# Chapter 10
# Tissue Distribution of Connexins

There are more than 20 known connexin proteins identified in the human and mice genome. However, not all the 20 connexins are expressed in all the cells. The expression of connexins has been shown to vary between tissues. Some of the connexins, for example, Cx43, show wide tissue distribution and while others are restricted to a particular cell type or tissue. The tissue distribution of connexins demonstrates their relevance in executing tissue-specific functions. Although connexins show similar, topological features, there exists appreciable amount of variability among different connexins. The variability involved in connexins allows for a great deal of diversity in gap junction formation. Each gap junction appears to confer some specificity for what type of molecules pass through it, based on either the charge or size of the molecule. Based on that specificity, it seems likely that even small amounts of a particular gap junction with a unique composition of connexins could be important for the movement of a particular metabolite or set of metabolites. Identifying what connexins are present in a particular tissue, even if only in small amounts, could thus be crucial for understanding their roles in cell communication as well as cell adhesion. These subtle variations in connexins and hence gap junctions are crucial for performing specialized function of different cell types and tissues. In the following paragraphs, the tissue distribution and tissue-specific function of connexins will be discussed.

## 10.1 Connexins in Vascular System

The vascular system is known to expresses many connexins, like Cx37, Cx40, Cx43, and Cx45. Both the smooth muscle cells and the endothelium cells express Cx37, Cx40, and Cx43. Using immunocytochemistry, Western blotting, and electron microscope studies, the presence of Cx37 and Cx40 has been unequivocally found in the endothelium. However, the expression of Cx43 in the endothelial cells is more controversial and appears to vary with vessel size, vascular region, and species.



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In the smooth muscles, connexin expression is not that well defined as that of the endothelial cells. Expression of Cx43 and Cx40 in the smooth muscle cells has been described, and recently, Cx45 has been reported as well. Moreover, in some instances, Cx37 that is usually thought to be an endothelial connexin has also been reported in smooth muscle cells. Physiological significance of connexins in the vasculature is to coordinate various functions by communicating between different cells. This communication between different cells of vasculature plays a central role in coordinating various cellular functions. Moreover, gap junctions formed between the smooth muscle and endothelium provide the pathway for the radial and longitudinal communication in the vascular system. Physiological significance of various vasculature connexins is briefly discussed below.

*Cx40:* The role of Cx40 has been demonstrated using Cx40 knock-out animals. Based on these studies, it has been shown that Cx40 is involved in the vasomotor tone regulation. Cx40-deficient animals develop hypertension and depict irregular vasomotion. The occurrence of hypertension in these animals has been correlated with the reduced density of the endothelial gap junctions, mediated by Cx40. The role of Cx40 in regulating the blood pressure has been attributed due to the formation of gap junctions between the renal endothelial cells and the renin-secreting cells of the afferent arteriole. Thus, the intercellular communication between the endothelial cells and the renal renin–angiotensin system plays an important role in the regulation of blood pressure. Moreover, deletion of Cx40 results in the elimination of a very rapid, non-decremented component of axial conduction induced by electrical stimulation or acetylcholine (Ach) stimulation. More studies are required to understand the molecular basis for this type of conduction. This will pave way for the development of various vasodilatation methods, enabling to communicate over long distances using Cx40 gap junction channels and thus might play a key role in the maintenance of vasomotor tone and blood pressure.

*Cx45:* The role of Cx45 in the vasculature has been ascertained using Cx45-deficient animals. Although the Cx45-deficient embryos show normal initiation of vasculogenesis, however, various defects are manifested, which include defective remodelling and organization of blood vessels and failure to form a smooth muscle layer surrounding the major arteries.

*Cx37:* Although Cx37 has been shown to be expressed in the vascular smooth muscles, deletion of these connexins does not show any defective vasculature or any defect related to the blood vessel development. Interestingly, simultaneous deletion of Cx37 and Cx40 is lethal with acute vascular abnormalities.

*Cx43:* Although Cx43 expression in vasculature is not that intense, it has been found to be important for the proper development of the vascular system. Cx43 controls cell proliferation and migration, and its expression in the smooth muscle cells is induced during mechanical injury. The role of Cx43 in cell migration, after mechanical injury, has been corroborated in the cultured endothelial cells, which resulted in the increased expression of the Cx43 expression, with the downregulation of the Cx37 expression, and no change in the Cx40. Moreover, endothelial cell-specific deletion of Cx43 causes hypotension, in contrast to the deletion of Cx40, which results in hypertension.

## 10.2 Connexins in the Heart

The heart is an electric organ and the expression of gap junctions in this organ is well suited for the physiological function of the heart. For the conduction of electric impulses in the heart, the intercellular gap junction channels between various cells are regarded indispensible. The presence of different connexins in the heart is crucial for the functional differences between various regions of the heart. Although the different connexins expressed in the cardiomyocytes form similar gap junction channels, these gap junctions also differ significantly in their channel properties in accordance to the region where they express.

The gap junction channel properties in the cardiomyocytes are highly dynamic and are targets of modulation by several conditions, for example, hypoxia/ischaemia. The short-term changes on the gap junctional conductance are mainly caused by phosphorylation. Moreover, the prolonged changes in the gap junctions are known to occur at the transcriptional level. Various growth-inducing factors, like epidermal growth factor (EGF), platelet-derived growth factor (PDGF), transforming growth factor b (TGFb), and tumour necrosis factor a (TNFa), are reportedly known to increase the expression of Cx43 in the neonatal cardiomyocytes. The increased expression has been attributed to the increase in the mRNA levels as well as in the decrease in the turnover of Cx43.

The expression of the connexins varies between different regions of the heart and during the development of the heart. Connexin43 (Cx43) constitute the major gap junction protein that is expressed in the cardiomyocytes of atrial and ventricular mammalian myocardium. In the mouse and rat, it is abundantly expressed in all the cardiac compartments with the exception of the sino-atrial (SA) and atrioventricular (AV) nodes, the His bundle, and proximal parts of both bundle branches, but it is expressed in more distal parts of the bundle branches. In humans, Cx43 is expressed in all parts of the ventricular conduction system. The role of the Cx43 in the normal functioning of the heart can be ascertained by the fact that many genetic alterations of the Cx43 in mouse lead to various abnormalities. For example, disruption of both the alleles of Cx43 in mouse embryos results in their death shortly after birth. The cause of death is due to the asphyxiation caused by the obstruction of right ventricular outflow. Moreover, Cx43 knock-out mice models have demonstrated the role of Cx43 during the development of the heart. The Cx43 null mice embryos show delayed looping of the heart tube and the formation of certain bulges, lined by smooth muscle marker cells. The abnormal bulges have been demonstrated to be formed due to the failure of cardiac crest cells to migrate to the tubular heart to form proper epicardium. The migratory failure of these cells has been attributed due to the lack of Cx43 gap junction communication. These studies have been corroborated by the transgenic mice that over-express Cx43 and show increased migration of cardiac crest cells, while the mice expressing low levels of Cx43 showed decreased migration of these cells. In addition, Cx43 null mice show abnormal development of coronary arteries and have reduced diameter, decreased expression of myosin, etc. A similar kind of manifestation has been observed in various human

cardiac abnormalities, including the coronary artery anomalies. Interestingly, various genetic mutations in human Cx43 have been associated with various coronary artery abnormalities, and the deficiency of Cx43 in the ventricular cardiomyocytes has been associated with arrhythmias and sudden death. One of the common conditions associated with the embryos of Cx43 null mice−/− is the arrhythmias, and when these hearts are exposed to acute ischaemia, they show high frequency of ventricular tachyarrhythmias as opposed to heterozygous Cx43+/− mice. Thus, reduction of Cx43 expression, and consequently the electrical coupling, may play a critical role in ventricular arrhythmogenesis. Remodelling of Cx43, during acute ischaemia, is known to be the primary cause of developing arrhythmogenesis.

The Cx40 is expressed in the fetal and neonatal ventricles; however, its expression goes down drastically during the adult stages. Low levels of Cx40 expression have also been found in the SA node and AV node. Cx40 is involved in the propagation of electrical impulse from the atria to the ventricles. The role of Cx40 has been ascertained using knock-out mice. Mice heterozygous for Cx40 deletion possess similar electrophysiological properties as compared to wild-type mice. However, Cx40 KO null mice (Cx40−/−) show many electrophysiological disturbances. For example, the Cx40 KO mice exhibit disturbed cardiac influx propagation at various levels of the CCS and an increased incidence of inducible atrial arrhythmias. The disturbances of impulse conduction are in agreement with the expression localization of Cx40 in the AV node, the His bundle, and the bundle branches. In addition to the alterations of the electric activity, Cx40 KO mice show other cardiac malformations. These defects include atrial and ventricular septation and in some cases hypertrophy. Moreover, deeper analysis of Cx40 heterozygous newborn mice showed bifid atrial appendages, ventricular septal defects, tetralogy of Fallot, and aortic arch abnormalities, whereas the Cx40 KO (Cx40−/−) mice showed double-outlet right ventricle, tetralogy of Fallot, and partial endocardial cushion defects. The defects in Cx40−/− indicate that Cx40 has a role in septum formation and in other cardiac development events. The expression pattern of Cx40 in the human heart is similar to that of the mouse heart. Thus, it is expected that the alterations in the Cx40 expression in humans will have similar consequences as that of Cx40 KO mice. Interestingly, dominant mutations in the transcription factors Tbx5 and Nkx2.5 that regulate the expression of Cx40 result in alterations in the cardiac electrophysiological and morphological phenotype resembling that of the Cx40-deficient mice.

As far as Cx45 is concerned, its expression has been observed in the SA node, AV node, and the ventricular conduction system. Moreover, low levels of expression have been also reported from the atria and the ventricles. The importance of Cx45 can be gauged by the observation that the Cx45 KO mice (Cx45−/−) die in utero on day 10.5 pc. The death is known to be caused by the defective vascular development and the block in the atrioventricular conduction. The Cx45 is regarded essential for the embryonic heart development. During the early stage of development, Cx45 expression is seen in most of the cardiac compartments, and in the later stages, its expression goes down and remains specific to particular regions. Hence, in adult heart, Cx45 expression remains confined to SA node and inter-ventricular septum.

## 10.3 Connexins in the Nervous System

Connexins were first described as the important molecular components of the electrical synapses in the central nervous system (CNS). Connexin expression is widely distributed in the central nervous system. Besides forming electrical synapses, connexins are also important as far as other physiological functions of the brain are concerned. In the following paragraphs, expression and functional analysis of different connexins in different cell types of the central nervous system will be discussed.

### *10.3.1 Neuronal Connexins*

Different techniques were instrumental in ascertaining the number of connexins, which are expressed in different regions of the central nervous system (CNS). Of these, electron microscopy and immunocytochemistry were very helpful in providing insights about the expression of different connexins in diverse regions of the CNS. Furthermore, with the use of different antibodies raised against various connexin proteins, it was possible to immuno-detect various connexins in the different regions of the CNS. Until now, more than eight different connexins has been detected in different neuronal cell populations. Cx32 is one of the major neuronal connexins expressed in the neurons. Moreover, Cx26, Cx36, and Cx43 have been detected in different neuronal subpopulation. Connexins have been shown to be widely expressed in different regions of the brain. These regions include the hypothalamus, striatum, inferior olive, hippocampus, olfactory bulb, retina, cerebral cortex, and cerebellum. Generally, it is regarded that the mature brain expresses less connexins as compared to the developing brain and there is a progressive decrease in connexin expression and gap junctional intercellular communication (GJIC) with the maturation of brain, although neuronal coupling persists in many brain regions in adults. Thus, it is regarded that there exists an inverse correlation between the connexin expression and neuronal differentiation. The observation is suggestive that gap junctional communication between different cells of the central nervous system allows passage of different signalling molecules between neurons and thus regulates the neuronal differentiation. Besides specific expression of connexin in different regions of the brain, there also exists neuronal specificity of connexin expression. Connexin expression and gap junction communication has been found to exist in different neuronal cell types, like cerebellar basket cells, pyramidal cells in cerebral cortex and hippocampus, medium spiny neurons in the striatum, dopaminergic neurons in the substantia nigra, and motor neurons in the spinal cord. Moreover, the GABAergic interneurons are interconnected through electrical synapses, and this includes interneurons from the cerebral cortex, cerebellum, and striatum and reticular neurons from the thalamus. Pyramidal cells of CA1 and CA3 regions of the hippocampus communicate with each other using ultrafast axo-axonal coupling, and this is achieved using gap junction communication mediated by connexins.

### *10.3.2 Glial Connexins*

In the CNS, glial cells outnumber the neuronal cells, and most of the glial cells are coupled through the gap junctions. Connexin expression remains more or less consistent in the glial cells during the development stages and persists throughout the differentiated stage. Connexin expression in various glial cell types is discussed below.

#### 10.3.2.1 Astrocytes

Astrocytes are the star-shaped glial cells with long processes. The syncytial organization of astrocytes is maintained through the gap junction communication. The interconnection of the astrocytic organization can be appreciated by injecting a low molecular weight tracer, like lucifer yellow, into a single astrocyte, and within no time, the dye appears in around 100 astrocytes. Using various gap junction-specific inhibitors, like carbenoxolone, the dye spread remains restricted to the injected astrocytes, thus confirming the role of the gap junction communication in maintaining the astrocytic organization. The presence of high abundance of gap junctions in the astrocytes allows direct intercellular diffusion of ions, nutrients, and signalling molecules between these cells. Cx43 is the most abundant connexin expressed in the astrocytes and thus constitutes the major connexin contributing to the gap junctional communication of the astrocytes. Moreover, low levels of Cx30, Cx40, and Cx45 have been also detected in the astrocytes. Cx43 expression in astrocytes starts very early during development, and the levels increase progressively during the adult stage.

#### 10.3.2.2 Oligodendrocytes

Oligodendrocytes constitute the myelin-forming cells of the central nervous system and are known to possess gap junctions. One of the major connexins expressed in the oligodendrocytes is Cx32. Moreover, Cx45 has also been detected in the oligodendrocytes. Immunofluorescence studies have shown that the Cx45 is expressed mainly in the cell soma and proximal processes of oligodendrocytes localized in the white and grey matter. Recently, a novel gap junction protein called Cx29 was detected in the oligodendrocytes. The expression of different connexins in the oligodendrocytes is indicative of the presence of heterologous gap junctions between the oligodendrocytes. In fact, the presence of homologous gap junctions between the oligodendrocytes is still debated, and accumulating evidence indicates that the majority of gap junctions in oligodendrocytes are heterologous.

#### 10.3.2.3 Microglial Cells

Microglia belongs to the class of glial cells that provide macrophagic function in the brain and spinal cord. The microglia constitutes the first line of immune defence in the central nervous system (CNS). The microglia constitutes 10–15 % of the total glial cell population within the brain. Under normal conditions, the microglia acquire a specific ramified morphological phenotype termed "resting microglia". Microglia provides scavenging function in the CNS against damaged neurons and infectious agents. The microglia perform an important function in the CNS by acting as antigenic presenting cells and thus activating various immune cells. Microglial cells are considered the most susceptible sensors of brain pathology. Upon any detection of signs for brain lesions or nervous system dysfunction, microglial cells undergo a complex, multistage activation process that converts them into the "activated microglial cell". For the activation, the microglia utilizes various communication mechanisms, and one such signalling communication is provided by the gap junction channels. Thus, it is regarded that the microglia uses gap junctional communication as an important means to achieve the activation state induced by specific factors in their microenvironment. Connexin expression in the microglial cells has been well documented. The expression of connexins in the microglial cells is highly dynamic. In the normal adult rat cerebral cortex, less than 5 % of microglial cells are found to be Cx43 immuno-reactive. Moreover, the in vitro cultures of microglial cells show low levels of diffused Cx43 levels. However, in both cases, Cx43 expression dramatically increases after the activation of microglial cells. The activation under in vivo conditions can be caused by the stab wound, while in culture after treatment with inflammatory cytokines. Moreover, under culture conditions, treatment with cytokines also shows increased cytoplasmic to membrane localization of the Cx43 and thus increased connectivity between the activated microglial cells by gap junctions. Besides Cx43, it is regarded that other connexins mediate gap junctional communication between the microglial cells. These observations are based on the fact that the microglia of Cx43 null mice retain the capability of gap junctional communication.

#### 10.3.2.4 Ependymal Cells

Ependymal cells are the specialized glial cells that form the lining of the ventricles and cerebral aqueducts. These cells are highly coupled with the gap junctions. Ultrastructural details of the ependymal cells show numerous gap junctions, with smaller gap junctional plaques localized at the apical margins and the larger plaques at the lateral membranes between the apposed cells. The efficient coupling is important for the synchronized activity of ciliated ependymal cells. Connexin proteins that are specific to the ependymal cells are Cx26 and Cx43.

#### 10.3.2.5 Meningeal Cells

The CNS is surrounded by three protective connective tissue sheaths of mesenchymal origin. Dura mater is the external meninge, whereas the two inner ones are the arachnoid and pia mater, or the leptomeninges, which send extensions into the neural parenchyma. Gap junctions are extremely abundant in the developing and adult meninges. Indeed, cultured leptomeninges are strongly coupled, even more than in astrocytes. Three connexin types, Cx26, Cx30, and Cx43, are expressed at high levels in meningeal cells which show strong punctate staining.

### *10.3.3 Gap Junctions Between the Glial Cell Types*

Besides forming gap junctions between its own cell types, numerous gap junctions are also known to exist between different glial cell types. For example, electron microscopy studies have indicated that there exist numerous gap junctions between astrocytes and oligodendrocytes. In addition, functional studies have demonstrated the occurrence of gap junction communication in the cocultures of astrocytes and oligodendrocytes. Gap junctions between these two glial cell types occur at specialized regions along the surface of these cells. The major connections occur between the two cell bodies, between soma and glial processes, and between astrocytic processes and the external foil of the myelin sheath of oligodendrocytes. The main connexins that contribute to these gap junctions are the Cx43 and Cx32.

### *10.3.4 Gap Junctions Between the Glial Cells and Neurons*

The existence of gap junction communication between glial cells and neurons is conditional rather than absolute. This type of heterocellular interactions occurs only under certain conditions. For example, in the cocultures of neurons and astrocytes, gap junction plaques are detected for a certain time window. During this time, it is regarded that the gap junctional communication is important for the neuronal differentiation. Although the connexins involved in this communication are not fully known, locus ceruleus glial cells express Cx26, Cx32, and Cx43, whereas neurons express Cx26 and Cx32. Accordingly, the formation of homomeric and/or heteromeric channels could be involved in heterocellular coupling.

The gap junctional permeability in neurons and astrocytes is highly dynamic. For the functioning of gap junctions in the brain in a dynamic manner, they are regulated by a number of bioactive molecules. Similar to other tissues, gap junctions in the CNS are subjected to long- and short-term regulation. Long-term regulation occurs over hours or days and operates at the transcriptional level. This is associated with changes in the expression of connexins and thus the number of junctional plaques. Short-term regulation occurs in minutes and deals with changes in opening

probability, time of opening/closure, and/or unitary conductance of functional channels already in place at gap junctions. Moreover, changes in the rate of gap junction internalization and connexin degradation may also occur. In addition, connexin expression and hence gap junctional communication in the astrocytes and in neurons is regulated by neurotransmitters, growth factors, peptides, cytokines, and endogenous bioactive lipids. Thus, like chemical synapses, electrical synapses, mediated by gap junctions in the brain, are subjected to some plasticity and are tightly modulated by neuronal products and secretions from other brain cell types, such as glia (astrocytes, microglia) and endothelial cells. Hence, it is suggested that any abnormal release of these compounds or signals results in the change of connexin expression. These changes are often responsible for various pathophysiological disorders of the brain. Numerous bioactive compounds, like monoamines, excitatory and inhibitory amino acids, and their derivatives, are known to regulate the gap junctional communication. The effect of bioactive amines is mediated through the generation of various secondary messengers and protein kinases, and these actions can be mimicked by direct activation of several transduction signalling pathways using various agonists. The use of various agonists and antagonists and their effect on the gap junctional communication has paved way for using various gap junction-specific drugs. In fact, alteration of gap junction communication between neurons has been observed in vivo after applying antipsychotic drugs or amphetamine withdrawal, suggesting that electrical synapses and neuronal connexin are considered as potential targets for drug therapies. Moreover, like chemical synapsis, synaptic efficacy at electrical synapses is potentiated using various signalling inputs. For example, use of electrotonic transmission at excitatory inputs to the goldfish Mauthner cell exhibits a long-term potentiation due to an increase in gap junctional conductance produced by a stimulation paradigm similar to that used in the hippocampus.

### *10.3.5 Functions of Neuronal Connexins*

#### 10.3.5.1 Electrical Transmission

The gap junction formed by the neuronal connexins is called the electric synapses. Most of the electrical synapses analyzed are characterized by their bi-directionality, voltage dependency, and a low average coupling coefficient. Electrical synapses are efficient in transmitting pre-potential spikes and after hyperpolarization phases, as compared to a single action potential. Electrical synapses act as low-pass filters, and their cut-off frequency explains the functional involvement at the neuronal network level. There are instances in the CNS where electrical and chemical synapses coexist and modulate each other's function. For example, coexistence of electrical and chemical synapses in inhibitory neuronal networks allows for enhanced timing of spike transmission (1–2 ms for mixed synapses versus 10–20 ms for electrical coupling alone). Electrical synapses are known to contribute to several functional network properties of the brain, like spike synchronization and network oscillations,

coordination and reinforcement of postsynaptic inhibitory potentials, and the detection of coincidence in the inhibitory networks. Electric synapses play a role in the gamma oscillation of the brain, and these are mediated by the Cx36. Targeted deletions of Cx36 show reduced synchrony of gamma oscillations (30–70 Hz) in the neocortical slices. However, ultrafast oscillations or "ripples" (~150 Hz) remained unaffected in such knock-out animals. Other connexins are known to be part of the electric synapses and play role in various physiological functions of brain. Besides their electrical role at neuronal synapses, gap junctions are also important for the metabolic and biochemical coupling by allowing the passage of various molecules. This contribution of gap junctions to non-synaptic interactions between neurons involves the passage of low molecular weight signalling molecules, such as secondary messengers (cyclic nucleotides), amino acids (glutamate), and metabolites (glucose, lactate). Passage of signalling molecules is regarded important for the proper maturation of the developing brain. For example, the passage of inositol trisphosphate (IP3) through the gap junction of developing visual cortex neurons is important for coordinating their neuronal activity.

#### 10.3.5.2 Homeostasis

Besides electrical transmission, gap junctions are critical for the neuronal homeostasis. During neuronal activity, increased concentration of potassium [$K^+$] in the external environment of neurons can induce shrinkage of the glial cells. Glial cells maintain the normal neuronal activity by rapid withdrawal of the increased potassium concentration from the extracellular environment. Glial cells achieve this potassium buffering by having gap junctions between themselves. Thus, gap junctions constitute an intercellular pathway that transfers $K^+$ from areas of high concentration to those having lower concentration. Gap junctions in the astrocytes are also involved in the homogenization of intracellular ionic concentrations. Inhibition of gap junction in cultured astrocytes results in the imbalance of intracellular $Na^+$ concentration.

#### 10.3.5.3 Energy Supply to Neurons

Gap junctions form channels through which various metabolites can easily pass. Glucose, glucose-6-phosphate, and lactate are well known to pass through the astrocytic gap junction channels. Astrocytes contain long processes that link the endothelial cells of blood capillaries at one end with the neuron at the other end. The astrocytes form important intracellular pathways for the transfer of energy metabolites from blood to neurons. Gap junctions contribute to these pathways by providing neurons with energy-producing compounds, since astrocytes are essential morphological intermediates located between blood capillaries and neurons. Moreover, during certain hypoxic conditions, high concentration of metabolites in the astrocytes provides important reservoirs to provide glucose and lactate to the neurons via gap junctions.

#### 10.3.5.4 Neuroprotection

The role of gap junctions in protecting neuronal cells from ischaemic insults is well established. The neuroprotective role of the connexins has been established by the studies showing that the closure of gap junction channels by uncoupling agents results in the increased neuronal vulnerability to ischaemic insults. Moreover, in agreement with this hypothesis, Cx43 heterozygote knock-out mice, ischaemia induced by the occlusion of the middle cerebral artery shows a large infarct volume as compared to that observed in the wild-type mice. These studies confirm the role of gap junctional communication in the neuroprotection. However, there are also some reports that the gap junction channels in the astrocytic network propagate death signals from the site of injury insults to other parts of the CNS.

#### 10.3.5.5 Regulation of Cell Volume

Regulation of cell volume is of primary importance for the normal functioning of the brain. Brain cells are endowed with the capability to regulate cell volume during certain pathophysiological conditions. Exposure of astrocytes to hyposmotic solution results in transient changes in their cell volume. However, immediate response of the astrocytes to the hyposmotic conditions is associated with an increased conductance of the gap junctions. Moreover, astrocytes restore their volume by losing ions and amino acids, and osmotically regulated water and gap junctional communication are important for such functions.

#### 10.3.5.6 Propagation of Intercellular Calcium Waves

It is well established that the astrocytes propagate intercellular $Ca^{2+}$ waves over long distances in response to stimulation and, similar to neurons, release transmitters, called "gliotransmitters", in a $Ca^{2+}$-dependent manner. Calcium signals and the occurrence of calcium waves in astrocytes provide these cells with a specific form of excitability. Various lines of evidence have shown that there exist different pathways for the transmission of $Ca^{2+}$ waves in the astrocytes. Some of these involve the direct communication between the cytosols of two adjoining cells through gap junction channels, while others depend upon the release of gliotransmitters that activate membrane receptors on neighbouring cells. Gap junction-mediated transmission of $Ca^{2+}$ waves was first identified in astrocytes. The conclusion was drawn from the use of gap junction communication inhibitors that impaired the $Ca^{2+}$ wave spread across the astrocytic network. This finding together with several other studies provided a strong basis in support of the view that the gap junction channels play a crucial role in the transmission of $Ca^{2+}$ signals between astrocytes. Besides playing a role in $Ca^{2+}$ wave propagation between the astrocytes, it is also established that the astrocytes signal to neurons through $Ca^{2+}$-dependent release of glutamate. Various studies have indicated that the Cx43 and Cx32 have the ability to pass

intercellular $Ca^{2+}$ waves between the astrocytes and the neurons. Interestingly, studies have pointed the role of $IP_3$ that can pass via gap junction channels and thus initiate the release of $Ca^{2+}$ in the neighbouring cells. Thus, it is regarded that $IP_3$ per se and not $Ca^{2+}$ results in the direct propagation of $Ca^{2+}$ waves. Besides direct gap junctional communication, connexin hemichannel-mediated $Ca^{2+}$ wave initiation has also been proposed. However, the involvement of connexin hemichannels in $Ca^{2+}$ wave propagation is regarded to be indirect rather than a direct effect. It is proposed that the purinergic receptor-mediated ATP release is controlled by connexins and the release of ATP initiates $Ca^{2+}$ wave.

### *10.3.6 Connexin Remodelling During Brain Pathologies*

Various central nervous system-associated injuries and pathologies are associated with the modulation of gap junctional communication and connexin expression. The regulation of connexin expression during brain pathologies is a cause or consequence of such conditions and remains to be elucidated. In the following paragraphs, modulation of connexin expression in various brain pathological situations will be discussed.

#### 10.3.6.1 Brain Inflammation

Brain inflammation occurs under various conditions, which include traumatic injury and brain diseases such as multiple sclerosis, ischaemia, and Alzheimer's disease. One of the hallmarks of brain inflammation is a condition called reactive gliosis. Reactive gliosis results in the proliferation of glial cells (astrocytes and microglia) to lesion site and is characterized by the glial fibrillary acidic protein (GFAP)-positive astrocytes. In addition, these conditions are accompanied by the modulation of gap junction communication. Reactive gliosis has been associated with modulation of Cx43 expression in the astrocytes and hence the gap junction communication. Moreover, during certain brain diseases, like Alzheimer's, an increased Cx43 immunoreactivity is observed at sites containing amyloid plaques. Moreover, during inflammation, activation of endothelin receptors by the endothelin results in the inhibition of gap junctional communication and hence propagation of calcium waves. Interestingly, during reactive gliosis, most of the components of the endothelin system (endothelin, endothelin receptors, endothelin-converting enzymes) are upregulated. In addition, an endothelin level is increased in several neurological disorders, such as Alzheimer's disease, subarachnoid haemorrhage, and ischaemia. The astrocytic gap junctional communication is also modulated by the release of nitric oxide, produced by inducible nitric oxide synthase and prostaglandin (PGE2), produced by the cyclo-oxygenase activation. All of these compounds contribute to the glia-mediated neuro-inflammatory response by affecting gap junction communication. The conclusion drawn from these observations suggests that during local inflammation in

the brain, pro-inflammatory cytokines, endothelins, etc., regulate the gap junctional communication of the astrocytes. This has been corroborated by the downregulation of Cx43 in the astrocytes under inflammatory conditions. Although the functional consequences of gap junction inhibition in the astrocytes during inflammation are not understood fully, it is regarded that inhibition of gap junctional communication may restrict the passage of active molecules to neighbouring astrocytes. This will reduce the spread of apoptotic signals within astrocytic networks and thus isolate intact tissues from primary lesion sites. However, it is pertinent to mention here that under certain cell-damaging conditions, increase in the gap junctional communication is known to dilute the damaging signal and thus contribute to the bystander effect and hence decrease neuronal vulnerability to oxidative stress. Therefore, reactive astrocytes with modified gap junctional communication are regarded as a key response in a dynamically changing environment that can modify neuronal functions and overall brain physiology.

#### 10.3.6.2 Epilepsy

Epilepsy is characterized by recurrent seizures and results from abnormal synchronous firing of neurons. Typically, it originates in networks that under normal conditions generate local or large-scale synchronized oscillations. Multiple factors contribute to this activity, including strong recurrent excitatory connections, the presence of intrinsically burst generated neurons, and ion regulation. Gap junctions or electric synapses play a critical role in the generation and propagation of various oscillatory waves in the central nervous system. Simulation and modelling of neuronal networks have supported the importance of GJIC in synchronized activity and how electrical coupling can modify frequency of oscillations and firing properties of neurons. Thus, gap junction-mediated electric synapses are crucial for synchrony and stabilization of bursting firing patterns of the neurons. The role of connexins or gap junctional communication has been demonstrated using various animal model studies. For example, Cx36-null mice show deficient synchronous activity of inhibitory interneuronal networks in neocortex. Moreover, these mice show impaired hippocampal gamma (~30–80 Hz) oscillations. Similarly, Cx32 knock-out mice show myelination defects with neuronal hyper-excitability. Recently, it has been demonstrated that very fast neuronal oscillations (VFOs, 140–200 Hz) are involved in the generation of seizures. These very fast neuronal oscillations have been shown to immediately precede seizure onset. Interestingly, studies have indicated that the axo-axonal gap junctions are involved in the generation and propagation of high frequency oscillations. It has been found that a very small number of gap junctions linking neurons are required to produce the appropriate oscillatory activity. These studies were confirmed by the electrophysiological detection of the axo-axonal gap junctions between CA1 pyramidal neurons. Moreover, in rat hippocampal, use of gap junction inhibitor carbenoxolone abolished very high frequency generated before epileptiform bursts. Similarly, in vivo evidence with the cat neocortex has shown that halothane (gap junction inhibitor) prevents the onset

of ripples observed during the epileptic seizures. These studies have confirmed the potential of gap junction inhibitors as pharmacological agents that may prove effective anticonvulsants.

#### 10.3.6.3 Brain Ischaemia

Ischaemic brain injury is one of the major causes of neurological malfunctioning. Brain ischaemia can engulf the major portion of brain and hence is named as global ischaemia, or it can be localized, called as focal ischaemia. Global ischaemia is usually caused when the blood supply to the brain stops temporarily, due to either cardiac arrest or systemic circulatory collapse. The resulting insults are of short duration with little or no blood flow changes. In contrast, focal ischaemic injury usually occurs if the blood flow to a particular region of the brain decreases, thus affecting its functioning. Few of the major outcomes of the focal ischaemia is that the cellular energy is depleted within minutes, there is a sudden loss of specific brain functions, and a core of dying tissue, the "infarct", develops. Besides neuronal damage, the ischaemic insult results in the swelling of astrocytes and malfunctioning of the glutamate uptake. Besides, the connexin expression in the astrocytes is modulated, and this results in the disturbed gap junctional communication. Since gap junction communication is pivotal for the normal functioning of neurons and brain homeostasis, hence, altered astrocytic gap junctions contribute to the neuronal death. Moreover, gap junction can act as a medium to propagate the secondary expansion of focal ischaemic injury. It has been observed that the astrocytic gap junctions remain open during ischaemia and mediate the propagation of cell death signals to the other parts of the brain. Modulation of gap junction communication after brain ischaemia is associated with the change in the expression of connexins. These changes can be either the upregulation of certain connexins or the downregulation of others. For example, in the hippocampus, global ischaemia induced an increase in Cx32 and Cx36 proteins, specific to the inhibitory interneurons of the CA1 region, whereas in CA3 region the expression of Cx32 and Cx36 in the neurons and the Cx43 expression in the astrocytes remain unchanged. It has been proposed that the increase of Cx32 and Cx36 expression in CA1 region contributes to the survival of the GABAergic neurons and increases their synchronized inhibitory synaptic transmission. Similarly, ischaemia of the forebrain, induced by bilateral carotid occlusion, produced increased Cx43 immunoreactivity at sites of mild injury, whereas regions exhibiting severe ischaemic injury showed a decreased Cx43 immunoreactivity.

## 10.4 Connexins in the Skeletal System

Skeletal systems have abundant gap junctions present in all the bone cells, with osteoblasts and osteoclasts having the highest number. The presence of abundant gap junctions in the bone cells is suggestive of their involvement in various bone

functions, including control of osteoblastic cell proliferation, differentiation, and survival. Although many connexins are known to express in the skeletal system, Cx43 forms the most abundant gap junctions in the skeletal system. Other connexins, which are expressed in the skeletal system, are Cx45 and Cx46. Besides, Cx40 have been shown to be present in the developing limbs, ribs, and sternum, but its expression goes down with the maturations, and in the adult skeletal system, its expression is not documented. Cx43 being the highly expressed connexin in the bone, its biological importance in the skeletal development has been established by numerous studies using human and mouse genetics. Mutational studies in mice with germ line null mutation of Cx43 indicated the hypo-mineralization of craniofacial bones and a severe delay in ossification of the axial and appendicular skeleton. In addition, numerous other skeletal abnormalities occur due to the absence of Cx43, like ossification defects and malformation of cranial ribs, vertebrae, and limbs. Moreover, osteoblast-specific deletion of Cx43 in the mice shows similar defects, excluding the craniofacial malformations or the ossification defects. In humans, the linkage of mutation of Cx43 locus to the human disease called oculodentodigital dysplasia (ODDD) provides the strongest evidence for a critical role of Cx43 in skeletal development. The molecular mechanisms of Cx43 action on the bone metabolism are still not well understood. However, recent studies have indicated that Cx43 mediate bone cell response to the hormonal stimulation. For example, the anabolic effect of parathyroid hormone (PTH) on the bone is attenuated in Cx43-deficient mice. Further analysis has shown that Cx43 deficiency results in the diminished production of PTH stimulated cAMP and hence decreased mineralization of the osteoblasts. In addition, as a mediator of hormonal stimulus, Cx43 also plays a role in the anabolic response to the mechanical stimulus. It has been observed that the mineral deposition rate at the mechanically stimulated endocortical surface of tibiae is significantly reduced in the conditionally Cx43 deleted mice relative to wild-type animals. Besides the involvement of Cx43 in regulating various physiological aspects of bone metabolism, its role in mediating the effect of various skeletal pharmacologic agents has been proposed. For example, the inhibitory action of bisphosphonate and alendronate on the apoptosis of osteoclasts has been shown to require Cx43. It has been observed that the anti-apoptotic action of bisphosphonates is independent of Cx43 gap junction communication. However, the role of Cx43 hemichannels has been proposed to be required for such effects. Cx43 is also known to modulate the expression of various genes in the osteoblastic cells. The transcription of $\alpha_1$(I) collagen and osteocalcin has been shown to be influenced by the expression of Cx43. Cx43 has been shown to exert its influence on the transcription of various genes by specific DNA promoter elements, known as “connexin response elements”. Similarly, Cx43 mediates its effect on the transcription of osteocalcin and α1(I) collagen genes through Cx43 response elements and the binding of Sp1/Sp3 transcription factors. Cx43 regulate gene expression either directly or indirectly by altering various signalling pathways. In osteoblasts, Cx43 alters ERK signalling, and this in turn modulates gene transcription from osteoblast gene promoters via decrease of ERK-dependent phosphorylation of Sp1 with preferential recruitment

of Sp3 to connexin response elements. Cx43 also mediate the transduction of mechanical signals in the bone cells. It has been found that the mechano-transduction is mediated by Cx43 hemichannels. Interestingly, Cx43 hemichannels have been found active in the osteocytic cells, where they mediate fluid flow-induced $PGE_2$ and ATP release.

## 10.5 Connexins in the Inner Ear

Connexin distribution in the ear is mostly restricted to the inner part of the ear. Several connexins have been identified in the rodent ear, which include Cx26, Cx30, Cx31, Cx32, and Cx43. By analogy, most of these connexins have been identified in the human ear. Of these, Cx26 is the physiologically most important connexins found in the inner ear. In the inner ear, connexins are localized in the epithelia and connective tissue of the cochlea, thus connecting these tissues with the gap junction network. In addition, gap junction plaques and intercellular gap junction communication exists in the organ of Corti. Besides forming homotypic gap junction, heterotypic gap junctions, formed of Cx26 and Cx30, have been identified in the cochlear tissue. Gap junctional communication in the cochlear tissue is non-selective to ions, but there exists some sought of selectivity as far as passage of secondary messengers and other molecules are concerned. Besides intercellular gap junctions, functional hemichannels have been reported to exist in the organ of Corti. Hemichannels are involved in the uptake of large anionic molecules and under special circumstances release ATP to the extracellular space. The cochlea in the inner ear is the sensory organ that transmits sound signals. The cochlea contains many cells, which include epithelial cells, fibrocytes, and the sensory receptor cells called as hair cells. The cochlea has three compartments, namely scala media, scala tympani, and scala vestibule, and these are filled with two types of solutions. The scala tympani and scala vestibule contains perilymph, having ionic composition similar to that of extracellular solution, whereas the scala media contains endolymph, which possesses a high concentration of $K^+$ (150 mM). One of the important properties of endolymph is the high positive potential (+80 mV), termed as endocochlear potential. The endocochlear potential is produced by the stria vascularis, a two-layered epithelium forming the wall of the scala media. The cellular components of the stria vascularis contain the potassium channel Kir 4.1 in the plasma membrane of intermediate cells and $K^+$ transporters in the basal membrane of marginal cells of the. The circulation of $K^+$ ions from endolymph to perilymph is thought to be mediated by the gap junctional network between the supporting cells and epithelial cells on the basilar membrane and between the fibrocytes of spiral ligament and epithelial cells of stria vascularis. The importance of gap junction communication in the cochlea can be ascertained by the fact that its disruption leads to several forms of non-syndromic and syndromic deafness.

## 10.6 Connexins in the Endometrium

Gap junctional communication plays an important role for the proper functioning of the endometrium. Cx43 and Cx26 are the two major connexins which are expressed in the endometrium. Endometrium is highly dynamic in terms of its growth properties, having non-pregnant cyclic phases and a pregnant phase. Both Cx43 and Cx26 play an important role during the cyclic phases of non-pregnancy and during early pregnancy. Cx26 and Cx43 expression during the cyclic phases of non-pregnancy is mostly at the transcriptional level, which is confirmed by the increased levels of Cx43 and Cx26 mRNA, however with low amounts of the corresponding proteins. Just prior to the preimplantation stage, when the endometrium is ready to receive the embryo, the transcription of both connexins is downregulated. Maternal progesterone hormonal signal is regarded responsible for the transcriptional suppression of Cx43 and Cx26. Estradiol on the other hand upregulates the expression of Cx26 and Cx43. The decreased gap junctional communication during the early pregnancy is important for the differentiation of the receptive epithelium of the endometrium. Interestingly in rats and guinea pigs, the use of anti-progesterone drugs during the first days of pregnancy has been shown to inhibit embryo implantation. With the growing pregnancy, an intimate contact between embryo and the endometrium is required for the successful outcome of the pregnancy. During placental formation and its penetration into the endometrium, connexin expression is upregulated and gap junctional communication is established between the endometrial and the placental cells. This intercellular communication, mediated by the gap junctions, is important for the successful implantation and the placental invasion. During implantation, induction of gap junction connexins in the endometrium occurs in response to embryo recognition. The first connexin whose expression is induced is Cx26, and this results in the decidualization of the stromal cells surrounding the implantation chamber. The implantation of the blastocyst is accompanied by the expression of Cx43. Thus, spatial and temporal expression of endometrial Cx26 and Cx43, in response to embryo recognition, is important for the successful implantation of the embryo. Embryo recognition by the endometrium is mediated by different signals, which include hormones, secondary messengers, growth factors, prostaglandins, and the mechanical stimulations. Additionally, Cx43 expression and the gap junction communication in the blastomeres of the embryo are important for compaction. In rat embryo, Cx31 has been shown to have similar spatio-temporal expression as that of Cx43. In the initial stage, both Cx31 and Cx43 show even distribution in the inner cell mass and the trophectoderm. However, during the later stages, Cx31 show expression in the cells of the ectoplacental cone, which invades into the maternal decidual tissue, whereas Cx43 shows expression in the embryo proper. During the differentiation phase, Cx26 expression is highly induced in the labyrinthine trophoblast and is responsible for the feto-maternal exchange. With the maturation of placenta, expression of Cx31 and Cx43 decreases with increasing trophoblast differentiation.

## 10.7 Connexins in the β Cells of Pancreas

The pancreas constitutes an important gland of the vertebrates. It is both an endocrine gland, producing several important hormones, including insulin and glucagon, and a digestive organ, secreting pancreatic juice containing digestive enzymes that assist in the digestion of nutrients in the small intestine. The endocrine part of the pancreas is made up of clusters of cells called islets of Langerhans. In humans, millions of these cells are dispersed in the pancreas and constitute 1 % of total volume of the pancreas. The islets of Langerhans play an important role in glucose metabolism and regulation of blood glucose concentration. The islets of Langerhans contain many different cell types, each specific for different functions. For example, α cells secrete glucagon hormone in response to the low blood glucose level, β cells secrete insulin in response to high blood glucose level, and δ cells secrete somatostatin that regulates the function of α and β cells.

β cells represent one of the important cell types of islets of Langerhans that perform crucial function of regulating blood glucose level by secreting an important hormone called as insulin. The clusters of the β cells require proper coordination for the secretion of insulin in response to high blood glucose level. This coordination integrates hundreds of β cells within each islet into a functionally homogeneous unit. β cells employ many mechanisms to achieve the proper coordination for the release of insulin. One of the important mechanisms for achieving the required coordination is the direct cell-to-cell coupling mediated by gap junction channels. Such channels help the cells to communicate with each other using various signalling molecules. The gap junction channels in the β cells are mostly made up of Cx36. Cx36 is a 321-amino acid protein with a long (99 amino acid) cytoplasmic loop containing an unusual stretch of 10 glycine residues and a short cytoplasmic COOH-terminal domain. The cytoplasmic loop and the carboxy-terminal domain of Cx36 contains potential recognition sites for various kinases, like casein kinases I and II, cAMP-dependent protein kinase, and calmodulin-dependent protein kinase II. Thus, Cx36 is a target of various kinases, consistent with the finding that, under certain conditions, the function of Cx36 is regulated by phosphorylation events. The gap junction channels formed by the Cx36 are mostly permeable to cationic species as compared to the negatively charged molecules. The importance of gap junction network between the β cells can be ascertained by the facts that the single cells (not in contact with other cells) show poor responsiveness to glucose stimulation, decreased basal expression of insulin, decreased pro-insulin biosynthesis, and less increase in the cytosolic calcium after glucose stimulation. However, the cells that are grown in contact with each other are very efficient in glucose responsiveness, insulin secretion, and cytosolic calcium increase. These observations are corroborated by using drugs that inhibit gap junction communication. For example, treating isolated islet cluster or the intact pancreas with carbenoxolone (gap junction inhibitor) resulted in decreased glucose responsiveness and insulin secretion, and these effects are reverted to normal when the carbenoxolone is washed away for the cells. Thus, the experimental evidence suggests that the gap junction channels between the cells

are important for mediating glucose-induced secretion of insulin. Gap junctional communication between the cells allows them to equilibrate various ions and molecules between the cells for the coordinated function. This is what the gap junction channels do in the β cell physiology. The cell-to-cell communication mediated by connexins is regarded advantageous for the tissues made of different cell types. This is because the cells in a cluster show minor differences in their structure and hence are functionally asynchronous. It has been found that the differences in the biosynthetic activity of β cell would result in the irregular responsiveness to the glucose stimulation for the secretion of insulin. In other words, the release of insulin will be less and not synchronous in response to high glucose stimulation. Hence, the Cx36 gap junction channels between the β cells nullify the biochemical disparity and allow the synchronous response to the glucose stimulation. Under such conditions, β cell clusters release significantly larger amounts of insulin than the uncoupled cells. The inhibition of gap junction coupling between the β cells is known to alter the basal and glucose-stimulated insulin secretion, the expression of insulin genes, and the regulation of cytosolic calcium. The Cx36-null mice (Cx36–/–) have proved instrumental in discerning the role of Cx36 for the proper functioning of β cells. Lack of Cx36 results in the failure of calcium wave synchronization between the β cells. Consequently, loss of the synchronization affects the simultaneous release of insulin upon glucose stimulation. Thus, cell-to-cell contact between the β cells is critical for the glucose-induced insulin secretion. These and many other studies support the evidence of the importance of Cx36 signalling for the coordinate release of insulin by the β cells, and thus, the altered insulin secretion of Cx36-null mice results in abnormal control of blood glucose levels. Interestingly, the phenotypic effects of Cx36-null mice, like glucose intolerance, loss of circulating insulin oscillations, and increased β cell apoptosis, resemble to various β cell-related pathological phenotypes found in human beings. Since the human β cells are also coupled by the gap junctions formed by Cx36, it is safe to argue that various diabetes-related issues in humans are the consequence of altered Cx36 signalling. The significance of Cx36 in the β cell physiology is not limited to coordinate the insulin secretion in response to glucose stimulus, but also, Cx36 has been shown to protect the islets of Langerhans from the autoimmune attack mediated by various pro-inflammatory cytokines in the islet environment. The protection offered by Cx36 to the pancreatic cells is established from the events that occur during type-1 diabetes. In type-1 diabetes, the islets of Langerhans are self-attacked by various immunological factors, resulting in the reduced cell mass and hence the insufficient secretion of insulin. The role of Cx36 in providing protection against the self-attacking molecules has been demonstrated using transgenic mouse models. For example, transgenic mice over-expressing Cx36 significantly protected the β cells against cytotoxic drugs and cytokines that were shown to induce cell death similar to the onset of type-1 diabetes. Interestingly, mice lacking Cx36 showed increased sensitivity to these molecules and develop symptoms similar to type-1 diabetes. The molecular mechanism responsible for such protection, mediated by Cx36, is still a mystery. Various studies have shown that both gap junction-dependent and -independent mechanisms are responsible for the protection.

The role of Cx36 has also been reported in type 2 diabetes. Type 2 diabetes is a multifactorial disease, and association between type 2 diabetes and mutations in the chromosome regions that harbour Cx36 has been found. Although mutational analysis of Cx36 in type 2 diabetic patients has not been established in humans, mice possessing inactivated Cx36 depict certain diabetic phenotypes similar to what occurs during the early onset of type 2 diabetes in humans. The complete understanding of the role of Cx36 in diabetes will pave way for the innovative therapies in order to improve β cell functioning and hence blood glucose regulation.