*^{G138R}/+ [Dobrowolski et al., 2008]; Fig. 1A, Table I). These strains serve as phenotypic mimics and thus useful models of a rare human disorder termed oculodentodigital dysplasia (ODDD), characterized by a spectrum of clinical features including, amongst others, craniofacial abnormalities, syndactyly, neurological problems, and cardiac defects [Paznekas et al., 2003, 2009]. To date, over 65 mutations in *GJA1* have been identified and reported to cause ODDD [Paznekas et al., 2003, 2009; Laird, 2008]. The functional effects of many of these *GJA1* mutations have been extensively studied and shown to act in a dominant negative manner, significantly reducing total and phosphorylated levels of Cx43 protein. Formation and function of Cx43 gap junctions are concomitantly significantly reduced, since a large portion of Cx43 remains trapped in intracellular structures, such as the golgi [Flenniken et al., 2005; Shibayama et al., 2005; Kalcheva et al., 2007; Dobrowolski et al., 2008; McLachlan et al., 2008]. Interestingly, however, the effect of the Cx43 mutant proteins on the activity of opened hemichannels is more variable [Lai et al., 2006; Dobrowolski et al., 2007]. The point mutation models discussed in this review are the *Gja1*^{Jrt}/+ and *Cx43*^{G138R}/+ mouse models (the only ones whose bone phenotypes have been reported upon).

Below and in Table II, we summarize the consequences of *Gja1* mutation and ablation in these various mouse models. Comparison across models highlights the complex roles and underlying mechanisms of Cx43 in bone cell lineage development, activities, and in the formation and maintenance of the skeleton.

BONE DEVELOPMENT AND SKELETAL HOMEOSTASIS

Functional Cx43 gap junctions and hemichannels are crucial for the processes of bone formation, maintenance, and healing. Loss or disruption of Cx43 gap junction formation and/or function results in varying degrees of osteopenia in all the mouse models studied. The osteopenic phenotype includes reduced bone mineral density [Lecanda et al., 2000; Flenniken et al., 2005; Chung et al., 2006; Zhang et al., 2011; Watkins et al., 2011; Zappitelli et al., 2013], changes in geometrical properties (e.g., decreased cortical thickness and increased marrow space) [Grimston et al., 2008, 2012; Watkins et al., 2011; Zhang et al., 2011; Bivi et al., 2012a; Zappitelli et al., 2013], reduced bone biomechanical properties (e.g., material and structural parameters) [Watkins et al., 2011; Zhang et al., 2011;

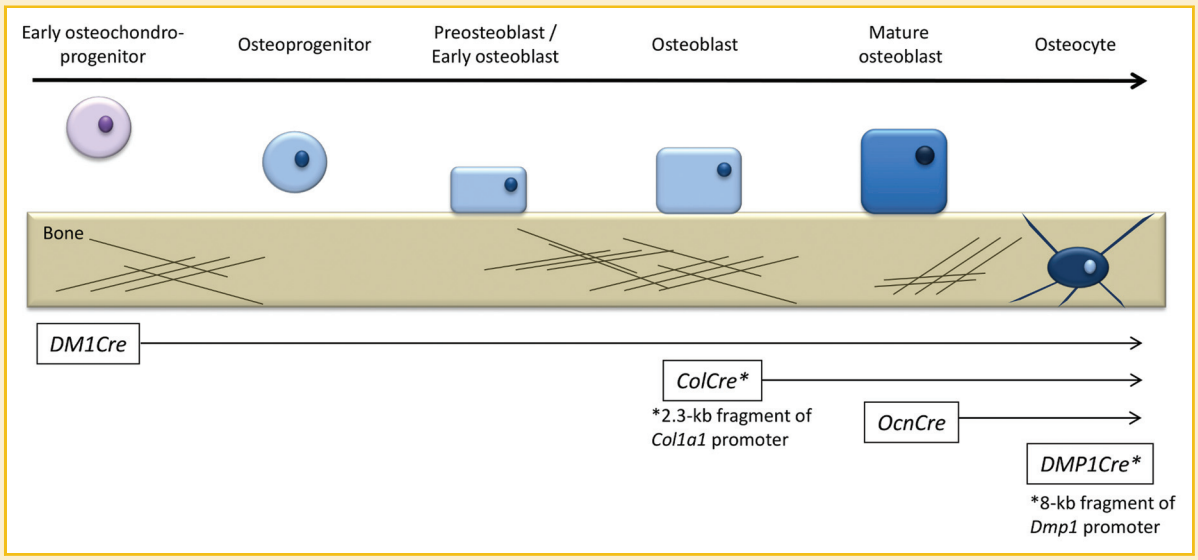

Fig. 3. Schematic of osteoblast lineage development and the different promoters used to drive Cre for differentiation stage-specific Cx43 ablation. The figure depicts in simplified form osteoblast differentiation from mesenchymal progenitor (osteochondroprogenitor) to terminally differentiated osteocyte. Displayed are the promoter-Cre constructs, which depict the point in the lineage development that Cx43 is disrupted in the conditional deletion mouse models. All cells in the body that express Cx43, which is amongst the most ubiquitously expressed of Cxs, are affected in the Cx43 global knockout and Cx43 point mutation mouse models.

Bivi et al., 2012a,b; Grimston et al., 2012; Lloyd et al., 2012; Zappitelli et al., 2013], and reduced ability to heal after a bone fracture [Loiselle et al., 2012]. The impact of Cx43 extends to bones formed via both endochondral ossification, for example, the long bones of the appendicular skeleton and intramembranous ossification, for example, the calvaria bones. Cx43 is important during initial formation and mineralization of early prenatal and neonatal bones, as its disruption can lead to shortened and/or misshapen bones and delayed mineralization, as in the $Cx43^{-/-}$, *DM1Cre;* $Cx43^{-/fl}$, *DM1Cre;*$Cx43^{+/fl(G138R)}$ and $Gja1^{Jrt/+}$ mice [Lecanda et al., 2000; Flenniken et al., 2005; Watkins et al., 2011]. While phenotypic traits paralleling those seen in human ODDD (e.g., craniofacial and other bone geometry anomalies, enamel hypoplasia, syndactyly amongst others) have been reported in some mouse Cx43 models, we have found no reports of low bone mass in patients with *GJA1* mutations. Whether this is due to interspecies differences in the importance of Cx43 in bone maintenance (e.g., other factors compensating for the loss of functional Cx43 channels), or reflects that the presentation of more severe symptoms (e.g., various neurological and cardiac symptoms, ocular abnormalities, conductive hearing loss) have precluded the testing or reporting of altered bone density is not yet clear [Paznekas et al., 2003].

While not yet reported in human ODDD or exhaustively studied in any of the mutant mice described to date, it is now known that Cx43 channels play a role in controlling both the composition and quality of the bone matrix. For example, at least certain mutations of Cx43 negatively affect the maturation of collagen fibrils [Bivi et al., 2012b], result in disorganization of collagen fibers in the matrix [Watkins et al., 2011], and alter the content of non-collagenous proteins, in particular bone sialoprotein (BSP) [Zappitelli et al., 2013], in bone matrix. The decreased maturation of collagen fibrils in *OcnCre;*$Cx43^{-/fl}$ mice decreases the strength of the bone material [Bivi et al., 2012b], while the disorganization of collagen fibers in the matrix of *DM1Cre;*$Cx43^{-/fl}$ mice is

TABLE I. Connexin 43 Missense Point Mutation Summary Chart

	$Gja1^{Jrt}/+$	$Cx43^{I130T}/+$	$Cx43^{G138R}/+$
Reference	Flenniken et al. [2005] and McLachlan et al. [2008]	Kalcheva et al. [2007]	Dobrowolski et al. [2008]
Mutation	G60S	I130T	G138R
Mutation location	First extracellular loop	Intracellular loop	Intracellular loop
Mutation described in human ODDD	No	Yes	Yes
Effect on			
Total Cx43 levels	Unaffected or ↓	↓	↓
p-Cx43 levels	↓	↓	↓
Gap junction formation	↓	↓	Unaffected or ↓
Gap junction function	↓	↓	↓
Hemichannel function	Unknown	↓	↑

The location and functional effects of the Cx43 missense point mutations shown in Figure 1A are described.

TABLE II. Connexin 43 Mutant Mice Summary Chart

	Cx43$^{-/-}$	*DM1Cre;Cx43*$^{-/fl}$; *DM1Cre;Cx43*$^{+/fl(G138R)}$	*ColCre;Cx43*$^{-/fl}$	*OcnCre;Cx43*$^{-/fl}$	*DMP1Cre;Cx43fl/fl*	*Cx43*^{G138R}/+	*Gja1*^{Jrt}/+
ODDD-like craniofacial abnormalities	yes [Lecanda et al., 2000]	not reported	no [Chung et al., 2006]	not reported	not reported	yes [Dobrowolski et al., 2008]	yes [Flenniken et al., 2005]
Body weight			↓ [Chung et al., 2006]	~ [Plotkin et al., 2008] or ↓ [Lloyd et al., 2012]			↓ [Zappitelli et al., 2013]
Bone development and skeletal homeostasis							
BMD		↓ BMD [Watkins et al., 2011]	↓ BMD & BMC [Chung et al., 2006] ~ BMD (tibial diaphysis) [Grimston et al., 2008]	~ BMD [Plotkin et al., 2008; Bivi et al., 2012a; Bivi et al., 2012b] ↓ BMD [Zhang et al., 2011; Lloyd et al., 2012]	~ BMD [Bivi et al., 2012a; Bivi et al., 2012b]		↓ BMD & BMC [Flenniken et al., 2005; Zappitelli et al., 2013]
Geometrical properties		↑ TtAr, ↑MaAr [Watkins et al., 2011; Grimston et al., 2012]	↑TtAr, ↑MaAr [Grimston et al., 2008]	↑TtAr, ↑MaAr [Zhang et al., 2011; Bivi et al., 2012a; Lloyd et al., 2012]	↑TtAr, ↑MaAr [Bivi et al., 2012a]		↓ TtAr, ↓ MaAr young: ↑MaAr/TtAr old: ~MaAr/TtAr [Zappitelli et al., 2013]
Cortical Bone		↓ CtTh [Watkins et al., 2011], ↓ CtBV [Grimston et al., 2012] ↑ porosity [Watkins et al., 2011; Grimston et al., 2012]	↓ CtTh [Grimston et al., 2008]	~[Zhang et al., 2011; Bivi et al., 2012a] or ↓ [Lloyd et al., 2012] CtTh ~porosity [Zhang et al., 2011] ↑porosity [Bivi et al., 2012a; Lloyd et al., 2012]	~CtTh ↑CtAr [Bivi et al., 2012a]		young: ↓ CtTh & ↓ CtAr/TtAr old: ↑CtTh, ~CtAr/TtAr [Flenniken et al., 2005; Zappitelli et al., 2013]
Trabecular Bone		~ [Watkins et al., 2011; Grimston et al., 2012]	↓ BV/TV, ↓ TbTh, ~TbN [Chung et al., 2006]	~ [Bivi et al., 2012a; Bivi et al., 2012b; Lloyd et al., 2012]	~ [Bivi et al., 2012a; Bivi et al., 2012b]	↓ BV/TV ↑TbSp [Dobrowolski et al., 2008]	↓ TbN & TbTh [Flenniken et al., 2005; Zappitelli et al., 2013] ↓ BV/TV until 8mo. [Zappitelli et al., 2013]
Calvaria Bone		1 mo. cKO: hypomineralized, hypoplastic bones 1 mo. cODDD: ~ mineralization ↓ size [Watkins et al., 2011]					
Biomechanical Properties		↓ ultimate force to failure ↓ yield force ↓ bone strength ↑moment of inertia [Watkins et al., 2011; Grimston et al., 2012]	~moment of inertia [Grimston et al., 2008]	↓ material properties [Zhang et al., 2011; Bivi et al., 2012b] ~ structural properties [Bivi et al., 2012b] ↑ultimate bending moment [Lloyd et al., 2012] ↑moment of inertia [Bivi et al., 2012a; Bivi et al., 2012b; Lloyd et al., 2012]	↓ material properties [Bivi et al., 2012a; Bivi et al., 2012b] ~ structural properties [Bivi et al., 2012b] ↑moment of inertia [Bivi et al., 2012a]		↓ mechanical strength [Flenniken et al., 2005] young: ↓ material properties all ages: ↓ structural properties ↓ moment of inertia [Zappitelli et al., 2013]
Bone matrix		disorganized collagen fibers, appearance of woven bone, ↓ mineralization [Watkins et al., 2011]		↓ collagen maturation [Bivi et al., 2012b] ~mineralization [Plotkin et al., 2008; Bivi et al., 2012a]	~ collagen maturation ↓ mineralization [Bivi et al., 2012b]		↑BSP content ~OCN & OPN content [Zappitelli et al., 2013]
Prenatal/Neonatal Skeleton							
Neural crest-derived skull elements	E16.5: delayed ossification E18.5: hypomineralized, smaller skull P0: open parietal foramen, smaller calvaria, flattened skull, thin & brittle bones [Lecanda et al., 2000]	P0 cKO: hypoplastic, hypomineralized & smaller P0 cODDD: hypomineralized [Watkins et al., 2011]	P0: no major skeletal abnormalities [Chung et al., 2006]				P3: delayed ossification, thin, porous [Flenniken et al., 2005]
Mesoderm-derived skull elements	E16.5: most elements displayed delayed ossification, reduced size, hypomineralization [Lecanda et al., 2000]	P0 cKO: hypoplastic smaller cODDD skull hypomineralization [Watkins et al., 2011]	P0: no major skeletal abnormalities [Chung et al., 2006]				P3: delayed ossification, thin & porous, open foramena [Flenniken et al., 2005]
Axial skeleton	E15.5- 18.5: delayed endochondral ossification	P0 cKO: shortened ribs [Watkins et al., 2011]	P0: no major skeletal abnormalities [Chung				

TABLE II. (*Continued*)

	Cx43$^{-/-}$	*DM1Cre;Cx43*$^{-/fl}$; *DM1Cre;Cx43*$^{+/fl(G138R)}$	*ColCre;Cx43*$^{-/fl}$	*OcnCre;Cx43*$^{-/fl}$	*DMP1Cre;Cx43fl/fl*	*Cx43*^{G138R}/+	*Gja1*^{Jrt}/+
	E16.5- 18.5: deformed ribs P0: normal mineralization [Lecanda et al., 2000]		et al., 2006]				
Appendicular skeleton	E14.5: delayed ossification, decreased size P0: morphologically normal [Lecanda et al., 2000]	P0 cKO: shortened tibia and femur [Watkins et al., 2011]	P0: no major skeletal abnormalities [Chung et al., 2006]				
Development and Activity of Osteoblast Lineage Cells							
Osteoprogenitors & BMSCs	↓ CFU-O (calvarial-derived) [Lecanda et al., 2000]	↑CFU-F, ↑CFU-O, ↑proliferation [Watkins et al., 2011]	↑CFU-F, ↑CFU-O, ↑proliferation [Gonzalez-Nieto et al., 2012]				↑CFU-F, ↑CFU-ALP, ↑CFU-O ~ proliferation, late culture: ↑OB marker expression [Zappitelli et al., 2013]
Calvaria							
In vivo							
In vitro	~ proliferation ↓ ALP ↓ mineralization ↓ *Col1a1 & Ocn* ↑*Opn* [Lecanda et al., 2000]		↓ ALP, delayed mineralization, ↓ Runx2, *Col1a1, Opn & Ocn* [Chung et al., 2006]		~ proliferation ↑apoptosis [Bivi et al., 2012a]		~proliferation ~ ALP ~mineralization, ~*Col1a1, Opn* ↓ *Bsp*, ↓ *Ocn* [McLachlan et al., 2008]
Cortical							
Ob	Neonatal long bone cultures: ↓ *Col1a1, Ocn & Opn* [Lecanda et al., 2000]	↑periosteal BF ↓ endosteal BF ~*Runx2, Opn, Bsp* ↓ *Osx, Alp, Col1al* [Watkins et al., 2011; Grimston et al., 2012]	↓ *Sost* [Gonzalez-Nieto et al., 2012]	↑periosteal BF [Bivi et al., 2012a] ↓ periosteal BF [Lloyd et al., 2012] ~ periosteal BF [Zhang et al., 2011] ~ endosteal BF [Bivi et al., 2012a; Lloyd et al., 2012] ↑endosteal BF [Zhang et al., 2011]	↑periosteal BF ↑endosteal BF [Bivi et al., 2012a]		~ObS/BS 2mo.: ↓ endosteal MAR 8mo: ↑endosteal MS/BS ~marker expression [Zappitelli et al., 2013]
Osy		~OsyN/BA ↓ *Dmp1 & Sost* [Watkins et al., 2011; Grimston et al., 2012]		↑apoptosis ↑empty lacunae [Plotkin et al., 2008; Bivi et al., 2012a; Bivi et al., 2012b]	↑apoptosis [Bivi et al., 2012a; Bivi et al., 2012b] ↑empty lacunae [Bivi et al., 2012a] ↓ SOST^{+} OsyN [Bivi et al., 2012a]		~OsyN/BA ↓ *Sost* (2mo.) [Zappitelli et al., 2013]
Trabecular							
Ob		~ObN/BS [Watkins et al., 2011]	↓ ObS/BS ~ MAR [Chung et al., 2006]			~ ObS/BS [Dobrowolski et al., 2008]	~ObS/BS ~BF ↑marker expression [Zappitelli et al., 2013]
Osy				~ apoptosis [Plotkin et al., 2008]			~ OsyN/BA [Zappitelli et al., 2013]
Serum Parameters		↑OCN [Watkins et al., 2011; Grimston et al., 2012]		↓ OCN [Bivi et al., 2012a] ~ PINP [Zhang et al., 2011]	↑OCN [Bivi et al., 2012a]		↑ALP [Zappitelli et al., 2013]
Development and Activity of Osteoclasts							
Cortical		↑endosteal OcN/BS ↑ endosteal resorption [Watkins et al., 2011]	↑endosteal OcN & OcS/BS [Grimston et al., 2011]	↑endosteal OcN & OcS/BS [Bivi et al., 2012a] ↑OcN/BS [Zhang et al., 2011]↑ endosteal resorption [Zhang et al., 2011; Bivi et al., 2012a]	↑endosteal OcN/BS [Bivi et al., 2012a]		2mo.: ↑ endosteal OcS/BS 4- 8mo.: ↓ endosteal OcS/BS [Zappitelli et al., 2013]
Trabecular		~ OcN/BS [Watkins et al., 2011]	~ OcS/BS [Chung et al., 2006]	↑OcN/BS [Zhang et al., 2011]			2mo: ↑OcS/BS 4mo: ~OcS/BS ~ expression of fusion/diff markers ↑*CtsK* [Zappitelli

TABLE II. (*Continued*)

	Cx43$^{-/-}$	*DM1Cre;Cx43*$^{-/fl}$*; DM1Cre; Cx43*$^{+/fl(G138R)}$	*ColCre;Cx43*$^{-/fl}$	*OcnCre;Cx43*$^{-/fl}$	*DMP1Cre;Cx43fl/fl*	*Cx43*^{G138R}*/+*	*Gja1*^{Jrt}*/+*
							et al., 2013] ~ BM- and spleen- derived osteoclastogenesis & resorption [Zappitelli et al., 2013]
In vitro (BM- and spleen-derived)		~ BM-derived osteoclastogenesis [Watkins et al., 2011]					
Serum Parameters		↑CTX [Watkins et al., 2011]		↑CTX [Zhang et al., 2011]	~ CTX [Bivi et al., 2012a]		~ CTX [Zappitelli et al., 2013]
RANKL/OPG		~*Rankl* ↓ *Opg* [Watkins et al., 2011]		long bones: ~*Rankl/Opg* MLO-Y4 cells: ↑*Rankl/Opg* [Zhang et al., 2011]	whole bone: ~*Rankl/Opg* ↓ OPG^{+} Osy (%) ~RANKL^{+} Osy (%) [Bivi et al., 2012a] MLO-Y4 & primary calvaria cells: ↑*Rankl*, ↓ *Opg* [Bivi et al., 2012a]		2mo.Tb: ~mRANKL 4mo. Tb: ↑mRANKL young: ~serum OPG old: ↑serum OPG ~ serum RANKL [Zappitelli et al., 2013]
Response to challenge or stimuli							
In vivo loading		Compressive loading: ↑periosteal response (BFR & MS/BS) ↑endosteal response (where BFR was further decreased versus WT*) ↑Tb BV/TV & TbTh [Grimston et al., 2012]	3-point bending: ↓ endosteal response (BMD, BFR, MAR, MS) [Grimston et al., 2008]	Cantilever loading: ↑periosteal response (BFR, MS) [Zhang et al., 2011]	Axial loading: ↑periosteal response (BFR, MAR) ~ endosteal response [Bivi et al., 2013]		
In vivo unloading			Botulinum toxin A muscle paralysis: No effect on Tb bone loss Attenuated loss of Ct structure (CtAr) and strength [Grimston et al., 2011]	Hind limb suspension: Attenuated loss of Tb bone (BV/TV, TbTh) No effect on loss of Ct bone (CtAr, CtTh),↑loss femoral strength, Preservation of BF no ↓ peri- & endosteal BF [Lloyd et al., 2012]			
Fracture Healing				↓ BF (↓ mineralized callus), ↓ remodelling (↓ OcN/BA), ↓ biomechanical properties [Loiselle et al., 2012]			
Response to treatments			Attenuation of anabolic action of PTH (e.g. attenuated response of BMC, BV/TV, MAR) [Chung et al., 2006]	Response to glucocorticoids (prednisolone): ~ [Plotkin et al., 2008] Response to bisphosphonates (alendronate)= ↓ protective effect against apoptosis, but the result of ↑BMD is unaffected [Plotkin et al., 2008] Response to bisphosphonates (alendronate): attenuated periosteal response, ~ endosteal response [Bivi et al., 2012a]			

The major skeletal consequences of Cx43 ablation and disruption by point mutations are shown.
↓ , decreased versus WT; ↑, increased versus WT; ~, unaffected versus WT; BMD, bone mineral density; BMC, bone mineral content; TtAr, total tissue area; MaAr, marrow area; CtAr, cortical bone area; CtTh, cortical thickness; BV/TV, bone volume/ tissue volume; Tb, trabecular; TbTh, trabecular thickness; TbN, trabecular number; TbSp, trabecular separation; BS, bone surface; cKO, DM1Cre;Cx43$^{-/fl}$; cODDD, DM1Cre;Cx43$^{+/fl(G138R)}$; Ct, cortical bone; Calv, calvaria bone; Ob, osteoblast; Osy, osteocyte; BF, bone formation; MAR, mineral apposition rate; MS/BS, mineralizing surface/bone surface; BA, bone area; ObN, osteoblast number; ObS, osteoblast surface; OsyN, osteocyte number; OcN, osteoclast number; OcS, osteoclast surface; BM, bone marrow; CFU-O, -ALP, -F, colony forming unit -osteoblast, -alkaline phosphatase, -fibroblast.

accompanied by a reduction in mineralization [Watkins et al., 2011]. The increased matrix-deposited BSP directly contributes to increased osteoclastogenesis and bone resorption in young *Gja1*^{Jrt}/+ mice [Zappitelli et al., 2013]. More work is needed to further assess the bone matrix—changes in make-up and quality—in Cx43 murine mutant models and potentially ODDD patients, and their impacts on the resultant bone phenotypes.

DEVELOPMENT AND ACTIVITY OF OSTEOBLAST LINEAGE CELLS

Although functional Cx43 channels are required for the normal processes of differentiation, function, and bone-forming activities of osteoblasts, understanding the exact role—or multiplicity of roles—of Cx43 in the osteoblast lineage is still evolving. For example, there have been discordant findings in terms of osteoblastogenesis and osteoblast function in various Cx43 mutant models. The discrepancies are, in part, related to the stage in the lineage development that Cx43 channels are disrupted. For example, disruption in early stage cells indicates that Cx43 has a role in stromal cell commitment, maintenance of precursor populations and/or in controlling the subpopulation makeup of the stroma. At these earlier stages, disruption of Cx43 channels results in increased mesenchymal progenitor and osteoprogenitor numbers, as seen in the *DM1Cre;Cx43*$^{-/fl}$, *ColCre;Cx43*$^{-/fl}$, and *Gja1*$^{Jrt/+}$ mice [Watkins et al., 2011; Gonzalez-Nieto et al., 2012; Zappitelli et al., 2013], suggesting that Cx43 may function to restrain progenitor numbers in the bone marrow, possibly by suppressing proliferation [Zhang et al., 2001] or by promoting apoptosis; this is in contrast to its role in osteocytes, where Cx43 is necessary to maintain viability (see below) [Plotkin et al., 2008; Bivi et al., 2012a].

It should also be noted that when Cx43 channels are disrupted very early in the lineage (e.g., in *Cx43*$^{-/-}$, *DM1Cre;Cx43*$^{-/fl}$ and $^{+/fl(G138R)}$, *Cx43 +/G138R* and *Gja1*^{Jrt}/+ mice), a wide spectrum of results have been reported from osteoblast dysfunction (reduced mineralization capacity and decreased expression of osteoblast markers) [Lecanda et al., 1998, 2000; Watkins et al., 2011; Grimston et al., 2012] to osteoblast activation (i.e., upregulated expression of osteoblast markers and increased bone formation markers) [Zappitelli et al., 2013]. Furthermore, osteoblasts from different skeletal locations (e.g., calvaria versus trabecular versus cortical bone-derived; endocortical- versus perisoteal-derived; and calvaria-derived versus bone marrow stromal-derived osteoblasts in vitro) are differentially sensitive to loss of Cx43. For instance, ablation of Cx43 typically results in upregulated osteoblast bone formation on periosteal surfaces of cortical bone and enhanced responsiveness to in vivo loading (e.g., *DM1Cre;Cx43*$^{-/fl}$ and *OcnCre;Cx43*$^{-/fl}$ mice) [Watkins et al., 2011; Bivi et al., 2012a; Grimston et al., 2012]. While the effect of Cx43 ablation on endosteal bone formation has been reported to be different in different models, osteoblastic responsiveness to mechanical loading on endosteal surfaces is attenuated in Cx43 knockout mice (e.g., *DM1Cre;Cx43*$^{-/fl}$ and *ColCre;Cx43*$^{-/fl}$ mice) [Grimston et al., 2006, 2008; Watkins et al., 2011; Zhang et al., 2011]. The differing responses to loading on the periosteal and endosteal envelopes of Cx43-deficient bone have been posited to arise due to decreased SOST production specifically by osteocytes close to the periosteal surface or from site-specific (periosteal vs. endosteal) cell autonomous alterations in sensitivity to mechanical load and mechanotransduction. While more remains to be done to understand the mechanisms, the results highlight the complex role that Cx43 has in osteoblastic and their accessory cells in different skeletal locations.

Finally, when Cx43 channels are disrupted at later stages (e.g., in MC3T3 cells, a preosteoblastic cell line, or in *OcnCre;Cx43*$^{-/fl}$ and *DMP1Cre;Cx43*$^{fl/fl}$ mice), osteoblastogenesis and osteoblast activity have been reported to be normal [McLachlan et al., 2008]. However, Cx43 appears to have a role in maintaining osteocyte viability specifically in the cortical bone compartment. Studies on the *OcnCre;Cx43*$^{-/fl}$ and *DMP1Cre;Cx43*$^{fl/fl}$ mice reveal that loss of Cx43 in mature osteoblast and osteocyte populations leads to increased osteocyte apoptosis [Plotkin et al., 2008; Bivi et al., 2012a]. Bivi et al. [2012a] suggested that the reduced osteocyte number results in changes in levels of osteocyte-derived factors (e.g., SOST, RANKL and OPG) that control bone formation and resorption, and that osteocytes undergoing apoptosis emit signals that act as chemoattractants to induce osteoclast recruitment and resorption, driving changes in the bone geometry of these Cx43 mutant mice. Nevertheless, it remains unclear why when Cx43 channels are also disrupted earlier, that is in osteoblast precursors (e.g., in the *DM1Cre;Cx43*$^{-/fl}$ or the *Gja1*^{Jrt}/+ mice), osteocyte numbers and apoptosis are unaffected [Watkins et al., 2011; Zappitelli et al., 2013].

DEVELOPMENT AND ACTIVITY OF OSTEOCLASTS

A variety of in vitro studies indicate that functional Cx43 gap junctions are essential for osteoclastogenesis (differentiation and fusion), osteoclastic bone resorption, and/or osteoclastic survival [Jones and Boyde, 1994; Ilvesaro et al., 2000, 2001; Ransjo et al., 2003]. For example, disruption of Cx43, with the use of antibodies, mimetic peptides (e.g., Gap 27), or pharmacological inhibitors, reduces osteoclastogenesis (formation and fusion of TRAP-positive osteoclasts), bone resorption (number and/or size of resorption pits) and osteoclastic survival rate in culture models. Such studies have suggested that Cx43 gap junction communication has a direct effect on osteoclast development, activities, and survival, perhaps by influencing signaling pathways downstream of RANKL [Matemba et al., 2006], other inducers of osteoclastogenesis, or factors controlling proliferation and/or apoptosis.

On the other hand, when Cx43 channels are disrupted in vivo, as in the mouse models described, osteoclastogenesis, and bone resorptive activities are significantly increased. For example, when Cx43 channels are disrupted globally, as in the *Gja1*^{Jrt}/+ mice, OcS/BS and expression of the osteoclast activity marker Cathepsin K are upregulated; however, no cell autonomous changes in osteoclast parameters are seen when *Gja1*^{Jrt}/+ bone marrow cells are differentiated into osteoclasts in vitro [Zappitelli et al., 2013]. Similarly, disruption of Cx43 at various stages of the osteoblast lineage, as in *DM1Cre;Cx43*$^{-/fl}$, *OcnCre;Cx43*$^{-/fl}$, and *DMP1Cre;Cx43*$^{fl/fl}$ mice, results in increased osteoclast numbers and bone resorption in bones, especially on endosteal surfaces [Watkins

et al., 2011; Zhang et al., 2011; Bivi et al., 2012a]. These results suggest that the effect of Cx43 disruption on osteoclast formation and function is indirect or secondary to communication from osteoblasts, osteocytes, and/or stromal cells either directly through gap junction intercellular communication, through signals emitted from apoptotic osteocytes acting as chemoattractants for osteoclasts and their precursors (as discussed above), or through changes to factors such as RANKL and OPG, or a combination of these.

That Cx43 gap junction communication affects the expression of RANKL and OPG, chemokines produced by osteoblasts, osteocytes, and stromal cells that are crucial to the formation and activation of osteoclasts, is now well established. Disruption of Cx43 in *DM1Cre;Cx43*$^{-/fl}$, *OcnCre;Cx43*$^{-/fl}$, *DMP1Cre;Cx43*$^{fl/fl}$, and *Gja1*^{Jrt}/+ mice causes changes in the RANKL-OPG signaling axis favoring an increase in the RANKL/OPG ratio [Watkins et al., 2011; Zhang et al., 2011; Bivi et al., 2012a; Zappitelli et al., 2013], promoting osteoclastogenesis and bone resorption, and contributing to the osteopenic phenotypes of Cx43 mutant mice. One exception is found in older *Gja1*^{Jrt}/+ mice, in which protection from old-age related bone loss has been attributed in part to an increased production of OPG [Zappitelli et al., 2013].

Given the results to date, it remains unclear whether osteoclasts and adjacent cells communicate directly via gap junction intercellular communication and/or whether osteoclasts rely on hemichannels to communicate (receive and emit signals) with nearby cells on the bone or within the stroma. Regardless, more work is needed to determine the precise direct and/or indirect mechanism(s) by which Cx43 gap junction communication regulates bone resorption.

Cx43 AND BONE CELL RESPONSE TO CHALLENGE OR STIMULI

Modulating the formation and function of gap junctions and hemichannels allows cells to modulate (propagate or diminish) signaling responses in networks of nearby and connected cells. Notably, osteoblastic cells respond to a variety of mechanical and hormonal signals, to bone endogenous (autocrine/paracrine) factors, such as cytokines and growth factors, and to exogenous factors, such as pharmacological agents, with changes to Cx43 expression. This leads to differences in functional coupling (passage of signaling molecules and ions) between cells or cells and their environment, in turn altering osteoblastic cell responsiveness to the same or other signals [Donahue et al., 1995b; Schiller et al., 1997], and resulting in an overall adaptive response of the skeleton to a particular stimulus.

Responsiveness to, for example, mechanical stress is needed to maintain the integrity of the skeleton. Ablation of Cx43 (e.g., in *DM1Cre;Cx43*$^{-/fl}$, *OcnCre;Cx43*$^{-/fl}$, and *DMP1Cre;Cx43*$^{fl/fl}$ mice) generally enhances the anabolic response to mechanical loading [Grimston et al., 2006, 2008; Watkins et al., 2011; Zhang et al., 2011]. The increased responsiveness has been linked to changes in signaling molecules, specifically to a decrease in SOST expression (in bones of *DM1Cre;Cx43*$^{-/fl}$ mice [Grimston et al., 2011]) or to an upregulation of β-catenin protein levels ("priming" osteocytes of *DMP1Cre;Cx43*$^{fl/fl}$ mice to respond to stimulation [Bivi et al., 2013]). Conversely, loss of Cx43 attenuates the catabolic response to unloading (e.g., in *ColCre;Cx43*$^{-/fl}$ [Grimston et al., 2011] and *OcnCre;Cx43*$^{-/fl}$ [Lloyd et al., 2012] mice). This phenomenon has also been linked to a reduction in SOST (i.e., the decreased number of SOST-positive osteocytes in *OcnCre;Cx43*$^{-/fl}$ mice [Lloyd et al., 2013]), or to the increase in baseline osteoclast numbers (in *ColCre;Cx43*$^{-/fl}$ mice [Grimston et al., 2008, 2011]) which may limit further osteoclast activation upon unloading. Regardless, these data suggest that disruption of Cx43 alters how bones perceive and respond to mechanical stimulation, possibly because Cx43 has a role in desensitizing the bone to mechanical signals either by controlling molecules that enhance sensitivity to such signals (e.g., SOST and β-catenin) or by directly controlling the viability/activities of cells that respond to mechanical stimulation (e.g., osteocytes, oteoblasts, and osteoclasts).

These studies also identify location-specific roles for Cx43—where the anabolic responses to loading are typically enhanced on periosteal surfaces but are more variable (attenuated or unaffected) on endosteal surfaces of Cx43-deficent cortical bones, and where the catabolic responses to unloading are attenuated in cortical bone but are more variable (attenuated or unaffected) in trabecular bone of Cx43-deficient mice. As noted earlier, understanding the location-specific response to mechanical signals in Cx43-deficient bone remains the subject of considerable effort. Below we discuss several likely possibilities for the variable responses, including: (A) location-specific changes in molecules regulating osteoblast and osteoclast formation and activity, (B) differences in how strain is distributed or felt at different skeletal sites and influenced by the altered bone architecture in Cx43-deficient bone, and (C) inherent location-specific differences in cell sensitivity to strain and mechanotransduction.

A. Signaling Molecules: As mentioned above, loss of Cx43 may increase osteocyte apoptosis (promoting release of chemoattractants that recruit osteoclasts and their progenitors) and modulate levels of osteocytic genes (factors such as SOST and OPG, that control bone turnover) at specific skeletal locations, for instance, preferentially closer to the periosteal versus endosteal envelope of cortical bone, as suggested by Bivi et al. [2012].
B. Strain Distribution: It is worth noting that bones experience different mechanical forces and pressures depending on location within the organism (e.g., impact during movement felt along the long bones versus strain during mastication in certain areas of calvaria bones [Behrents et al., 1978]), depending on how load/strain is distributed/felt in different parts of an individual bone (e.g., different amounts of force felt on the periosteal vs. endosteal surfaces during stimulation [De Souza et al., 2005]), and the magnitude and type of strain/unloading (e.g., 3-point bending vs. cantilever loading vs. compressive loading, and muscle paralysis vs. hind limb suspension). Further compounding these factors is that skeletal architecture in Cx43-deficient mice is significantly altered, as discussed above, and the impact of these changes on how load is felt and distributed must be taken into account. Such differences may be critical to outcome when one considers that mechanical stimulation itself induces mechanosensing cells such as osteoblasts and osteocytes to upregulate Cx43 channel formation and intercellular communication [Ziambaras et al., 1998; Cheng et al., 2001].
C. Cell Sensitivity: Some studies suggest that there may be cell-inherent site-specific difference in response to loading/unloading

by Cx43 deficiency, in particular that periosteal cells are more sensitive to mechanical strain than those on/near endosteal surfaces, or that signaling through Cx43 may serve to specifically inhibit osteoblast activity on periosteal surfaces and osteoclast activity on endosteal surfaces [Zhang et al., 2011]. Llyod et al. [2012] suggest that Cx43 deficiency attenuates the loss of cortical bone more consistently than trabecular bone during unloading in *ColCre;Cx43*$^{-/fl}$ and *OcnCre;Cx43*$^{-/fl}$ mice because Cx43 has a more significant/prominent role in the structural development of, and activities of cells localized in the cortical over trabecular bone compartment. In this regard, it is interesting to note that all of the Cx43 models discussed in this review exhibit prominent cortical bone phenotypes, while changes in trabecular parameters are much more variable.

Functional Cx43 channels are also necessary to modulate the response of osteoblasts to PTH treatment, thus treatment with PTH upregulates Cx43 expression and gap junction intercellular communication in osteoblastic cells [Schiller et al., 1992; Donahue et al., 1995b; Civitelli et al., 1998]. Cx43 can alter the transcriptional regulation of particular genes′ promoter elements via select signaling pathways, like MAP-kinase and protein kinase C-δ, which may be required to integrate and enhance PTH-activated signals or transduce them across the osteoblast network [Stains and Civitelli, 2005; Niger et al., 2012]. Not surprisingly, loss of Cx43 attenuates the bone anabolic actions of PTH, as demonstrated in the *ColCre;Cx43*$^{-/fl}$ mice [Chung et al., 2006]. Chung et al. [2006] also observed some site-specific differences in Cx43 sensitivity to PTH actions in *ColCre;Cx43*$^{-/fl}$ mice, revealing that loss of Cx43 attenuates PTH response on trabecular bone to a greater extent than cortical bone, and on endosteal surfaces to a greater extent than periosteal surfaces. The site-specific and envelope-specific in vivo actions of PTH in weight bearing bones may be related to the cooperative and synergistic relationship between the effects of PTH and mechanical stimulation [Ma et al., 1999; Sekiya et al., 1999], both of which require the presence of functional Cx43 channels (which also have location-specific roles, as discussed above) to modulate and amplify cellular responses (e.g., increases in osteoblast numbers and activity).

Finally, Cx43 channels are also critical in transducing the pro-survival signals which are generated from local/endogenous factors, systemic hormones (e.g., PTH [Jilka et al., 1999]), mechanical stimulation [Bakker et al., 2004; Plotkin et al., 2005], and pharmacological agents (e.g., bisphosphonates). For instance, Cx43 hemichannels are essential transducers of the cell survival signals and anti-apoptotic effects of bisphosphonates [Plotkin and Bellido, 2001; Plotkin et al., 2002] and ablation of Cx43 inhibits the protective effects (e.g., the anti-apoptotic effect on osteoblasts and osteocytes) of these drugs, as seen in the *OcnCre;Cx43*$^{-/fl}$ mice [Plotkin et al., 2008].

AGE-RELATED CHANGES IN Cx43 CHANNEL FORMATION AND FUNCTION

The formation and responses of Cx43 channels to stimuli decline as a function of age [Asumda and Chase, 2011; Genetos et al., 2012]. Cx43 gap junction formation is significantly lower in older versus younger bone marrow mesenchymal stem cells and hematopoietic stem cells [Rosendaal et al., 1994; Krenacs and Rosendaal, 1998; Asumda and Chase, 2011]. This is interesting vis-a-vis the proposed role of Cx43 in developing bone marrow, during cell division and progenitor proliferation, and in repopulation of the bone marrow during regeneration [Rosendaal et al., 1994; Krenacs and Rosendaal, 1998], but cause versus effect relationships remain to be rigorously determined. However, it has been shown that Cx43 gap junction intercellular communication in response to at least one hormonal signal, that of PTH, decreases as a function of age [Genetos et al., 2012], and ablation of Cx43 in mice results in an attenuated response to PTH [Grimston et al., 2008]. The decrease in Cx43 gap junction formation and function with age may explain, at least in part, why the adult skeleton is less able to adapt to the physical demands placed on it. Also the decreased responsiveness of cells to hormonal and mechanical signals with age, due to decreased formation and function of Cx43 channels, likely contributes to the presence of osteopenia and osteoporosis with increasing age. The early-onset osteopenic phenotypes displayed by the Cx43 mutant mouse models further support the link between reduced Cx43 channel formation/function and osteopenia/low BMD.

CONCLUSION

The development of Cx43 mutant mouse models has provided significant support for and new understanding of the critical role(s) that Cx43 channels play in multiple aspects of skeletal development, turnover and function. Cx43 channels are both generated in response to stimuli (e.g., mechanical, hormonal, and other [cytokines, growth factors]) and essential in the propagation of these and other signals between cells in the skeleton and the cells and their environment for such critical cell functions as bone cell differentiation-survival-apoptosis, bone formation-resorption, and the composition and quality of the matrix formed.

Beyond elucidating critical roles for Cx43-containing gap junctions and hemichannels in development and turnover of the skeleton, the genetically engineered mouse models discussed are providing insights into the cellular and molecular mechanisms by which Cx43 functions in bone. Further, it is now clear that the formation and function of Cx43 channels and their impact on cellular activities vary as a function of skeletal site, age of the organism, and stage of lineage development of osteoblastic cells. The basis or bases of these variations will require additional studies but are likely to be multifactorial. For example, as indicated above, mechanical load modulates Cx43 expression in osteoblastic cells, and Cx43 channels mediate different cellular responses (bone formation and resorption) based on the different forces generated and felt, leading to changes in skeletal architecture, bone mass, and mechanical properties of the bone. As also noted above, PTH upregulates Cx43 expression and, in the skeleton′s role as the body′s reservoir for calcium, Cx43 channels mediate the responses of osteoblasts to PTH, a hormone that regulates calcium homeostasis by promoting osteoblastogenesis, bone formation, and RANKL production. Furthermore, the skeletal site-specific actions of PTH—predominantly in the weight bearing long and vertebral bones

[Iida-Klein et al., 2002]—arise due to the cooperative actions of mechanical stimulation and PTH (both of which require functional Cx43 channels) to synergistically increase site-specific bone formation [Ma et al., 1999; Sekiya et al., 1999; Turner et al., 2006].

As briefly discussed, an area deserving more attention is understanding Cx43 influences on osteoblast and osteocyte activity, including expression of osteoblast-associated genes and proteins such as BSP, OCN, and FGFs, as well as resultant changes in matrix quality and composition (e.g., matrix content of SIBLING proteins). This not only impacts bone parameters (e.g., bone density, architecture, and material properties), but through bone's endocrine functions can affect other target organs. For instance, the production of FGF23 by osteocytes can control phosphate homeostasis by affecting resorption in the kidneys, and the production of OCN by osteoblasts or its release from the bone matrix by osteoclasts can affect insulin production/secretion by pancreatic beta cells [Lee et al., 2007; Ferron et al., 2010; Fulzele et al., 2010].

Finally, although the cause versus effect relationships have yet to be fully elucidated, bone density and Cx43 gap junction channels and their responses to stimuli decline as a function of age. It is possible that this decrease in gap junction intercellular communication disrupts the equilibrium between bone formation and resorption required in order to maintain skeletal integrity. Thus, Cx43 may be a potential therapeutic target in the future, but its ubiquitous expression means that any methods targeting Cx43 expression or channel function would have to be targeted to the bone tissue with a high amount of specificity. Also, the importance of Cx43 in mediating the cellular responses to osteoporotic treatments like bisphosphonates or PTH, means that Cx43 may be an important target in patients identified as "non-responsive" to current treatments. In the meantime, it is important that we gain further understanding about the complex roles of Cx43 in bone.

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