Chapter 6
Transcriptional Regulation of the Connexin Gene

As described previously, alternate splicing and differential promoter usage can give rise to various transcripts of a particular connexin gene. Some of these transcripts are ubiquitously expressed, while some are tissue specific. The transcription of connexin genes is controlled by of various transcription factors and other biological substances. The transcription factors play an important role in regulating the expression of connexin gene. The types of transcription factor(s) determine whether a particular connexin is expressed ubiquitously or in cell type-specific manner. In the following sections, the role of various transcription factors and other signalling molecules that regulate the ubiquitous or tissue-specific expression of connexin genes will be discussed.

6.1 Regulation by Ubiquitous Factors and Signal Transduction Pathways

The basal level of connexin transcription is established by the RNA Pol II promoter elements and the basal transcription factors. The ubiquitous expression of connexins is determined by the various housekeeping transcription factors. Some of these transcription factors are discussed below.

6.1.1 Sp1 and Activator Protein 1 (AP-1)

The Sp family of transcription factors constitute the ubiquitously expressed proteins. Sp1 is an important member of Sp family of transcription factors that recognizes GCGG sequences, called as GC boxes. DNA-binding domain of Sp1 consists of three zinc fingers, which recognize and bind the GC box. Sp1 regulates the basal

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transcription of several connexin genes, such as Cx32, Cx40, Cx43, and Cx26. In addition to Sp1, Sp3 is known to bind the P1 promoter elements and control the expression of rat Cx40 and Cx43. The cumulative effect of Sp1 and Sp3 binding is required for the transcription of various connexin genes. The P1 promoter element of Cx32 possesses two binding sites for Sp1 transcription factor, and the binding regulates only its basal transcription and not the tissue-specific expression. The binding of Sp1 and Sp3 to the promoter elements of connexin gene is regulated by various signalling molecules and signalling pathways. Moreover, during certain pathophysiological conditions, the Sp1/Sp3 transcription factors are known to be involved in the deregulation of connexin expression. For example, high blood glucose concentration downregulates Cx40 expression in the vascular endothelial cells and that results in the impaired endothelial capillary network formation. The decreased expression of Cx40 has been attributed due to high glucose-induced downregulation of Sp1 transcription factor.

Activator protein 1(AP1) is a basal transcription factor, which activates the transcription of its target genes. AP1 acts as dimer and consists of either Jun proteins (Jun homodimer) or Fos proteins (Fos homodimer) or the combination of Jun with Fos (Jun–Fos heterodimer) or even heterodimer of these proteins with other proteins. AP1 transcription factor forms a characteristic DNA-binding domain called leucine zipper. AP-1 binds to a consensus sequence [TGAC (T/G) TCA] in the promoter and induces transcription of the gene. One or more AP-1-binding sites have been identified in the mouse, rat, and human Cx43 proximal promoters P1. The rat Cx43 promoter contains two AP-1-binding sites, whereas the mouse and human promoters each have one AP-1-binding site. Although the AP1 regulates the basal transcription of various connexin genes, however, under certain circumstances, it induces the expression of certain connexin genes. For example, during the onset of labour, AP-1 transcription factor is necessary for the upregulation of Cx43 expression.

The AP-1 transcription factors are the target of various signalling molecules and pathways that in turn results in the regulation of connexin expression. For example, stimulation of urinary bladder smooth muscle cells by the basic fibroblast growth factor (bFGF) upregulates Cx43 transcription via extracellular signal-regulated kinase (ERK) 1/2-AP-1 pathway. The binding of AP1 to the Cx43 promoter is regarded essential for the upregulation of Cx43 transcription upon stimulation with bFGF. Similarly, TGF-beta induces Cx43 gene expression in normal murine mammary gland epithelial cells. The signalling pathway that activates c-Jun/AP-1 is essential for mediating TGF-beta1-induced Cx43 gene expression.

6.1.2 Cyclic AMP

Cyclic AMP (cAMP) is a ubiquitous secondary messenger, which is formed in response to some extracellular stimuli that activate G protein-coupled adenylate cyclase. The cAMP in turn activates protein kinase A (PKA) and that mediates various

functions of cAMP by phosphorylating various target proteins. The adenylate cyclise converts ATP to cAMP at the inner surface of plasma membrane. The cAMP modulates the expression of various genes by binding and thus activating certain proteins known as cAMP response element binding (CREB) proteins. These proteins recognize certain sequence elements in the DNA, known as cAMP response elements (CRE). The promoter elements of various connexins contain cAMP response elements and thus are target of cAMP-mediated regulation. Cx43 promoter element I contains putative cAMP response element, and the expression of Cx43 is enhanced when cells are treated with the cAMP analogue 8Br-cAMP. The signalling mediated by cAMP employs short-term and long-term effects on the regulation of Cx43. The short-term effects include the increased redistribution of Cx43 to the cell membrane, and the long-term effects include increased transcription of Cx43 gene.

6.1.3 Retinoids

Like cAMP, retinoids are known to induce gap junctional intercellular communication and upregulate Cx43 expression in normal and preneoplastic cells. Treatment with the synthetic retinoid tetrahydrotetramethylnapthalenylpropenyl-benzoic acid (TTNPB) is known to cause an approximate tenfold elevation of Cx43 gene transcripts. However, the molecular mechanism for the upregulated expression of Cx43 remained to be understood. It has been hypothesized that retinoic acid increased Cx43 expression in cells by increasing transcription and by stabilizing Cx43 transcripts. The role of retinoic acid on the expression of Cx43 has been studied with reference to human endometrial stromal cells. It has been shown that the retinoic acid upregulated Cx43 mRNA and protein expression with the concomitant increase in the gap junction intercellular communication in the human endometrial stromal cells. The endometrial stromal cells express retinoic acid specific nuclear receptors and the binding of retinoic acid to these receptors results in the upregulation of the transcription of Cx43 gene. In addition to increasing Cx43 mRNA and protein levels, retinoic acid also enhances the dephosphorylation of Cx43, so that the nonphosphorylated form increases with respective to phosphorylated forms. The decrease in the phosphorylation status of Cx43 increases the gap junction communication between the cells.

Similar studies in human skin epidermis have shed more light on the regulation of connexin expression by retinoic acid. Human keratinocytes are connected by many gap junctions, and these junctions are mostly made from Cx26 and Cx43 proteins. It is well known that connexins are involved in keratinocyte differentiation and retinoic acid provides the necessary link by regulating the expression of connexins during cell differentiation. The mechanism by which retinoic acid modulates the differentiation of the epidermis is still not well understood. However, it is regarded that retinoic acid changes the expression of connexins and modulates the gap junction mediated cell–cell communication.

6.1.4 Wnt Pathway

Wnt genes encode a large family of secreted glycoproteins, which play important roles in directing cell fate and cell behaviour and in tumorigenesis. Wnt signalling pathway results in the activation of certain transcription factors, like TCF/LEF proteins. Nuclear localized β-catenin/TCF complexes regulate the transcription of target genes by binding to the consensus sequence A/TA/TCAAAG, known as the TCF/LEF binding site. Various studies have demonstrated the co-localization of Wnt and connexin expression. Connexins are known to have an important role in regulating cell growth and differentiation. Connexin genes are regarded as the targets of Wnt signalling. Interestingly, rat Cx43 promoter P1 contains two TCF/LEF binding consensus sequences in opposite orientations. Two similar TCF/LEF motifs are also found in the human and mouse Cx43 promoters. One of the prime targets of Wnt signalling is the expression regulation of Cx43 in cardiomyocytes. Wnt signalling and Cx43 expression have been shown to be important for the normal development of the heart. Besides regulating expression of Cx43, several lines of evidence suggest that the Wnt signalling also modulates the gap junction channel activity of Cx43. The molecular mechanisms involved in the Wnt signalling and Cx43 are still emerging. One such mechanism involves the Wnt-dependent accumulation of β-catenin near the membrane and the transcriptional activation via a β-catenin/TCF nuclear complex. Since Cx43 is known to interact with β-catenin, the activation of Wnt signalling results in the enhanced interaction between the Cx43 and the β-catenin as part of a complex within the junctional membrane. The sequestration of β-catenin by Cx43 serves to negatively regulate its own transcription, as well as other β-catenin/TCF-dependent transcriptional targets. These findings are corroborated by the observations demonstrating the upregulation of β-catenin levels in various human cancers that act as an important oncogenic signal for the neoplastic transformation of the cells. Since Cx43 is known for its tumour suppressor activity, hence its sequestration by increased β-catenin levels may serve an important event for deregulating the growth of the cells. In addition to the role in regulating cell growth, Wnt–β-catenin–Cx43 signalling is also involved in human cardiomyopathies. During cardiomyopathies, expression of Cx43 is either downregulated or abnormally localized in the cell. The abnormal expression of Cx43 has been linked with decreased β-catenin activation of Cx43 promoter. These observations indicate that the alterations in Wnt signalling influence β-catenin function, which in turn influence gap junction channel gene expression in the heart. These facts are confirmed by the downregulation of β-catenin and concomitant decrease in the Cx43 abundance during various forms of heart disease.

6.2 Regulation by Tissue-Specific Factors

The expression of some connexins shows differential spatial and temporal expression. These connexins are only expressed in certain cell types, and many connexins show development regulation. Besides ubiquitous transcription factors, tissue-specific

expression is mediated by additional cell type-specific transcription factors and the signalling pathways that activates such factors. Some of the cell type-specific transcription factors and the signalling molecules involved in tissue-specific expression of connexins are discussed below.

6.2.1 Homeodomain Transcription Factors

Homeodomain-containing proteins are the family of transcription factors that regulate gene expression during development. The homeodomain is 60 amino acid motif that recognizes a specific stretch of 180 bp DNA sequence in the promoter region of various genes, known as homeobox. Nkx2.5 is a transcription factor, which is expressed mostly in cardiac tissue and is critical for the proper development of heart. This protein factor belongs to the family of homeodomain containing transcription factors. Nkx2.5 shows a wide range of expression in the cardiac tissue from drosophila to humans. Cx43 is the main connexin that is expressed in cardiac tissue, and the promoter element P1 of Cx43 contains consensus binding site for Nkx2.5 transcription factor. Nkx2.5 activates the expression of Cx43 and under certain conditions may repress it. Moreover, Nkx2.5 is also known to bind the promoter element of another cardiac connexin, Cx40, and activates its expression. In addition, some studies have pointed out the role of Nkx2.5 in the regulation of cardiac-specific Cx45. Another homeodomain transcription factor that is known to influence the transcription of connexins is Shox2. Shox2 binds the promoters of Cx40 and Cx43 and activates their transcription. In mouse cardiac cells, Cx40 transcription is further regulated by homeodomain-only protein (Hop). The Hop encodes a 73 amino acid protein that contains a domain (the 60-amino acid homeodomain) homologous to those seen in homeobox transcription factors. Unlike all other known homeobox transcription factors, Hop does not directly bind DNA. It is expressed in the embryonic heart and plays an important role in development of the adult cardiac conduction system. Various studies have demonstrated that the expression of Hop is required for the proper expression and localization of Cx40 in the cardiac conduction system.

6.2.2 T-Box Transcription Factors (Tbx5, Tbx2, Tbx3)

T-box transcription factors form a large family of proteins that possess a helix–loop–helix type DNA-binding domain. They are regarded critical for various developmental fates. This family of proteins possesses both transcriptional activators (Tbx5) and transcriptional repressors (Tbx2 and Tbx3). The helix–loop–helix domain of these proteins recognizes the same sequence on the DNA, and hence both activators and repressors bind the same sequence. Thus, the outcome of the binding of T-box transcription factors on the gene expression is determined by the relative

concentration of activators and repressors. Several T-box binding sites have been identified in rat and mouse Cx40 and Cx43 promoters. The T-box protein expression coincides with the expression of Cx40. Tbx5 is a T-box transcription factors that is known to bind at multiple sites on the Cx40 promoter. Tbx5 haplo-insufficent mice show marked decrease in the Cx40 mRNA. Besides Tbx5, two other T-box transcription factors are known to influence the expression of Cx40. These include Tbx-2 and Tbx-3 that are known to act as transcriptional repressors. These facts are based on the observations that there exists an inverse relation between the expression of Tbx-2 and Cx40 mRNA in various regions of the developing heart. For example, during mouse embryonic stages 9.5–14.5, the presence of Tbx2 in the cardiac chamber of the embryos is inversely related with the expression of Cx40 and Cx43. Similar to Tbx2, the Tbx3 shows an inverse relation with the expression of Cx43 and Cx40 during cardiac development. The Tbx3 specifically interacts with the TBE-containing DNA region of the Cx43 promoter, indicating that Tbx3 directly represses Cx43.

6.2.3 GATA Family

GATA transcription factors belong to the category of transcription factors that possess two zinc finger DNA-binding domains. They recognize the DNA with a consensus sequence of (A/T) GATA (A/G). Based on their expression profile, the GATA proteins have been divided into two subfamilies, GATA-1, GATA-2, and GATA-3 and GATA-4, GATA-5, and GATA-6. The GATA-1, GATA-2, and GATA-3 genes are prominently expressed in haematopoietic stem cells, where they regulate differentiation and specific gene expression of T-lymphocytes, erythroid cells, and megakaryocytes. GATA-4, GATA-5, and GATA-6 genes are expressed in various mesoderm- and endoderm-derived tissues such as the heart, liver, lung, gonad, and gut, where they play critical roles in regulating tissue-specific gene expression. GATA transcription factors specifically regulate the expression of connexins in various tissues. Rat Cx40 promoter contains putative binding sites for GATA-4, and it has been shown that GATA-4 expression induces the transcription of Cx40.

6.2.4 HNF-1

HNF-1 is a liver-specific transcription factor, which regulates the expression of many liver-specific genes. Two isoforms, HNF-1α and HNF-1β, are known, and these show developmental expression regulation. The HNF-1α is highly expressed in adult liver, whereas the HNF-1β is expressed during the embryonic development. The HNF-1 functions either as a homodimer or as a heterodimer. The promoter region of the mouse Cx32 gene contains two putative binding sites for HNF-1. HNF-1 is

mainly responsible for the cell-specific expression of Cx32. These observations are based on the fact that the HNF-1α null mice show significantly decreased Cx32 expression.

6.2.5 Oestrogen

Oestrogen is a steroid hormone, which directly influences the expression of many genes by binding and subsequent activation of oestrogen receptor. Oestrogen receptors constitute the widely studied nuclear receptor. There are two subtypes of the oestrogen receptor, which are known as ER-α and ER-β, and they are the products of two different genes and show tissue-specific expression. In addition to the nuclear receptor-mediated action, oestrogen also influences various physiological processes by influencing various other signalling pathways. Oestrogen influences the expression of connexins, in particularly the Cx43. As described above, oestrogen exerts its effect by binding to nuclear receptors, and the complex binds specific DNA sequences in the promoter region of target genes, called as oestrogen response elements. The role of oestrogen on the connexins transcription has been studied with reference to the Cx43 upregulation during the onset of labour. It is well established that the Cx43 plays a critical role during the onset of labour by increasing the myometrial cell coupling and their coordinated synchronous contraction. This phenotypic effect is strongly correlated with the dramatic increase in both mRNA and protein levels of Cx43 in the myometrium. Interestingly, rat Cx43 promoter has been shown to possess oestrogen response elements, and these elements are responsive to the oestrogens, thus responsible for the increase of Cx43 expression.

6.2.6 Thyroid Hormone

The role of thyroid hormone in regulating the expression of connexins has been studied with reference to Cx43. The role of thyroid hormone in regulating Cx43 transcription was deduced from the observation that the treatment of rat liver samples with thyroid hormone resulted in elevated Cx43 mRNA. Subsequent sequence analysis of the Cx43 upstream promoter element leads to the discovery of thyroid hormone response elements located at positions −480 to −464. Additionally, it is regarded that the −480 position in the rat connexin43 forms stronger complexes with thyroid hormone receptor alpha/retinoid X receptor alpha heterodimers than with vitamin D receptor/retinoid X receptor alpha heterodimers. The physiological relevance of the thyroid hormone in regulating connexin expression has been studied in Sertoli cell of testis. It is well known the thyroid hormones regulate Sertoli cell proliferation and differentiation in the neonatal testis. Further studies have shown that thyroid hormone inhibits Sertoli cell growth and proliferation by inducing the expression of Cx43. The upregulation of Cx43 expression was associated with

increased gap junction communication between the cells. These studies were further corroborated by the inhibitors of gap junction coupling that were shown to significantly reverse the inhibitory effect of thyroid hormone on the Sertoli cell growth and proliferation.

The importance of thyroid hormone regulation of connexins expression is evident from the association of hypothyroidism with the expression of various connexins in the thyroid cells. The outcome of iodine deficiency-related hypothyroidism is the decreased gap junction communication between the thyroid cells. Similarly, in autoimmune-related hypothyroidism, the thyroid cells show reduced expression of Cx43, Cx32, and Cx26. Moreover, the thyroid-stimulating hormone (TSH), produced in the hypothalamic region of the brain, regulates thyroid hormone production by increasing the connexin function of thyroid cells. Hence, thyroid hormone acts as an important signalling molecule that regulates the expression of various connexins. Moreover, the expression of connexins is regarded essential for the proper release of thyroid hormone from the thyroid gland.