Journal of Oral Biosciences 55 (2013) 29–33

Contents lists available at SciVerse ScienceDirect

Journal of Oral Biosciences

journal homepage: www.elsevier.com/locate/job

Review

Biological roles of gap junction proteins in cartilage and bone development

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ARTICLE INFO

Article history:
Received 16 November 2012
Received in revised form
5 December 2012
Accepted 17 December 2012
Available online 12 February 2013

Keywords:
Connexin
Pannexin
Gap junction
Cartilage
Bone

ABSTRACT

Cell–cell and cell–matrix interactions are essential for cell differentiation, function, and maintenance of skeletal tissue. Gap junction proteins, composed of connexin (Cx) and pannexin (Panx) families, mediate these interactions and play an important role in cell–cell communications. Cx and Panx share similar protein structures, but have evolved differently. The Panx family was initially identified by its sequence homology to the invertebrate gap junction innexin family. The Panx family comprises three members, Panx1, 2, and 3. Panx1 is expressed in many organs, such as the eyes, thyroid, prostate, kidneys, and liver, but its expression is especially strong in the central nervous system. Similarly, Panx2 is expressed mainly in the central nervous system. Panx3 is expressed predominantly in skeletal tissues, including cartilage and bone. In this review, we describe the expression and functions of Cxs and Panx3 in cartilage and bone.

Contents

1. Introduction

Bone is a mineralized connective tissue that has numerous important functions, including mechanical protection for organs, mineral storage, and hematopoiesis [1,2]. Bone is formed by two different processes: endochondral ossification and intramembranous ossification [3]. Endochondral ossification contributes to the formation of most bones in the body, and its process involves a series of events. These processes include mesenchymal condensation, formation of cartilage and apoptosis of mature hypertrophic chondrocytes, and removal of mineralized cartilage with vascularization followed by replacement with bone-forming cells, and subsequently, trabecular bone formation. In contrast, the process of intramembranous ossification involves bone formation by osteoblasts in the absence of a cartilaginous scaffold. Gap junctions play a critical role in the differentiation of chondrocytes and osteoblasts. Chondrocytes express connexin (Cx) 43 and pannexin (Panx) 3 [1,2], and osteoblasts express Cx43, Cx45, Cx46, and Panx3 [1,3]. Here we review the recent findings on gap junction proteins in cartilage and bone development.

2. Connexins and pannexins

Gap junctions, which are specialized cell–cell interactions, comprise an intercellular transmembrane channel that connects the cytoplasm of neighboring cells through the docking of

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http://dx.doi.org/10.1016/j.job.2012.12.001

hemichannels. Hemichannels that mediate the cell–matrix interaction are formed by gap junction proteins, and they function as a single transmembrane channel [4]. Gap junctions in vertebrates are composed of members of the Cx and Panx families. Although the sequences of Cxs and Panxs are different, they share similar protein structures [5]. Both Cxs and Panxs have characteristic structures consisting of four hydrophobic transmembrane domains spaced by two extracellular loops, an intracellular loop and intracellular amino (NH_2) and carboxyl (COOH) termini (Fig. 1). This structure is required for the formation of a hexameric membrane pore complex and the hemichannel, which is the basic structure of all gap junction proteins [6]. Cxs assemble as hexamers to form a single channel, which is called the connexon [7]. On the other hand, Panx1 and Panx2 form hexameric and octameric channels, respectively [8,9]. The number of Panx3 oligomers has not been analyzed, but it is predicted that Panx3 forms a hexameric channel [8]. Panx oligomers are often called pannexons; each docked connexon or pannexon forms a gap junction channel. Undocked connexons or pannexons can function as hemichannels in the plasma membrane. Functional hemichannels have been shown for several Cxs, such as Cx23, Cx46, Cx43, and all Panxs. Bruzzone et al. [10] suggested that Panx1 has the ability to form functional intercellular channels in a paired *Xenopus* oocyte. However, many studies have indicated that Panx1 acts as a single membrane channel, allowing communication with the extracellular space [11–16]. Cxs have three conserved cysteine residues in each extracellular loop, with the exception of Cx23, which has two cysteine residues and forms a hemichannel, while Panxs have two conserved cysteine residues in each of their loops (Fig. 1). It has been suggested that these cysteines may be involved in the formation of gap junction channels and their properties [9,12,17]. This structural property may support the hypothesis that the major function of Panxs is to act as single membrane channels. However, innexins, the invertebrate gap junction proteins, have two conserved cysteine residues in each extracellular loop, and they can form gap junctions [18]. Furthermore, sequence analysis of Panx1 and Panx3 proteins revealed that *N*-linked glycosylation occurs at asparagine 254 in the second extracellular loop of Panx1, and at asparagine 71 in the first extracellular loop of Panx3 [19]. Further, Panx2 encodes a potential *N*-linked glycosylation site at asparagine 86 in its first extracellular loop [20]. Glycosylation of Panxs is important for proper trafficking, stability, and function of these proteins at the cell surface [8,19]. This glycosylation is thought to disturb the docking of two pannexons [21]. However, *N*-glycosylation-deficient Panx1 and Panx3 mutant proteins can reach the cell surface and form functional channels, indicating that Panxs can form functional channels even in the absence of glycosylation [20,22]. Panxs are expressed in many different tissues and cell types, and their cell surface expression levels vary for the different locations. These observations suggest that the cell-specific expression, function, and channel properties of Panxs may be mediated by post-translational modifications, such as glycosylation and phosphorylation.

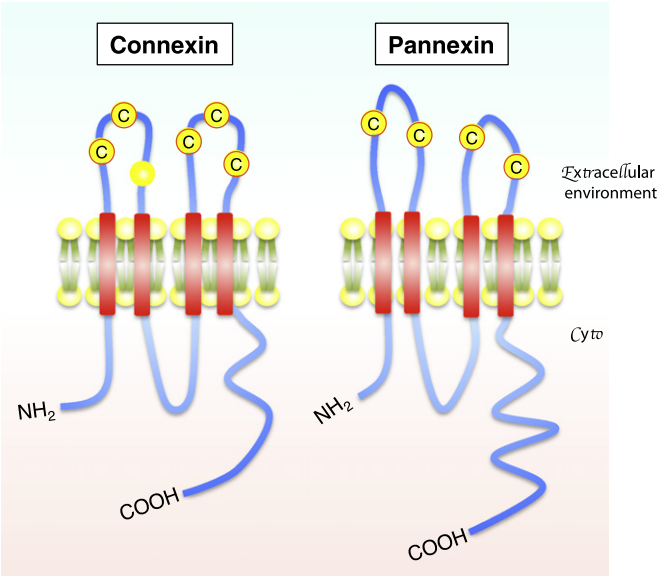

Fig. 1. Structures of connexin and pannexin. Both connexin and pannexin family proteins consist of four transmembrane domains, two extracellular loops and amino- (NH_2) and carboxyl- (COOH) terminal cytoplasmic tails. Further, each extracellular loop in connexin and pannexin comprises three and two cysteine residues (C), respectively.

3. Connexins in cartilage development

Cxs have been shown to play critical roles in skeletal development in humans and animals [23,24]. In the initiation of endochondral ossification, mesenchyme condensation is required for the precise positioning and formation of the cartilaginous skeletal model [25]. The condensing cells differentiate into chondrocytes, which proliferate and produce type II collagen, forming the proliferative zone. Chondrocytes then cease proliferating in the prehypertrophic zone, and they differentiate into type X collagen-producing hypertrophic chondrocytes in the hypertrophic zone. Mature terminally differentiated hypertrophic chondrocytes mineralize the surrounding cartilage matrices, eventually die by apoptosis, and are replaced with osteoblasts to form the trabecular bone [25]. In these processes, Cx43 transcripts can be detected in the limb mesenchyme condensation [26]. In a developing chick wing, blocking Cx43 expression using antisense oligodeoxynucleotides causes limb patterning defects, including deletion of the anterior digits [27]. A gap junction blocker, 18 alpha glycyrrhetinic acid (18α-GCA), inhibits BMP-2-mediated mesenchymal cell condensation and chondrogenic differentiation in in vitro micromass cultures of chondrocytes from chick embryos [28]. These results indicate that Cx43 is required for mesenchyme condensation and that it regulates limb patterning and morphogenesis. In humans, patients with oculodentodigital dysplasia (ODDD) associated with Cx43 gene mutations have limb phenotypes that include syndactyly of the hand and foot, hypoplasia or aplasia of the middle phalanges, and abnormalities in the craniofacial elements and dentition [23]. However, the long bones in Cx43-null mice are similar in size compared tithe wild-type mice [1,24], suggesting that Cx43 is dispensable for growth plate formation. The reason for the apparent discrepancy between these observations is not clear. When we examined the expression of Cx43 mRNA and protein in the growth plate of mouse embryos by in situ hybridization and immunostaining, we observed very low levels of Cx43 expression in growth plate chondrocytes. There are large numbers of chondrocytes embedded in the growth plates of cartilage matrices, so it may be difficult to form gap junctions between neighboring cells. In contrast, in articular cartilage, Cx43 is expressed in the outer layer of the knee joint articular cartilage [29]. Stimulation of rabbit articular chondrocytes in a primary culture with interleukin-1b induced a dose-dependent upregulation of Cx43 [30].

4. Connexins in bone development

The expressions and functions of Cxs have been studied in several osteoblastic cell lines, as well as in primary cultures