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Structure and function of gap junction proteins: role of gap junction proteins in embryonic heart development

BHAVESH K. AHIR[1] and MARGARET K. PRATTEN*[,2]

[1]National Center for Computational Toxicology (B205-01), U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA and [2]School of Life Sciences, Queen's Medical Centre, University of Nottingham, Nottingham, UK

ABSTRACT Intercellular (cell-to-cell) communication is a crucial and complex mechanism during embryonic heart development. In the cardiovascular system, the beating of the heart is a dynamic and key regulatory process, which is functionally regulated by the coordinated spread of electrical activity through heart muscle cells. Heart tissues are composed of individual cells, each bearing specialized cell surface membrane structures called gap junctions that permit the intercellular exchange of ions and low molecular weight molecules. Gap junction channels are essential in normal heart function and they assist in the mediated spread of electrical impulses that stimulate synchronized contraction (via an electrical syncytium) of cardiac tissues. This present review describes the current knowledge of gap junction biology. In the first part, we summarise some relevant biochemical and physiological properties of gap junction proteins, including their structure and function. In the second part, we review the current evidence demonstrating the role of gap junction proteins in embryonic development with particular reference to those involved in embryonic heart development. Genetics and transgenic animal studies of gap junction protein function in embryonic heart development are considered and the alteration/disruption of gap junction intercellular communication which may lead to abnormal heart development is also discussed.

KEY WORDS: *intercellular communication, heart development, embryogenesis, teratogenicity, embryotoxicity*

Introduction

The complex events underlying embryonic development and homeostatic balance require a flow of information (cell-to-cell communication) between cell and tissue subsystems (Levin, 2002). There are two modes of cell-to-cell communication which depend on both extracellular and intracellular pathways to coordinate intercellular communication within tissues or cells: (a) the extracellular pathway uses the secretion of signal transduction substances like hormones, neurotransmitters and growth factors into the extracellular spaces. (b) the intracellular pathway occurs within the limiting plasma membrane of a group of cells and is mediated by specialised cell surface membrane structures (termed gap junctions) that permit the intercellular exchange of ions and low molecular weight molecules (John *et al.*, 2003).

Intercellular communication via gap junctions

Intercellular communication through gap junctions has vital roles in cell differentiation, survival, metabolism, morphogenesis, and mutagenesis (Levin, 2002; Levin and Mercola, 1999; Lo, 1996; Lo and Gilula, 1979; Perkins *et al.*, 1997). Gap junction (GJ)* proteins play an important role in direct communication between cells of many tissue types. GJs are specialised intercellular membrane-spanning domains that allow the passage of small molecules (<1kDa) including second messenger (e.g. cyclic- adenosine monophosphate (c-AMP), inositol triphosphate) or ionic signals from one cell to another (Sohl and Willecke, 2004). The name "gap" derives from the 2-3 nm gap between the plasma membrane of the two apposing cells connected by such channels (Wei *et al.*, 2004). These GJ channels are comprised of a series of transmembrane proteins called connexins (Cxs), where six such proteins form a hemichannel which docks with a compatible hemichannel of a neighbouring cell. Thus, each gap junction channel is composed of a pair of hexamers termed connexons (hemichannels) that, in turn, are comprised of six subunits termed Cxs (Coppen *et al.*, 2003).

Abbreviations used in this paper: Cx, connexin; GJ, gap junction; GJIC, gap junction intercellular communication.

***Address correspondence to:** Margaret K. Pratten. School of Life Sciences, Queen's Medical Centre, University of Nottingham, Nottingham NG7 2UH, UK, Phone: 44-(0)-1158-230174. Fax: 44-(0)-1158-230142. E-mail: margaret.pratten@nottingham.ac.uk

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In this article, the current biology of GJ proteins (including the biochemical and physiological properties) and the structure and function of GJ proteins are reviewed. Special attention is paid to the involvement of gap junction intercellular communication (GJIC) in embryology with particular references to those involved in embryonic heart development and the alteration and/or disruption of GJIC is described, which may lead to abnormal heart development

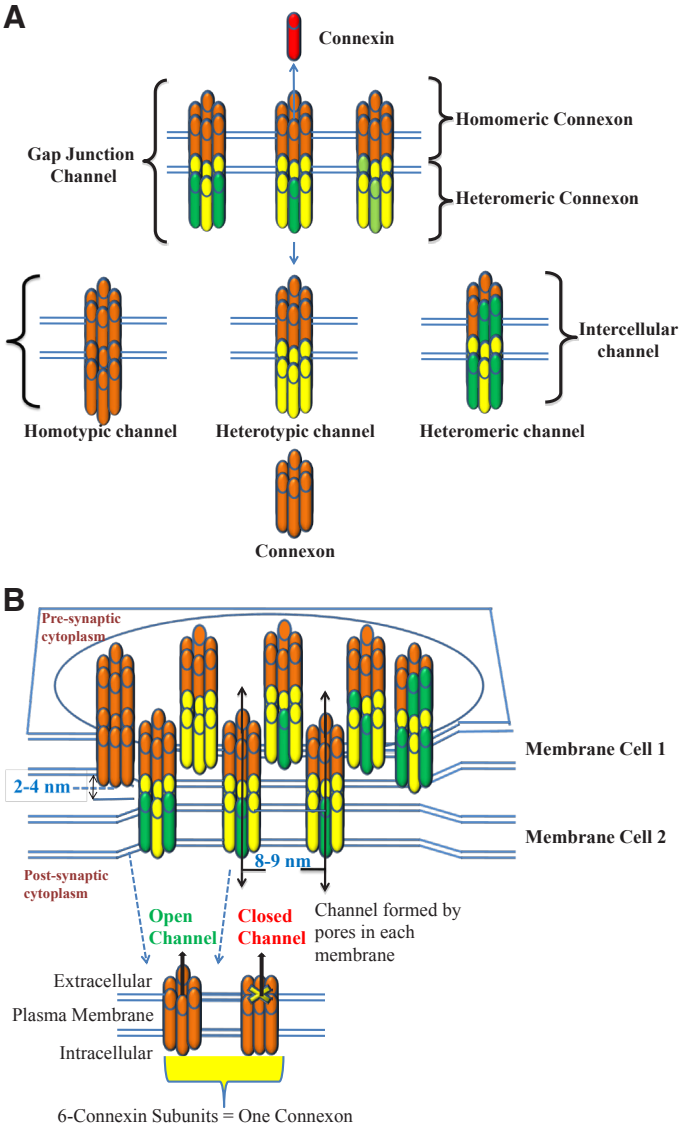

Fig. 1. Assembly of connexins to gap junctions. (A) *Structure of Gap junction (GJ) intercellular channels. Six Cx gap junction proteins are assembled to form a connexon. A connexon from one cell docks with a connexon from an apposing plasma membrane to form an intercellular GJ channel. Oligomerization of different types of connexons may form homotypic or heterotypic or heteromeric channels, containing multiple Cxs.* **(B)** *Schematic illustration of groups of intercellular channels (termed a gap junctional plaque). Part of a gap junction plaque showing multiple intercellular channels interconnecting two cells and the composition of an individual channel from two half channels (connexons) which are made of connexin proteins. Six connexin sub units of the hemichannel (connexon) may co-ordinately change configuration to open and close the hemichannel.*

Biochemical and physiological properties of connexins

Connexins (Cxs) are a multigene family of proteins. To date, 20 Cx genes have been identified in the mouse genome (Table 1) and 21 in the human genome (Table 1), however, this figure is constantly changing as new Cxs are being identified (Dbouk *et al.*, 2009; Sohl *et al.*, 2003; Sohl and Willecke, 2004; Verheule and Kaese, 2013) Currently, Cxs are named either according to the molecular mass of the polypeptide predicted from the cDNA sequences or from evolutionary considerations, for example a 43 kDa Cx protein is referred to as either $\alpha 1$ connexin or connexin43. Using the former nomenclature system, Cx proteins have been sub-classified or subdivided into at least three subgroups referred to as Group I (α-connexins), Group II (β-connexins), Group III (γ-connexins) or Group IV (δ-connexins) according to sequence similarity and length of cytoplasmic domain (Kumar and Gilula, 1996; Willecke *et al.*, 2002). The oligomerisation of Cx to connexon occurs in the Golgi apparatus (GA), after their synthesis in the endoplasmic reticulum (ER) (Musil and Goodenough, 1993). Cxs inserted into the ER membrane assemble into oligomeric structures composed of six protein subunits arranged concentrically around a central channel, these are connexin hemichannels (CxHCs) often called a connexon (Fig. 1A) (Evans and Martin, 2002; Saez *et al.*, 2003). Connexons may be comprised of a single Cx protein, termed homomeric, or contain different types of Cx proteins, termed heteromeric connexons, and two identical connexons can form a homomeric or a heteromeric channel can be generated by two connexons having two or more different Cx subunits. Furthermore, the docking of two homomeric connexons composed of the same Cx protein yields a homotypic intercellular channel, whereas the oligomerization of two homomeric Cxs comprised of different Cxs forms a heterotypic intercellular channel (Rackauskas *et al.*, 2007). Both homomeric/heteromeric (Fig. 1A) forms of channels exist *in vitro* and *in vivo*, and these homo or heterotypic channels provide greater complexity in the regulation of gap junction intercellular communication (Laird, 2005).

Cxs are fairly ubiquitous in most mammalian tissues, and are being found in other vertebrates (White *et al.*, 2004; Willecke *et al.*, 2002). Invertebrates display direct cell-to-cell communication via a family of proteins termed innexins, which play a Cx-like role, even though they lack primary amino acid sequence homology to Cxs (Phelan, 2005; Phelan and Starich, 2001). In addition, three innexin related proteins, termed pannexins, have been identified in the genome of higher vertebrates, although it is still not clear if they form intercellular channels (Bruzzone *et al.*, 2003; Koval *et al.*, 2014) As already mentioned, CxHCs, are delivered to the plasma membrane, where they diffuse laterally into cell-contact regions to dock head-to-head with partner Cxs present on adjacent cells to produce a channel (Fig. 1B) (Bruzzone *et al.*, 1996; Evans *et al.*, 2006; Goodenough *et al.*, 1996; Kumar and Gilula, 1996). The GJ generated directly couples the cytoplasm of adjacent or neighbouring cells and underpins the integration and coordination of cellular signalling, metabolism and physiological functions including cell differentiation, growth and proliferation, electrical activation of tissue (e.g. contraction of heart or smooth muscle cells), neuronal signalling, hormone secretion, auditory function and wound healing (Bruzzone *et al.*, 1996; Goodenough *et al.*, 1996; Kardami *et al.*, 2007; Kumar and Gilula, 1996). Thereby, they can provide both electrical and metabolic coupling between excitable (e.g. smooth

TABLE 1

TABLE OF KNOWN HUMAN AND MOUSE CONNEXIN (CX) GENES AND THEIR EXPRESSION

Human Connexin (Gene Symbol)	Mouse Connexin (Gene Symbol)	Representative tissue/organ	Representative cell type
hCx23 (GJE1)	*mCx23 (Gje1)*	*Nd*	*Nd*
hCx25 (GJB7)	*Nd*	*Nd*	*Nd*
hCx26 (GJB2)	*mCx26 (Gjb2)*	*Breast, cochlea, liver, kidney, pancreas, intestine, placenta and skin*	*Hepatocytes, keratinocytes*
hCx30.2/Cx31.3 (GJC3)	*mCx29 (Gjc3)*	*Brain*	*Oligodendrocytes, schwann cells*
hCx30 (GJB6)	*mCx30 (Gjb6)*	*Brain, cochlea, skin*	*Keratinocytes*
hCx30.3 (GJB4)	*mCx30.3 (Gjb4)*	*Skin, kidney*	*Keratinocytes*
hCx31 (GJB3)	*mCx31 (Gjb3)*	*Cochlea, placenta, skin*	*Keratinocytes*
hCx31.1 (GJB5)	*mCx31.1 (Gjb5)*	*Skin*	*Keratinocytes*
hCx31.9 (GJD3)	*mCx30.2 (Gjd3)*	*Testis*	*Smooth muscle cells*
hCx32 (GJB1)	*mCx32 (Gjb1)*	*Liver, nervous,*	*Hepatocytes, schwann cells,*
Nd	*mCx33 (Gja6)*	*Testis*	*Sertoli cells*
hCx36 (GJD2)	*mCx36 (Gjd2)*	*Retina, nervous, pancreas*	*Neurons, pancreatic beta cells*
hCx37 (GJA4)	*mCx37 (Gja4)*	*Blood vessels, lung, skin*	*Endothelial cells, granulose cells*
hCx40 (GJA5)	*mCx40 (Gja5)*	*Heart, skin*	*Cardiomyocytes, endothelial cells, keratinocytes*
hCx40.1 (GJD4)	*mCx39 (Gjd4)*	*Developing muscle*	*Myocytes*
hCx43 (GJA1)	*mCx43 (Gja1)*	*Heart, skin*	*Many cell types*
hCx45 (GJC1)	*mCx45 (Gjc1)*	*Heart, skin*	*Cardiomyocytes, smooth muscle and neuronal cells*
hCx46 (GJA3)	*mCx46 (Gja3)*	*Eye (lens)*	*Lens fiber cells*
hCx47 (GJC2)	*mCx47 (Gjc2)*	*Brain*	*Oligodendrocytes*
hCx50 (GJA8)	*mCx50 (Gja8)*	*Eye (lens)*	*Lens fiber cells*
hCx59 (GJA9)	*Nd*	*Nd*	*Nd*
hCx62 (GJA10)	*mCx57 (Gja10)*	*Eye (retina)*	*Horizontal cells*

[Nd: not detected]

and cardiac muscle) and non-excitable cells (e.g. endothelial cells, fibroblasts and adipocytes). Signals are therefore able to travel distances through a monolayer via GJs without being exposed to the extracellular milieu (Bennett *et al.*, 1991; Bruzzone *et al.*, 1996; Kumar and Gilula, 1996). GJ channels also provide a low resistance intercellular pathway for the conduction of electrical impulses in synchronous beating cardiomyocytes and the voltage mediated signals across the heart (Kanno and Saffitz, 2001). It is difficult to attribute specific cellular functions to connexons due to the lack of specific inhibitors for Cxs or GJ intercellular channels. Connexon channels are regulated by complex mechanisms which are sensitive to stimuli such as calcium, pH, voltage, phosphorylation and dephosphorylation (Harris, 2001). Connexon channels have very similar structures; however, the physiological properties are thought to be dependent upon the Cx composition in the connexon channel. Each channel can be comprised of different combinations of Cx proteins, dependent upon the types of Cx protein found within the particular cells (Johnson *et al.*, 1973; Sandow *et al.*, 2003; Woodward *et al.*, 1998). These connexon channels can form across similar cell types, e.g. one cardiomyocyte to next cardiomyocyte (homocellular) or between different cell types e.g. cardiomyocyte–to-endothelial cell (heterocellular).

Most tissues and cell types express two or more different Cx proteins. For example, keratinocytes express at least Cx26, Cx30, Cx30.3, Cx31, Cx31.1 andCx43 (Table 1) (Di *et al.*, 2005; Goliger and Paul, 1994; Kretz *et al.*, 2003). Likewise, heart cells (cardiomyocytes) express Cx40, Cx43 and Cx45 (Table 1) (Beyer *et al.*, 1995; Moreno, 2004; Oyamada *et al.*, 1994) and hepatocytes (liver cells) express Cx26 and Cx32 (Hennemann *et al.*, 1992; Paul, 1986). Collectively, co-expression of multiple Cx proteins within the same cell or tissue type allows for possible compensatory mechanism to overcome the mutation or loss of one Cx protein type., For example the loss of Cx26, found in both keratinocytes and hepatocytes, leads to deafness, but no liver diseases have been reported suggesting that the co-expressed Cx43 may compensate for the loss of Cx26 in liver cells (Laird, 2006). Cx polypeptides have been found within the ER and in the GA suggesting that the trafficking of Cx proteins to the cell surface is through the secretory pathway. This was further confirmed using methods that interfere with this pathway using drugs, e.g. Brefeldin-A or carbonyl cyanide m-chlorophenylhydrazone, or by using temperature control methods (Laird *et al.*, 1995; Segretain and Falk, 2004). After leaving the GA, Cxs travel via vesicular carriers along microtubules to the plasma membrane (Lauf *et al.*, 2002). However, evidence suggests that some Cxs, including Cx43, may be trafficked in a microtubule independent manner (Duffy *et al.*, 2002).

Communicating junctions are found in most mammalian cell types with the exception of skeletal muscle fibres, certain neurones, circulatory blood cells (though some blood cells express GJ proteins and GJ like structures) and spermatozoa (Sertoli cells and Leydig cells) (Bruzzone *et al.*, 1996; Mok *et al.*, 1999; Risley *et al.*, 1992).

Intercellular communication between endothelial cells is crucial for cell survival and tissue homeostasis. There are three types of Cxs (Cx37, Cx40 and Cx43) expressed by human vascular endothelial cells. It was first suggested that the major Cx expressed by vessel walls was Cx43 (Bruzzone *et al.*, 1996; Delorme *et al.*, 1997; Isakson *et al.*, 2006). However, previously reported evidence suggests that Cx expression and distribution may be species and tissue specific, with difference in expression between cells in culture and *in situ* (Delorme *et al.*, 1997).

Compositions or structure of connexins

Cx proteins form connexon hemichannels at the cell membrane which are hexameric, transmembrane proteins with both the C and N terminals residing within the cytoplasm (Fig. 2). The six diverse subunits of Cx are symmetrically organised in the plane of the membrane bilayer, and were first identified in 1977 (Caspar *et al.*, 1977). Each Cx protein is folded into an 'M' shape and it traverses the plasma membrane four times. The transmembrane structure of a generic connexin protein consists of four hydrophobic membrane-spanning domains (M1-M4), two conserved extracellular domains (E1-E2), and three distinct intracellular domains, the NT (amino) and CT (carboxyl)-termini and one variable cytoplasmic loop (CL) facing the cytoplasm (Fig. 2) (Goodenough *et al.*, 1996). The extracellular regions, E1 and E2 are loops which interact with the connexon channels of adjacent cells (Yeager and Gilula, 1992) via highly conserved cysteine residues (©-cysteine), which are involved with the regulation of connexon-connexon interactions, channel formation and voltage gating (Solan and Lampe, 2009). There are regions between the transmembrane (TM) domains M2 and M3 and the C-terminal that are highly variable between connexons of differing molecular weight mass, which are thought to be

involved in regulation (White *et al.*, 1995). Cx proteins differ mainly in the amino acid sequences of the intracellular loop (cytoplasmic loop) and carboxyl terminal tail (CT) (Goodenough *et al.*, 1996). Investigation of the four transmembrane spanning domains of the Cx proteins have identified that a tilting action of these regions can close the gap junction channel, untilted Cx protein monomers being open channels. Within the extracellular loops (E1 and E2) there are three highly conserved (except Cx31) cysteine (C) residues (Fig. 2), first loop: [C-X_6-C-X_3-C] and second loop [C-X_5-C-X_5-C]. Opposing C residues on the loops are thought to form disulphide bridges to stabilise the docking of two connexon hemichannels to form the conduit channel (Sohl and Willecke, 2004). Disulphide bridges in between the cysteines within E1 and E2, crossing the space between E1 and E2, create the β-barrel conformation required for interaction between the two opposing Cxs (Evans *et al.*, 2006).

Overview of the life cycle of connexins

Cxs are synthesised by membrane bound ribosomes and transported from ER (endoplasmic reticulum) to the plasma membrane (Zhang *et al.*, 1996). The oligomerization of Cx to connexon occurs in the Golgi apparatus, termed the trans-Golgi network (TGN) depending upon the Cx types (e.g. Cx43 and Cx46 do not seem to be oligomerised into connexons while they remain in the ER but are most likely to oligomerise in the TGN) (Musil and Goodenough, 1993). Misfolded Cx proteins tend to be translocated from ER membranes and are likely subjected to the proteosomal degradation pathway. In some transformed cell lines or cells with defective protein trafficking features, Cx may be able to bypass the normal secretory route and enter into lysosomes for lysosomal degradation (Fig. 3) (Evans *et al.*, 2006). With the help of microtubules, transport vesicles are thought to deliver closed connexons to the cell surface and cadherin based cell adhesion events facilitate their docking at sites of intercellular contact, forming a GJ channel (Evans *et al.*, 2006). They then diffuse laterally into cell-contact regions to dock head to head with partner Cxs present on adjacent cells to form GJ plaques (Fig. 3, thin light blue colour arrow) (Laird, 2006). Previously reported evidence suggests a role for tight junction associated scaffolding proteins, like zonula occludens-1 (ZO-1); interaction of ZO-1 with multiple Cxs may play a role in regulating the size of the GJ plaque (Giepmans, 2004), and for the exchange of small molecules including second messengers (e.g. cyclic-AMP, inositol triphosphate) (Martin and Evans, 2004). One mechanism of GJ internalisation is via the formation of annular junctions, where GJ plaques and fragments of GJ plaques are internalised into one of the two neighbouring cells as a double membrane bound structure. There are other mechanisms of GJ disassembly into small aggregates and internalisation using more classical pathways involving clathrin, caveolae and endosomes have not been ruled out. There is thus some evidence to suggest that the GJs and Cxs are degraded by both a ubiquitin-dependent proteosomal pathway and a lysosomal pathway (Laing *et al.*, 1997; Qin *et al.*, 2003; VanSlyke *et al.*, 2000; VanSlyke and Musil, 2005). Internalisation and degradation of GJs are dynamic mechanisms with reports of Cxs having a rapid turnover rate. Cx43 has been identified as having a half-life of 1.5-5 hours, making it a good cell modulator (Laird *et al.*, 1995). Although this is a rapid protein turnover (Laing *et al.*, 1997), communication is not thought to be controlled entirely by protein turnover/degradation, as there are factors such as pH, changes in voltage, and Cx phosphorylation which can gate Cx channels (Laird, 2005). The regulation of GJ assembly and turnover is likely to be vital in the control of intercellular communication (Solan and Lampe, 2009).

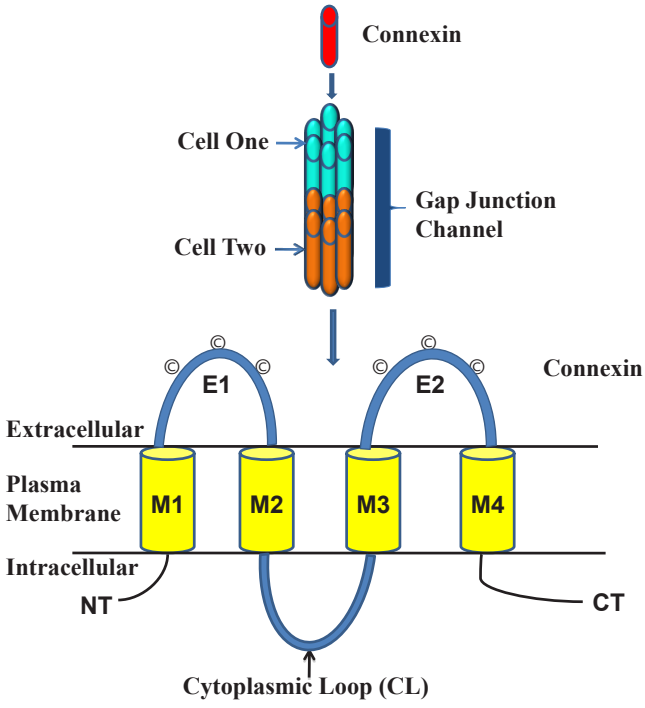

Fig. 2. Topological model of a single connexin (Cx) protein. *Gap junctions (GJs) are grouped together in plaques at the membrane surface, and are made up of twelve Cx proteins, organised as hemichannels (two hexameric connexons). The Cx structure composed of two extracellular loops, designated E1 and E2, four transmembrane-spanning domains (TM), one cytoplasmic loop (CL), one amino-(N) terminus (NT) region, and one carboxy-(C) terminus (CT) tail region. Each extracellular loop (E1 and E2) contains three conserved cysteines (C).*

Regulation or function of gap junction proteins: connexin phosphorylation

Cxs may undergo various types of biochemical and post-translational changes, including phosphorylation, hydroxylation, acetylation, disulfide bonding, nitrosylation, and palmitoylation. Among these post-translational modifications, the most studied and well understood is phosphorylation of Cxs on various residues (Solan and Lampe, 2009). These phosphorylation mechanisms are crucial in regulation and the proper control of formation of GJ channels. Phosphorylation of the Cx proteins causes alteration in GJ intercellular communication due to a conformational or structural change of the protein, which often results in a translocation of the Cx protein to the cytoplasm instead of forming a GJ plaque with the cell plasma membrane (Musil and Goodenough, 1991). At least 9 Cxs (Cx31, Cx32, Cx37, Cx40, Cx43, Cx45, Cx46, Cx50, and Cx56) have been shown to be phosphospecific proteins. Furthermore, many of the family of Cx protein not only contain “consensus phosphorylation sequences”, but also have been shown to be phosphorylated in the CT (Carboxyl-terminal) region that is located in the cytoplasm. Phosphorylation of the NT

(Amino-terminal) region of Cxs, that is also cytoplasmically located, has not been reported (Solan and Lampe, 2005). The exception to this is Cx26 which has a short C-terminal domain and is thought to remain unphosphorylated. Phosphorylation of Cx have been shown to involve different kinases (Fig. 4) such as v-Src (avian sarcoma (Schmidt-Ruppin-A-2)) viral oncogene, Protein kinase-C (PKC) and mitogen activated protein kinases (MAPKs) in *in vitro* cell cultures or tissues (Lampe and Lau, 2000; Musil and Goodenough, 1991).The effect of phosphorylation on GJ channel gating is very specific and selective. For example, the phosphorylation of Cx43 on different residues by the same kinase may result in opposite effects with respect to inhibiting or enhancing GJ intercellular communication (GJIC) (El-Sabban *et al.*, 2003). Fig. 4 shows a schematic illustration of the Cx43 protein denoting some of the known phosphorylation sites on the C-terminal domain region.

Treatment of cells with 12-O-tetradecanoylphorbol-13-acetate (TPA) (also known as phorbol ester), a potent tumor promoter which stimulates PKC, has been shown to reduce the number of GJs shown by freeze fracture electron microscopy (Laing *et al.*, 1997). Cx43 assembly is blocked and half-life shortened on TPA treatment (Lampe, 1994). Moreover, TPA and epidermal growth factor (EGF) are potent inhibitors of GJIC and their activation has been associated with phosphorylation of Cx43 at different sites consequently decreasing the amount of GJs formed within the cell membrane (Sirnes *et al.*, 2009).

TPA has been shown to induce rapid phosphorylation of Cx43 and inhibition of GJIC in a number of cell types. Interestingly, TPA mimics diacylgylcerol (DAG), an endogenous activator of PKC, which is formed by phospholipase-C (PLC) mediated cleavage of phosphatidylinositol 4, 5-bisphosphate (PIP_2) (Fig. 5). TPA binds to PKC to activate it instead of DAG (Leithe and Rivedal, 2004; Nishizuka, 1986). Cleavage of PIP_2 forms IP_3 (inositol-1,4,5-triphosphate) which in turn increases free Ca^{2+} levels in the cytoplasm and then Ca^{2+} increases PKC activity and cause a decrease in GJIC not only because of rapid conformational change of GJs but also due to the phosphorylation of Cx43 proteins by PKC in the long term (Dhein, 2004; Herve and Dhein, 2006). Fig. 5 demonstrates the phosphorylation of Cx43 on serine residues via PKC pathway (Fig. 5).

In vitro, GJ activity can be altered by PKC activation, leading to down regulation of intercellular coupling and an increase in the phosphorylation of Cxs, which is thought to block GJIC activity (Cruciani and Mikalsen, 2002). Phosphorylated isoforms of Cx43 run much slower on SDS-PAGE gels and it generally found to be phosphoserines, which can be dephosphorylated by phosphoserine specific phosphatase enzymes (Herve *et al.*, 2004; Lampe and Lau, 2000).

Several studies have indicated that activation of the protein kinase-A (PKA) pathway via dibutryl-cAMP increased serine 364 (S364) phosphorylation of Cx43 which leads to an increase in GJIC (Fig. 6) and the number of Cx43 positive plaques (TenBroek *et al.*, 2001). However, some cell types do not alter the phosphorylation status of Cx43 in response to dibutryl-cAMP. Recent evidence indicated that the cAMP dependent PKA pathway did not phosphorylate Cx43 directly, but the enhanced assembly of Cx43 GJIC was totally dependent upon the basal phosphorylation of S364 by unknown kinases (TenBroek *et al.*, 2001).

Sequence analysis of the Cx43 protein has shown that there are various other functionally important serine phosphorylation sites in the C-terminal domain which can be phosphorylated by PKC and MAPK (Warn-Cramer *et al.*, 1996). It is known that PKC activates the MAPK pathway via rapidly accelerated fibrosarcoma (RAF) kinase as well as the epidermal growth factor (EGF) receptor via v-Src viral oncogene (Sirnes *et al.*, 2009). Moreover, growth factors such as Insulin like growth factor (IGF), Fibroblast like growth factor (FGF) and Platelet derived growth factor (PDGF) and oncogenes (Ras, Raf, v-Src) are the ligands for tyrosine kinases which in turn activate a PKC dependent downstream pathway and decrease GJIC (e.g. neurotransmitters in the brain can cause alterations to GJIC which may be related to possible involvement of protein kinases) (Lampe and Lau, 2000;

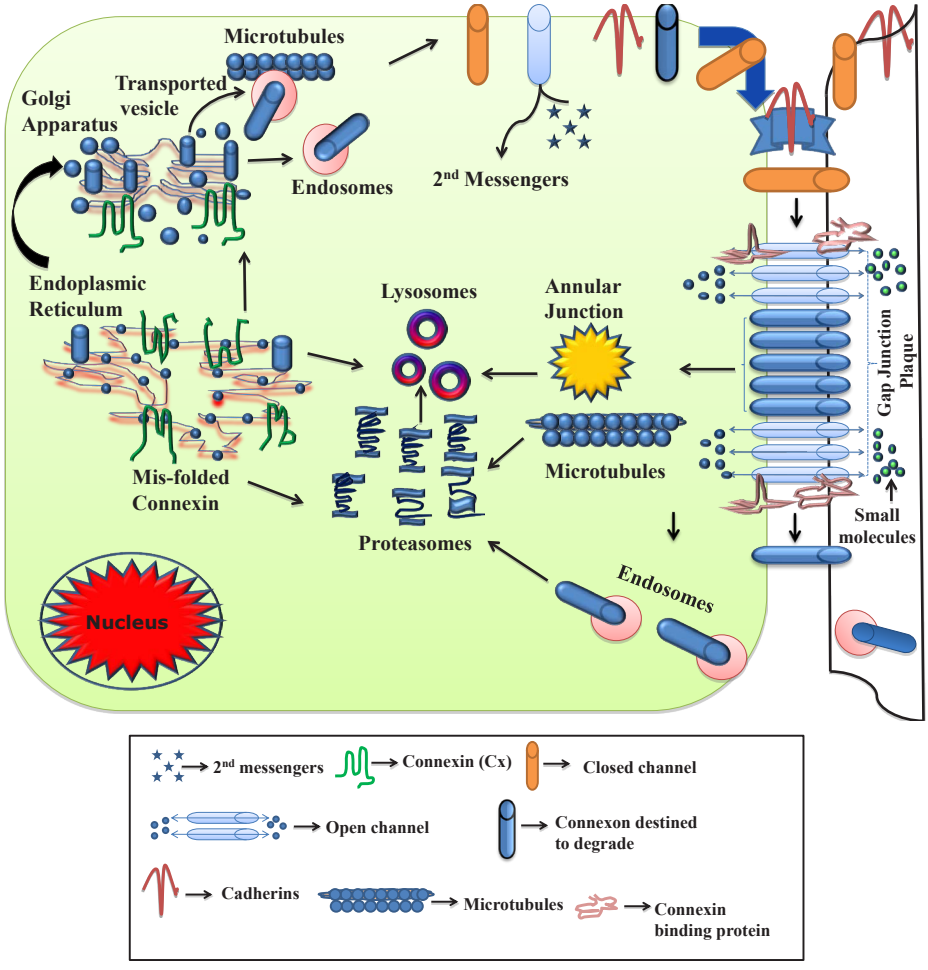

Fig. 3. Life cycle of a connexin (Cx). *Figure is adapted and modified, with permission from Laird, (2006).*

Lampe and Lau, 2004; Warn-Cramer and Lau, 2004).

GJIC in some cells has been shown to be inhibited by a protein tyrosine kinase (pp60$^{v\text{-}src}$ and p130$^{gag\text{-}fos}$) encoded by the viral oncogene v-src. These tyrosine kinases also cause phosphorylation of Cx43 which causes a decrease in GJIC (Fig. 7) (Crow *et al.*, 1990; Swenson *et al.*, 1990).

The involvement of gap junction proteins in embryogenesis

During gametogenesis, the growth and maturation of the oocyte involves movement of nutrients and cAMP from follicle cells to oocytes via GJs (Granot and Dekel, 1998). Furthermore, GJs are present between the oocyte and surrounding follicular cells to regulate follicular growth and the maturation in the mammalian ovary which suggest that they may pass regulatory signals between cells in other developing tissues (Brower and Schultz, 1982; Eppig, 1982; Heller *et al.*, 1981).

GJs are also expressed during the early blastula stage (Dorresteijn *et al.*, 1982; Magnuson *et al.*, 1977), and persist throughout embryogenesis (Bennett *et al.*, 1981). GJs significantly play an important role in preparing the uterus for embryo implantation as well as in the control of trophoblast invasion (Grummer *et al.*, 1996). Studies using the mouse preimplantation embryo have shown that Cx43 mRNA expression starts from the 4-cell stage onwards leading to a well coupled 8-cell stage (De Sousa *et al.*, 1993; Ruangvoravat and Lo, 1992). Cx mRNA expression patterns very distinct during early mouse embryonic development with Cx30, Cx31, Cx36, Cx43, Cx45 and Cx57 being expressed from the 2 to 4-cell stage, and Cx30.3, Cx31.1 and Cx40 from the 8-cell stage (Davies *et al.*, 1996; Houghton *et al.*, 2002). In addition to

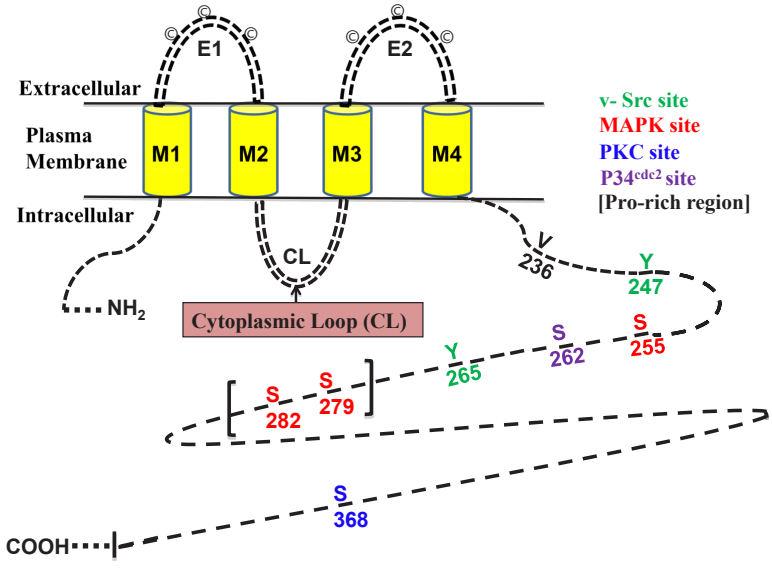

Fig. 4. Schematic diagram of Cx43 phosphorylation sites. *The numbers denoting the phosphorylation sites which are found on the C-terminal domain region, and are targeted by different known kinases are represented by different colours (v-Src site, green; MAPK site, red; PKC site, blue; P^{34cdc2} site, purple; protein rich domain corresponding to the P274-P284 region is bracketed). Figure is adapted and modified, with permission from Lampe and Lau, (2000).*

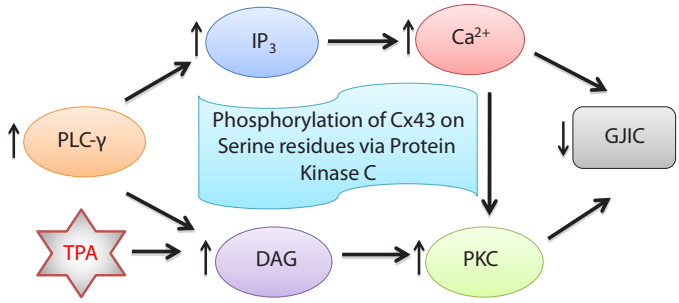

Fig. 5. Schematic diagram of Cx43 phosphorylation on serine residues via the protein kinase C (PKC) pathway. *[GJIC, Gap junction intercellular communication.*

mouse embryonic development, Cx43 and Cx31 are expressed in the inner cell mass and trophectoderm in the preimplantation of rat embryo (Grummer *et al.*, 1996), whereas human embryos express predominately Cx43 protein in their GJs prior to embryo implantation (Hardy *et al.*, 1996). It has been previously shown that the establishment of GJIC between the blastomeres has occurred at the 8-cell stage, and this is a precondition to maintain compaction (Lee *et al.*, 1987). Moreover, GJIC was first found to be turned on at the late 8-cell stage in mouse embryos, at which time all the blastomeres become linked via these GJ proteins channels (Lo and Gilula, 1979). Hence, these results also suggest that good GJIC is essential for compaction and early embryonic development (Becker and Davies, 1995; Kidder and Winterhager, 2001).

GJ proteins have long been speculated as playing an important role in development by forming morphogen gradient compartments, which can regulate cell growth, patterning, and differentiation (Caveney, 1985; Lo, 1996; Lo and Gilula, 1979). Even though morphogen gradients in development are well described, there is still little evidence for GJs playing a role in the formation of such gradients. It has been previously shown by Levin and colleagues that alteration or inhibition of GJIC may modulate left-right patterning in *Xenopus* embryos and chick embryo models (Levin, 2002; Levin and Mercola, 1998; Levin and Mercola, 1999; Levin and Mercola, 2000; Vandenberg *et al.*, 2014). These animal model experiments are consistent with the finding from the earlier report that mutation in Cx43α1 can cause visceral atrial heteroataxia (VAH) in humans (Britz-Cunningham *et al.*, 1995). This VAH syndrome involves fundamental perturbation of left-right patterning and is characterised by complex cardiac malformations in addition to visceral organ defects. The role of GJIC in regulating early embryogenesis have been studied in several embryo models, including squid (Potter *et al.*, 1966), amphibians (Slack and Palmer, 1969), molluscs (de Laat *et al.*, 1980), Fundulus (Bennett *et al.*, 1978), chick (Sheridan, 1968), and the mouse (Lo and Gilula, 1979).

Several studies have reported that GJs play an important role in early stages of embryonic muscle cell differentiation in vertebrate skeletal muscle (Blackshaw and Warner, 1976; Chow and Poo, 1984). Multiple GJ proteins found to be expressed during the embryonic phase of neurogenesis. Cx26 and Cx43 are known to be expressed by radial glia and neuronal progenitor cells found within

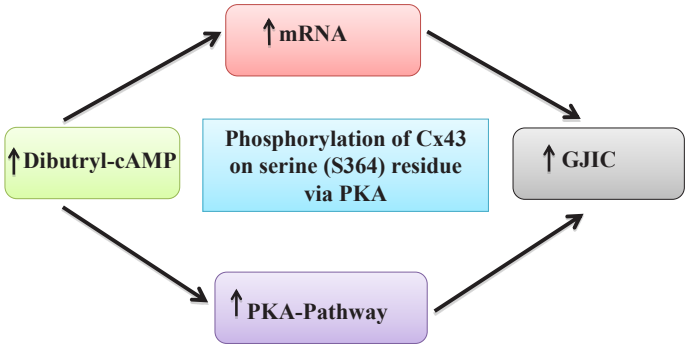

Fig. 6. Schematic diagram of Cx43 phosphorylation on serine residues via the protein kinase A (PKA) pathway. *GJIC, Gap junction intercellular communication.*

the ventricular zone of the developing brain (Bittman *et al.*, 1997; Nadarajah *et al.*, 1997). Several studies have demonstrated the expression of Cx genes in embryonic (Nishi *et al.*, 1991) and extra-embryonic tissues (Kalimi and Lo, 1989) of the gastrulating mouse embryo (Ruangvoravat and Lo, 1992). Cx43 is abundantly and differentially expressed during vertebrate embryonic development as shown in mouse (Dahl *et al.*, 1996; Ruangvoravat and Lo, 1992), and human embryos (Hardy *et al.*, 1996). However, it has been shown to be expressed at certain stages of development in many regions of the embryo, for example it is expressed in the spanning domain of the midbrain, hindbrain junction, in the telencephalon, and within the mouse embryo limb bud mesenchymal cells (Dahl *et al.*, 1996; Laird *et al.*, 1992). Furthermore, the role of Cx43 has also been implicated in morphogenesis of the embryonic chick limb bud formation during embryonic chick development (Dealy *et al.*, 1994; Makarenkova and Patel, 1999). Previously, it has been shown during mouse embryonic development that GJ are coupled in embryonic tissue formation and assumed to trigger intercellular pathways for chemical and/or electrical developmental signals and to define the boundaries of developmental compartments (Kalimi and Lo, 1988).

In invertebrates, for example in *Drosophila melanogaster*, mutants have been isolated for some of the innexin family members and functions have been assigned to *innexin-2* (*kropf*) which play an important role in embryonic epithelial organisation and morphogenesis (Bauer *et al.*, 2002; Bauer *et al.*, 2004), as well as *innexin-4* in the germ cell differentiation process (Tazuke *et al.*, 2002).

Several studies have reported the importance of GJ mediated intercellular communications during vertebrate patterning and embryonic development. For example, in the early amphibian embryos, perturbation of GJIC can lead to various developmental and embryological defects (Levin and Mercola, 1998; Warner *et al.*, 1984).

Recent studies have suggested that the *in vitro* differentiation systems using embryonic stem (ES) cells and induced pluripotent stem (iPS) cells also provide useful models to study GJ proteins (Cxs) expression and GJIC during the early stage of cellular differentiation in embryonic development (Pebay and Wong, 2014; Woodward *et al.*, 1998). Furthermore, it has been shown using human iPS cells that GJIC is re-established during reprogramming to pluripotency (Sharovskaya *et al.*, 2012). In addition to the role of GJ proteins in ES cells, Cx43 expression was found to be highly enriched in undifferentiated human iPS cell lines during and after the reprogramming (Ke *et al.*, 2013).

The second part of this review paper attempts to look in greater detail, the possible of role of GJ proteins and GJIC in embryonic heart development.

Role of gap junction proteins in embryonic heart development

The development of embryonic heart is a complex process and it undergoes both profound and dynamic alterations in size, structure and function as development proceeds. Accordingly, as development proceeds, intercellular gap junction communication in the developing heart is most likely to be adapted for various important functions including metabolic coupling, cellular electrical activity, homeostatic interchange of ions and other small cytoplasmic molecules and the passage of other signalling molecules which are potentially involved in developmental steps (Gourdie, 1995) (Fig. 8). The contraction of the heart is a dynamic process, which is functionally regulated by the coordinated spread electrical excitation through cardiac muscle (Gourdie, 1995). The synchronised contraction (functional syncytium) of myocytes in cardiac muscle cells requires the formation of structurally and functionally integral GJs (Delorme *et al.*, 1997). In the heart, they mediate the propagation of electrical activity that allows synchronous contraction of the cardiac muscle chambers, and contribute to coordination of function between cells of the arterial wall (Wei *et al.*, 2004).

Cardiac muscle cells are connected by three types of specialised junctions (GJ, fasciae adherens, and desmosomes), which are located in a specialised plasma membrane structure, called the intercalated disc (ID) (Kostin *et al.*, 1999), which acts to form zones of electrical and mechanical attachment between myocytes (Severs, 1990). The IDs between individual cardiomyocytes, therefore, have two main functions in synchronised contraction of the embryonic heart: (a) to ensure mechanical coupling or attachment between myocytes and (b) to spread fast coordination of electrical activity (electrical impulses) throughout the heart. However, improper mechanical coupling or attachment between cardiomyocytes leads to a heart pump dysfunction, whereas improper electrical coupling may lead to abnormal heart development and consequently development of cardiac arrhythmias due to abnormal conduction of the electrical activity (Noorman *et al.*, 2009).

Most tissues including the heart, express more than one Cxs, and six Cx proteins Cx37, Cx40, Cx43, Cx45, Cx46, and Cx50 have been identified in the mammalian heart (Gros and Jongsma, 1996). The expression pattern of Cxs in the embryonic heart is developmentally regulated (Fig. 8). In mammalian hearts, cardiomyocytes most abundantly express Cx40, Cx43 and Cx45. Therefore, in this review paper we have focused more on these three Cxs (Cx40, Cx43, and Cx45) isoforms and their role in embryonic heart development (Fig. 8) (Dhein, 2004). The expression

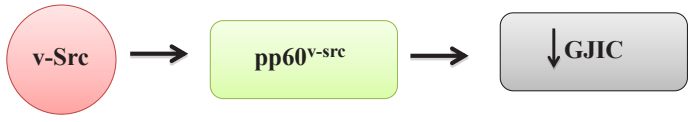

Fig. 7. Schematic diagram of Cx43 phosphorylation on tyrosine residues via the protein kinase A (PKA) pathway. *GJIC, Gap junction intercellular communication.*

of other cardiac Cxs such as Cx50 is found only in heart valves whereas Cx37 has been found mainly in endothelial cells of the endocardium, aorta and coronary vessels of the developing heart (van Veen *et al.*, 2001), although recently one study has reported that Cx37 deficient mice did not develop venous and lymphatic valves (Munger *et al.*, 2013). Previously, Gros *et al.* (2010) reported thatCx30 is expressed at low abundance in the mouse sinoatrial node (Gros *et al.*, 2010). Moreover, double knockout ($Cx37^{-/-}$ and $Cx40^{-/-}$) mice showed a higher percentage of atrial septal defects (ASDs) and ventricular septal defects (VSDs) (Simon *et al.*, 2004).

The expression patterns of Cxs (Cx40, Cx43 and Cx45) in developing embryonic heart follow remarkable spatiotemporal differences and demonstrate distribution differences in the cardiac system (Fig. 8) (Kostin *et al.*, 1999). Cx43 is the first gap junction protein identified in the heart (Beyer *et al.*, 1989; Beyer *et al.*, 1987) which is also expressed in virtually all myocytes of the working myocardium (atrial and ventricular) regardless of the stage of development (Gros and Jongsma, 1996; Vink *et al.*, 2004), and is principally responsible for electrical synchrony in the heart (Martin and Evans, 2004). Furthermore, Cx43 is found to be present in myocardial conduction tissues and coronary and aortic smooth muscle cells (Fromaget *et al.*, 1990). However, other Cxs are more specific in their involvement in heart development; Cx40 is prominently expressed in the atrium and conduction system, specifically in the His-Purkinje system (Gros *et al.*, 1994). Cx45 is expressed throughout the whole heart but has been detected predominately during early development of the heart (Dhein, 2004; Vink *et al.*, 2004). Quantitative gene and protein expression analyses suggest that the spatial and temporal distributions of Cx43 mRNA (Van Kempen *et al.*, 1996) and protein immunolocalization (Gourdie *et al.*, 1992; van Kempen *et al.*, 1991) reveal a striking regionalization of Cx43 within the developing rat heart. Moreover, the Cx43 transcriptional level is found to be very high within atrial and ventricular myocardium (Van Kempen *et al.*, 1996). This is consequent with marked increases in the densities of Cx43-puncta staining within the inner trabeculated muscle of the ventricles, but was absent at the sub-epicardial myocardium, the crest of the interventricular septum, the atrioventricular junction, and the outflow and inflow regions of the developing embryonic heart (Fig. 8) (Gourdie *et al.*, 1990; Gourdie *et al.*, 1992; van Kempen *et al.*, 1991; Van Kempen *et al.*, 1996). Studies have reported that these three Cxs are involved in both, cardiac morphogenesis and propagation of electrical activity: Cx43 also plays important role in the development of the outflow tract and coronary arteries (Kruger *et al.*, 2006) whereas Cx40 is involved in the development of the outflow tract and septa. Studies have reported that Cx40 generates channels with high conductance rate and Cx45 forms voltage sensitive channels with very low conductance rate for propagation of electrical activity in the embryonic heart (Wei *et al.*, 2004). Furthermore, mutation or alteration of Cxs (Cx40, Cx43 and Cx45) can lead to cardiac malformations in cardiac specific Cx knockout mice; Cx40 knockout mice die during gestation with atrioventricular septation defects and outflow tract malformation, while Cx45 knockout mice show conduction block and endocardial cushion defects during gestation (Wei *et al.*, 2004). Results from Cx deficient ($Cx40^{-/-}$/$Cx43^{-/-}$ and $Cx43^{-/-}$) mice also indicated a role of Cx40 and Cx43 in the looping process of cardiac morphogenesis (Kirchhoff *et al.*, 2000).

Disruption of Cx43 demonstrates right ventricular outflow tract obstruction, causing cyanosis and death at birth in mice (Vinken *et al.*, 2006). Despite these congenital heart defects, however, the hearts of these Cx43-/- mice still beat rather rhythmically, though not synchronously throughout the tissue, suggesting that other gap junction proteins in addition to Cx43 may partially compensate for the loss in GJIC (Vink *et al.*, 2004). It has been previously shown that a homozygous deletion of Cx40 combined with a heterozygous deletion of Cx43 causes cardiac malformations in mice (Kirchhoff *et al.*, 2000), whilst heterozygous deletion of Cx45 (Kruger *et al.*, 2006) results in neonatal death. The Cx43 deletion causes additional atrial defects and the Cx45 further delayed atrioventricular conduction. In addition, mouse models that are homozygously deficient (double knockout $Cx40^{-/-}$ and $43^{-/-}$) die much earlier than Cx43 knockout mice, around ED12

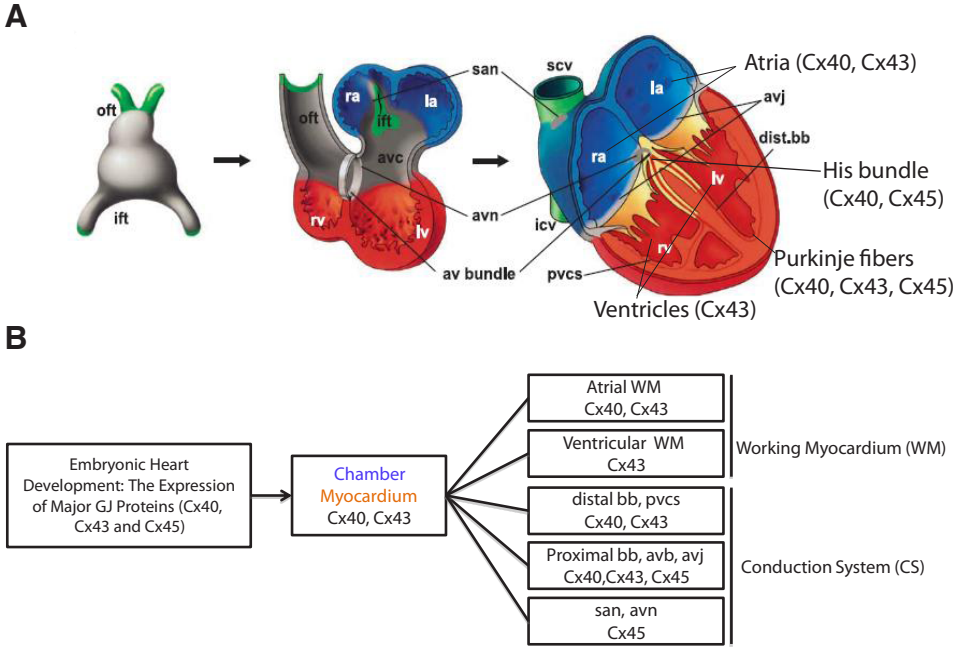

Fig. 8. Schematic representation and overview of embryonic heart development. (A) *The primary myocardium is in gray, and the chambers and ventricular conduction system are in red and blue, respectively. The mesenchyme at connections between the heart and the body is represented in green.* **(B)** *Schematic diagram showing the developmental relationships between components of the embryonic heart, three main cardiac GJ proteins (Cx40, Cx43, Cx45) expression are shown in each of the components of embryonic heart development. (Abbreviations: avc, atrioventricular canal; ift, inflow tract; la, left atrium; lv, left ventricle; oft, outflow tract; ra, right atrium; rv, right ventricle; avb, atrioventricular bundle; avj, atrioventricular junction; avn, atrioventricular node; bb, bundle branches; pvcs, periventricular conduction system (Purkinje Fibers); san, sinoatrial node; s(i)cv, superior (inferior) caval vein; WM, working myocardium; and CS, conduction system;). Figure is adapted and modified, with permission from (Christoffels* et al.*, 2004).*

(ED: Embryonic Day), with an abnormal rotation of the ventricles (Simon *et al.*, 2004). However, the role of Cx45 mediated intercellular communication is essential for the remodelling of the vascular system and the development of endocardial cushions. Loss of Cx45 leads to a conduction block at the atrioventricular canal, the outflow tract at mouse embryonic day 9.5 (ED9.5), and endocardial cushion defects with a lethal outcome at ED10, suggesting that Cx45 is important for the first contractions of the early embryonic heart (Dobrzynski and Boyett, 2006; Kruger *et al.*, 2006). These findings are consistent with the reports of other groups that the expression pattern of Cx45 is unique in the atrioventricular canal and outflow tract of the developing embryonic heart (Alcolea *et al.*, 1999; Delorme *et al.*, 1997).

Embryonic stem cell and gene targeting studies suggest that each of the three cardiac Cx (Cx40, Cx43 and Cx45) genes is required for heart conduction in the respective knockout mouse models (Gutstein *et al.*, 2001; Kirchhoff *et al.*, 2000; Kirchhoff *et al.*, 1998). Further to their roles in the embryonic heart conduction system, analysis of Cx43α1, Cx40α5, and Cx45α7 knockout mice models demonstrated that these Cx genes also play an essential role in heart morphogenesis. Kirchhoff *et al.,*2000) reported in his first study that 16% of new-born homozygous Cx40α5 knockout mice were dead at birth due to atrioventricular septal defects. In a second study, he showed that 33 % of homozygous Cx40α5 knockout mice were found to have outflow tract malformations consisting of double outlet right ventricle or Tetralogy of Fallot (Gu *et al.*, 2003). In addition, the analyses of the Cx45α7 and Cx43α1 knockout mouse models show that these two Cx genes are essential for the development of the endocardial cushion and outflow tract morphogenesis, as well as development of coronary arteries respectively (Kirchhoff *et al.*, 2000).

Knockout mice deficient in Cx45α7 die because of heart failure at ED10.5, demonstrating a cardiac looping defect, reduced trabeculation, disrupted formation of endocardial cushions, and a conduction block (Kumai *et al.*, 2000). It has been suggested by another study that Cx45α7 knockout mice die from abnormal vascular development (Kruger *et al.*, 2000). It has been previously shown by Gros *et al.* (2004) that the most common congenital malformations, including double outlet right ventricle, bifid atrial appendages, Tetralogy of Fallot, ventricular septal defect, aortic arch abnormalities and partial endocardial cushion defects, are observed using the Cx40 knockout mouse (Cx40-/-) model (Gros *et al.*, 2004). Further studies in mice demonstrated that both homozygous Cx43 knockout and Cx43 over expression have exhibited developmental cardiac defects including pulmonary outflow tract obstruction and conotruncal heart defects (Reaume *et al.*, 1995; Ya *et al.*, 1998). It has been shown that single knockdown of Cx40 slows atrioventricular conduction, however it is found to be normal in double knockout mice ($Cx40^{-/-}$ and $Cx30.2^{-/-}$), suggesting that the balance between Cx30.2 (the mouse ortholog of human Cx31.9 (Belluardo *et al.*, 2001)) and Cx40 is essential for the determination of atrioventricular conduction in mice (Schrickel *et al.*, 2009). Furthermore, there is a study with the $Cx40^{-/-}$ mouse model to show the importance of this Cx40 in generation of the mature apex to base activation of the developing heart (Sankova *et al.*, 2012).

Taken together, these transgenic and Cx knockout mouse models have confirmed that GJ proteins play an important role in embryonic heart morphogenesis including cardiac conduction systems. Although there have been a large number of studies on embryonic heart development, specifically on the involvement of Cxs, from knockout mouse models, as described and mentioned above, there are some important differences in cardiovascular electrophysiology and in general and spatiotemporal GJ protein distribution in the developing human and mouse hearts (Kaese and Verheule, 2012; Verheule and Kaese, 2013). Nevertheless, the knowledge obtained from these knockout Cx models with reference to embryonic heart development provide a wealth of valuable molecular insights.

Functions of gap junction intercellular communication

As stated before, GJs provide a mechanism for cell to cell communication and the coordination of groups of cells. They are involved in many forms of intercellular signalling both in excitable and non-excitable cells. Previous studies on GJ regulation and functions have shown that these GJIC mechanisms fall into five general categories according to their respective functions: (a) speed, (b) synchrony, (c) switching, (d) symbiosis and (e) stimulation/suppression. In heart cells, GJs assist in the mediated spread of rapid electrical impulses that stimulate the coordinated contraction of the cardiomyocytes (Gourdie, 1995). GJs are also present in neuronal systems, where electrical synapses are used in neuronal pathways requiring high speed, synchronous neuronal signalling and a switch between neuronal pathways which occurs in areas of the eye, inner ear and brain (Nagy *et al.*, 2004). In the lens of the eye (non-excitable cells), GJs allow for symbiotic interactions between highly differentiated, functionally composed cells and more active, renewable cells, which perform cellular functions for both cell types (Gong *et al.*, 2007; Kistler *et al.*, 1999). Interestingly, studies have shown that GJs might also serve as tumour suppressors, since tumour cells tend to decrease GJIC, which in turn increases the lack of growth control and promotes differentiation of tumour cells (Holder *et al.*, 1993; Wu *et al.*, 2007).

The deletion of the Cx43 (*Gja1*) gene in mouse embryo delays the migration of the neural crest cells that contribute to cardiac morphogenesis, leading to an obstructed right ventricular outflow tract, impaired blood supply to lungs, and perinatal death (Lo *et al.*, 1997; Reaume *et al.*, 1995). Developing mouse embryos lacking both Cx43 and Cx32 survived to term but died shortly afterwards due to the same congenital heart defects observed with the Cx43 deficient mice (Houghton *et al.*, 1999; Reaume *et al.*, 1995). Moreover, mutations of Cx43 in humans have been reported in patients affected by Oculodentodigital Dysplasia (ODDD), an autosomal dominant syndrome characterised by craniofacial and limb dysmorphogenesis, spastic paraplegia, and neurodegeneration (Paznekas *et al.*, 2009). In humans, Cx32 mutations result in X-linked Charcot-Marie-Tooth syndrome (Bergoffen *et al.*, 1993), and Cx47 mutations lead into a central demyelinating condition called Pelizaeus-Merzbacher-Like-Disease (Uhlenberg *et al.*, 2004). Additionally, GJ proteins play an important role in the vascular system; studies using Cx-mimetic peptides to selectively blockade GJIC in rabbit iliac arteries suggest that Cx40 and Cx43 are required for endothelium-derived hyperpolarization (EDHF)-type signalling via propagation of both myoendothelial GJs and as well as GJs joining smooth muscle cells (Chaytor *et al.*, 2005). Furthermore, Cx40 mutation in humans causes idiopathic atrial fibrillation, and these result in reduced GJIC either through impaired Cx trafficking or inability to form plaques (Gollob *et al.*, 2006). Previously,

it has been demonstrated vascular disruption in mice lacking the endothelial GJ proteins and the double knockout of Cx37 and Cx40 mice die perinatally with dramatic vascular abnormalities (Simon and McWhorter, 2002).

The relation between gap junction intercellular communication and teratogenicity - effects on embryogenesis

The effect of GJs in embryonic development is quite well understood and studied, though the mechanisms underlying these effects are not fully understood. There appears to be a link between teratogenicity and carcinogenesis, in that many carcinogenic compounds are also teratogenic in nature, whereas teratogenic compounds are not necessarily implied to be carcinogenic (Trosko *et al.*, 1982). The compounds which stimulate GJIC, such as retinoic acid and vitamin D, can also suppress tumour formation (Tanmahasamut and Sidell, 2005). Inhibition of GJIC by several toxic compounds has been postulated to be a factor in the tumor promotion phase of carcinogenesis and teratogenesis, as well as immune, reproductive, neurological and cardiovascular dysfunction through loss of homeostatic control (el-Fouly *et al.*, 1987; Trosko *et al.*, 1998). Untimely or chronic disruption of GJIC during embryonic development could lead to embryotoxicity or teratogenicity, since many chemicals known to be tumor promoters, teratogens, or neurotoxins modulate GJIC (Trosko *et al.*, 1998). A range of nongenotoxic carcinogens has tested positive for inhibitory effects on GJIC *in vitro*. These compounds include the pesticides such as dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethylene (DDE), dieldrin, lindane, and heptachlor, the peroxisome proliferators such as clofibrate, nafenopin, Wy-14,643, and di-2-ethylhexyl-phthalate. In addition, several other classical tumor promoters and pharmaceuticals in rodents, such as the phorbol ester 12-O-tetradecanoylphorbol 13 acetate (TPA), polychlorinated biphenyl, and phenobarbital have been shown to affect GJIC (Swierenga and Yamasaki, 1992). It has been previously shown that ethylene glycol and monoalkyl glycol act as teratogens by inhibiting GJIC in Chinese hamster V79 cells (Loch-Caruso *et al.*, 1984). Moreover, TPA and monoalkyl glycol have shown to inhibit GJIC in normal human embryonic palatal mesenchyme (HEPM) cells, suggesting the HEPM cells are suitable to study disruption of GJIC as a mechanism responsible for teratogenesis (Welsch and Stedman, 1984; Welsch *et al.*, 1985).

It has been previously shown that GJIC is stimulated by teratogenic compounds like retinoic acid and thalidomide, exhibiting a similar pattern of congenital malformations due to their teratogenicity/embryotoxicity which include congenital heart defects, craniofacial malformations, limb defects, ear malformations, facial palsy, absent or shrunken eyes, cataract formation, ocular movement dysfunctions, kidney malformation, mental retardation and central nervous system defects (Mehta *et al.*, 1989; Nicolai *et al.*, 1997; Onat *et al.*, 2001). As mentioned above, the role of GJ is essential and vital not only for the eye, ear, brain, heart and central nervous system to perform their normal physiological functions in a normal adult, but also essential for their formation and regulation in embryonic development (Goodenough *et al.*, 1996). Moreover, thalidomide and retinoic acid cause limb and heart defects in embryos, whereas Cx43 plays a very crucial role in the developing limb (Dealy *et al.*, 1994; Makarenkova and Patel, 1999), and in the embryonic heart development (Dasgupta *et al.*, 1999; Fromaget *et al.*, 1990). It has been demonstrated that disruption of GJIC in embryonic heart development causes congenital cardiovascular defects (Dasgupta *et al.*, 1999; Severs *et al.*, 2004). As a consequence, disruption of GJ and GJIC could be a possible key mechanism explaining the mode of action of these two teratogens and their derivatives.

Conclusions

There are large numbers of GJ genes actively transcribed in the mammalian genome which also suggests that there is an evolutionary pressure that exist to maintain this high degree of GJ biological complexity. Moreover, GJ proteins and their long evolutionary history have permitted adaptation of GJIC with several important functions and multiple regulatory processes. Formation of GJIC is an essential mechanism in coordinating growth and development and tissue compartmentalization during embryonic development (Bennett *et al.*, 1981; Caveney, 1985; Levin, 2007; Lo, 1996; Lo and Gilula, 1979). Although there are numerous examples that clearly demonstrate a requirement of GJs in embryonic development, how GJIC function during embryogenesis remains largely unknown. This review paper seeks to describe the molecular insights and the functional role of GJ proteins in embryology in two major parts. In first part of this review paper we have described the general cell biology, structure, biochemical and physiological properties, molecular mechanisms and function of GJ proteins. In second part, we have described the role of GJ proteins and the importance of GJIC in embryology with particular emphasis on embryonic heart development. It also proposes a potential link between teratogenic molecules and perturbations or changes in connexion expression.

Conflict of Interest

There are no competing financial interests.

Acknowledgements

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