Review

Transcriptional control of myocardial connexins

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Received 6 October 2003; received in revised form 17 November 2003; accepted 10 December 2003

Time for primary review 20 days

Abstract

Rapid spreading of the electrical impulse throughout the heart is essential for coordinated contraction and is mediated by electrical coupling of cardiomyocytes through gap junction channels composed of connexin40 (Cx40), connexin43 (Cx43) or connexin45 (Cx45). Each of these connexin proteins has a characteristic developmental and regional expression pattern in the heart. Alterations in this pattern may result in abnormal cellular coupling and consequently contribute to irregularities in cardiac rhythm. Indeed, alterations in cardiac connexin expression have been correlated with cardiovascular disease for which the molecular mechanisms, however, are largely unknown. Transcription factors and their target elements in the genome regulate the expression of genes during development and in response to extracellular signals in a cell type-specific and quantitative manner. Altered transcriptional regulation of gene expression is a characteristic feature in the development of cardiac disease which may influence the connexin expression pattern as well. In this review, we will summarize what is known on transcriptional regulation of the Cx40, Cx43 and Cx45 genes in general, with an emphasis on the heart.

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Keywords: Connexins; Gap junctions; Gene expression

1. Introduction

Gap junctions are a collection of specialized plasma membrane channels (gap junction channels), which connect the cytoplasms of adjacent cells, thereby providing both electrical and metabolic coupling. Each gap junction channel consists of two hemichannels (connexons), both composed of six gap junction proteins (or connexins, Cx), that align head to head on apposing cell surfaces to form an intercellular channel [1,2]. In the mammalian heart, gap junctions mediate the rapid conduction of the electrical impulse that precedes coordinated contraction. This electrical impulse originates spontaneously in the sinoatrial node, which is located in the right atrial wall, and sequentially proceeds via atria, atrioventricular node and ventricular conduction system (comprised of His bundle, right and left bundle branches, and Purkinje fibers) towards the ventricles. The products of several connexin genes, e.g. connexin40 (Cx40), −43 (Cx43), −45 (Cx45), −37 (Cx37), −46 (Cx46), and −50 (Cx50) have been detected in heart tissue, but cardiomyocytes predominantly express Cx40, Cx43 and Cx45 (for review, see Ref. [3]). In this review, we will focus on these three myocardial connexins.

The distribution of Cx40, Cx43 and Cx45 in distinct regions of the adult and developing heart has been studied extensively in different mammalian species. The major gap junction protein in the heart is Cx43 which is found, irrespective of the stage of development, in the myocytes of both atria and ventricles. In adult heart, species-specific differences in Cx43 expression have been reported with regard to the ventricular conduction system: in e.g. humans Cx43 is expressed in all parts of the conduction system, whereas in mouse and rat it is restricted to the distal areas (distal part of bundle branches and Purkinje fibers) [3–9]. Regarding its presence in the sinoatrial and atrioventricular nodes, opposite results have been obtained [3,10,11]. In the adult mammalian heart, Cx40 is expressed in the myocytes of atria (with the exception of the rat) and the ventricular conduction system, but not in ventricular myocytes. How-

Abbreviations: E, exon; CNS, conserved noncoding sequence; CDS, protein-coding sequence; UTR, untranslated region; EST, expressed sequence tag; TIS, transcription initiation site; bp, base pair; kb, kilobase

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ever, during fetal and neonatal development Cx40 is detected in ventricular myocytes as well, but its expression decreases and disappears soon after birth [3–9,12,13]. As for Cx43, opposite results have been reported for the expression of Cx40 in sinoatrial nodal cells [3,10,11]. Early in mouse embryogenesis Cx45 is expressed in low amounts in all cardiac compartments, but is then downregulated during further development and finally restricted to some specialized regions in the adult heart. These regions include the ventricular conduction system, sinoatrial and atrioventricular nodes, interventricular septum and small focal parts of the ventricular free walls [10,14–17]. Thus, the developmental and regional expression pattern appears to be characteristic for each connexin and suggests the presence of distinct but tight control mechanisms.

Disruption of cardiac connexin gene expression has profound effects on normal cardiac development and/or function, as is evidenced by gene knockout studies in mice [18–26]. Complete ablation of Cx43 or Cx45 in homozygous null mice leads to premature death due to cardiac malformation of the right ventricular outflow tract [23] or a cushion defect [25], respectively. Mice heterozygous for a Cx43 or homozygous for a Cx40 null mutation develop normally but display abnormalities in cardiac conduction which correlate with a higher incidence of arrhythmias [18–21]. Although in these studies the alterations in connexin expression levels are mostly stretched beyond (patho)physiological limits, the severity of the phenotypes suggests that even less dramatic changes may have a clear impact on cardiac function as well. Cardiac diseases such as atrial fibrillation [27,28], hypertrophy and heart failure [12,29–31] all have been associated with such alterations in the distribution and/or level of connexin expression, but the underlying molecular mechanisms have not yet been elucidated.

Gene expression during development or in response to (patho)physiological signals is frequently controlled at the level of transcription and transcriptional control is expected to play a major role in determining the developmental and cell type-specific expression pattern of cardiac connexin genes as well. A characteristic feature in the development of e.g. cardiac hypertrophy is altered transcriptional regulation [32,33]. Moreover, in several forms of heart disease alterations in connexin protein and RNA levels appear to be correlated [28,29,34]. Therefore, it is expected that the altered regulation of connexin expression observed in the etiology of heart disease is also, at least partially, determined at the level of transcription.

The total transcriptional expression profile of genes, both in time and in space, is controlled through the interaction of transcription factors with specific DNA elements present in the regulatory region of a gene. This gene regulatory region is believed to be composed of independent regulatory modules, each of which consists of a unique combination of transcription factor binding elements and is responsible for part of the total transcriptional program of the gene [35–39]. Therefore, in order to understand the molecular mechanisms controlling cardiac connexin gene expression in health and disease insight into the nature of the involved DNA elements and the transcription factors that interact with them is essential. In this review, we will provide an overview of what is known on Cx40, Cx43 and Cx45 gene structure and on the regulatory elements and transcription factors involved in their expression in the heart and other tissues.

2. Gene structure and comparative genomics

In recent years, the genomic organizations of the Cx40, Cx43 and Cx45 genes have been elucidated by comparison of mRNA and genomic sequences and determination of the transcription initiation site (TIS) [40–49]. The details of the gene structures are summarized in Table 1 and a schematic representation of the organization is shown in Fig. 1. In general, cardiac connexin gene structure is rather simple.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Species</th>
<th>Reference</th>
<th>Chromosomal localization</th>
<th>Exon size (bp)</th>
<th>Intron size (kb)</th>
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</thead>
<tbody>
<tr>
<td>Cx43 mouse [43]</td>
<td>10</td>
<td>E1</td>
<td>189</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>rat [44]</td>
<td>20</td>
<td>E1</td>
<td>195</td>
<td>8.5</td>
<td></td>
</tr>
<tr>
<td>human [45,46]</td>
<td>6</td>
<td>E1</td>
<td>191</td>
<td>11.0</td>
<td></td>
</tr>
<tr>
<td>Cx45 mouse [47,48]</td>
<td>11</td>
<td>E1</td>
<td>359/360</td>
<td>1.4</td>
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<tr>
<td>rat n.a.</td>
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<tr>
<td>E2</td>
<td>73</td>
<td>1.5</td>
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<tr>
<td>E3 5′-UTR</td>
<td>23/21</td>
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<tr>
<td>E3 CDS</td>
<td>1191</td>
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Chromosomal localization was determined by performing a Blast analysis of the appropriate connexin protein coding sequence (CDS) against the corresponding genome database at the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/). Sizes of exon/intron sequences were deduced, if possible, from the indicated references, whereas nomenclature of exons is according to Fig. 1. 5′-UTR = 5′-untranslated region; n.d. = not determined; n.a. = not available.
For all three genes, a small part of the 5'-untranslated region (UTR), the complete coding and 3'-UTR sequences are localized on one exon, whereas the 5'-UTR is divided over one (Cx40, Cx43) or two (Cx45) exons. Different splicing patterns (dotted lines) result in the occurrence of RNA species derived from the following exons: (A) Cx40, E1(A)/E2 (human, rat, mouse) or E1B/E2 (human); (B) Cx43, E1/E2 (human, mouse, rat); (C) Cx45, E1/E2/E3 or E2/E3 (mouse). For Cx40, E1 (mouse, rat) and E1A (human) refer to equivalent regions.

For mouse Cx40, only one transcript has been identified in lung RNA that initiated at the beginning of E1(A) and contained sequences derived from exons E1(A) and E2 (Fig. 1A) [40]. The same transcription start point for the Cx40 gene was also used in rat (adult and neonatal lung, neonatal atrium and the rat aortic smooth muscle cell line A7r5) and human (right atrial appendage) cell types [41,42]. However, investigation of a human placental expressed sequence tag (EST) database suggested the existence of an alternative Cx40 transcript which initiated at the beginning of E1B and consisted of sequences derived from exons E1B and E2, but which lacked sequences from E1(A). Primer extension analysis indeed confirmed the occurrence of this transcript in the human trophoblast cell line BeWo, whereas quantitative real-time RT-PCR analysis revealed expression of both transcripts in the four chambers of the human heart, although transcript E1B/E2 appeared to be expressed at relatively low levels [42]. Thus far, alternative Cx40 transcripts have not been reported in mouse or rat cells.

Fig. 1. Schematic structure and splice pattern of the Cx40 (A), Cx43 (B) and Cx45 (C) genes. Exon (E) sequences are visualized as boxes, whereas the shaded parts represent protein-coding sequences. Positions of reported transcription initiation sites (TIS) and regions with promoter activity (double-headed horizontal arrows) are indicated. For all three genes, the complete coding sequence and 3'-UTR are located on one exon, whereas the 5'-UTR is divided over two (Cx40, Cx43) or even three (Cx45) exons. Different splicing patterns (dotted lines) result in the occurrence of RNA species derived from the following exons: (A) Cx40, E1(A)/E2 (human, rat, mouse) or E1B/E2 (human); (B) Cx43, E1/E2 (human, mouse, rat); (C) Cx45, E1/E2/E3 or E2/E3 (mouse). For Cx40, E1 (mouse, rat) and E1A (human) refer to equivalent regions.

The transcription initiation site for the Cx43 gene has been determined in mouse, rat and human heart tissue and in mouse myometrium [43–46]. For all three species, transcription initiated at the beginning of E1 (Fig. 1B) and the transcript consisted of E1/E2-derived sequences. Up to now, no alternative TIS have been reported.

For Cx45, the organization of the gene has only been reported for the mouse and appears to be different from the Cx40 and Cx43 genes [47,48]. Alignment of mouse genomic DNA and F9 embryonal carcinoma cDNA sequences revealed the presence of three exons and two introns (Fig. 1C). 5'-RACE analysis on F9 and adult mouse kidney RNA indeed confirmed the existence of E1/E2/E3 and E2/E3 transcripts in these cell types but the exact transcription start point remains unclear due to heterogeneity in the 5'-end of the RACE products. Both Cx45 mRNA molecules appeared to be present in the heart as assessed by RT-PCR analysis [47].

Regions in the genome with important biological functions (e.g. protein-encoding and gene-regulatory sequences) are often conserved between evolutionary distant species and, for specific genes, may be uncovered by orthologous genomic sequence comparison [50,51]. We have used the preprocessed whole-human/whole-mouse genome comparison (VISTA Genome Browser; (http://pipeline.lbl.gov/) for the identification of conserved sequences in 31 kb of the Cx40, Cx43 and Cx45 genes (Fig. 2). For Cx40, alignment of the appropriate human chromosome 1 and mouse con-
tig1660 (mapped to chromosome 3) sequences revealed high inter-species conservation between coding (E1(A), E1B and E2) and several noncoding regions (Fig. 2A, black arrows). In particular, the regions directly upstream of E1(A) and E1B, which direct Cx40 promoter activity as will be discussed later on, are highly conserved. Interestingly, Cx40 transcript E1B/E2 has thus far only been identified in human tissue but the strong human/mouse sequence conservation upstream of E1B warrants further investigation towards its role in the mouse as well. Alignment of the Cx43 gene region on human chromosome 6 and mouse contig5116 (mapped to chromosome 10) showed a similar high conservation between coding (E1 and E2) regions and identified several conserved noncoding sequences (CNS) (Fig. 2B, black arrows). As for Cx40, the Cx43 proximal promoter region, which is located directly upstream of Cx43 E1, is one of these CNS. Human (chromosome 17)/mouse (contig5703, chromosome 11) comparative analysis of the Cx45 gene revealed conservation of E2 and E3 sequences and at least one CNS but failed to identify the corresponding E1 sequence in the human genome, indicating that this sequence is not well conserved during evolution (Fig. 2C). Considering the high degree of conservation of proximal promoter regions in the Cx40 and Cx43 genes, it remains to be seen whether mouse Cx45 E1 is preceded by a functional regulatory region. Altogether, this type of comparative genomics may aid in the uncovering of regions involved in transcriptional control of cardiac connexin genes.

3. Transcriptional control of myocardial connexins

The transcriptional regulation of the Cx40 and Cx43 genes has primarily been investigated in vitro by mapping and characterization of the proximal promoters in cell lines or cultured primary cells using promoter/reporter and transcription factor/DNA binding assays. However, phenotypic analysis of mice, in which genes for transcription factors were either overexpressed or disrupted, has provided further insight into their gene regulation. A summary of what is known on Cx40 and Cx43 proximal promoter control is schematically represented in Fig. 3. The promoter of the Cx45 gene, in contrast, has not been identified yet and consequently will not be discussed here.
3.1. Cx40

The mouse Cx40 proximal promoter was mapped in Cx40-expressing smooth muscle- and cardiomyocyte-derived cell lines as the region in between positions -293 and +319, relative to the position of the TIS (defined as +1) (Fig. 3A). Moreover, a strong negative regulatory element was noticed in the (+121, +319) region. However, the nature of the transcription factors and DNA binding sites involved were not investigated [40]. Overexpression of a presumably dominant-negative mutant of the homeoprotein Nkx2.5 in mouse hearts resulted in a dramatic reduction of endogenous Cx40 RNA and protein, suggesting a positive role for Nkx2.5 in Cx40 gene regulation [52]. Bruneau et al. [53,54] also reported a dramatic decrease in endogenous Cx40 protein and RNA expression in the hearts of mice heterozygous for the T-box family transcription factor Tbx5. Indeed, Tbx5, alone or in synergy with Nkx2.5, was shown to activate the mouse promoter contained within a 1010-bp fragment (Fig. 3A) and to bind to at least two T-box binding elements present in this region. However, the exact Nkx2.5- and Tbx5-binding sites responsible for the synergistic activation were not determined. Mice deficient in the transcription factor HF-1b (also known as Sp4) displayed a decrease and mislocalization of endogenous Cx40 protein in cardiac Purkinje cells. Whether this decrease is a direct or indirect effect of HF-1b on Cx40 gene transcription remains to be determined [55].

The rat Cx40 proximal promoter has been mapped and extensively characterized in Cx40-expressing (rat thoracic aorta smooth muscle cells, rat primary neonatal ventricular cardiomyocytes) and -nonexpressing cell types (mouse neuroblastoma cells) [56,57]. The (-175, +85) region was in all instances able to fully direct reporter gene expression, for which five Sp1/Sp3 binding sites (A–E in Fig. 3A) were responsible. Each of these sites was shown to contribute to promoter activity and to bind the transcription factors Sp1 and Sp3 equally well. Trans-activation assays in cells, deficient for transcription factors of the Sp-family, revealed that both Sp1 and Sp3 were able to activate the rat Cx40 promoter. Random disruption of two of the Sp1/Sp3 binding sites almost completely abolished promoter activity. Altogether these results indicate that rat Cx40 proximal promoter activity is, in a cell type-independent manner, determined by the transcription factors Sp1 and Sp3.

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Fig. 3. Summary of regulatory elements involved in transcriptional control of the Cx40 (A) and Cx43 (B) proximal promoter regions. Numbering of regulatory regions is relative to the position of the reported TIS, which is defined as map position +1.
Expression of the human Cx40 gene is controlled at the transcriptional level through two different promoters (Fig. 3A). An approximately 1.2-kb region around E1A (equivalent to the mouse and rat proximal promoter) was shown to direct promoter activity, although at low levels, in human umbilical cord vein endothelial cells (HUVECs) [42]. Activity of this promoter region was confirmed independently in rat thoracic aorta smooth muscle cells using a 275-bp human DNA subfragment [58]. Since this subfragment was equivalent to the rat (−175, +85) region and the mouse proximal promoter, and since four of the five Sp1/Sp3 binding sites within this region are evolutionarily conserved (A, B, C, and E in Fig. 3A), it is likely that Sp1 and Sp3 similarly determine mouse and human proximal promoter activity. Interestingly, the human Cx40 gene contains an additional promoter (1.9-kb fragment around E1B, Fig. 3A) which appears to be active preferentially in throphoblast-derived cells [42]. The molecular mechanism for this cell type-specific behaviour has not been resolved yet.

The transcription factors Sp1 and Sp3 are ubiquitously expressed in a large number of mammalian cell types [59] and presumably provide, together with the general RNA polymerase complex, for a basal level of Cx40 transcription in various cell types. Transcriptional activators or repressors, in cooperation with Sp-family factors and the general transcription machinery, may determine whether Cx40 is expressed in particular cell types or not. Tbx5 and Nkx2.5 may serve such an activating role in the heart since disruption of their expression levels interferes with endogenous cardiac Cx40 protein and RNA levels. Especially the involvement of T-box transcription factors is interesting since this family constitutes both activators (e.g., Tbx5) and repressors (e.g., Tbx2) recognizing the same binding element, several of which are known to be expressed in the mammalian heart [60]. Recently, Habets et al. [61] showed in the developing heart that cooperative action of Nkx2.5 and Tbx2 inhibited ANF gene expression and proposed a potential mechanism for region-restricted ANF expression. In this model, ANF is not expressed in regions where Tbx2 counteracts the activating function of Tbx5; the role of Nkx2.5 is then to restrict the T-box factor effects to cardiac genes. Although the involvement of Tbx2 in Cx40 gene regulation has not been established yet, it is possible that a similar mechanism determines regional-specific expression of Cx40 as well. However, alternative mechanisms for the regional-specific expression of Cx40 are possible as well. As mentioned above, disruption of the HF-1b locus in mice has a negative effect on endogenous Cx40 expression levels. HF-1b is a member of the Sp-gene family that recognizes similar DNA-binding elements as Sp1 and Sp3 [62], and which is preferentially expressed in the mouse cardiac conduction system and ventricular myocytes [55]. Considering the importance of Sp-binding elements in the Cx40 proximal promoter for transcriptional activity, a direct role for HF-1b as an activator of the Cx40 gene in the cardiac conduction system cannot be excluded.

3.2. Cx43

The proximal promoters for the mouse, human and rat Cx43 genes have been mapped in several Cx43-expressing cell types to an evolutionarily conserved region of approximately 150 nucleotides up- and downstream of the TIS (Figs. 2 and 3B) [44,63–67]. Within this region, four evolutionarily conserved Sp-binding sites (1–4, Fig. 3B) and one AP1-binding element (site 2, Fig. 3B) are located; the rat Cx43 promoter contains an additional AP1-binding element (site 1, Fig. 3B) which is absent in the mouse and human genes. In myometrial smooth muscle cells, both a positive and a negative regulatory DNA element have been identified in the mouse Cx43 promoter, which were capable of binding to as yet unidentified nuclear proteins [63]. For the human Cx43 proximal promoter, it was demonstrated by promoter/reporter assays and Sp1/AP1 overexpression studies that both Sp1 and AP1 are necessary as transcriptional activators for optimal promoter activity [64]. Sp-binding element 4 and AP1-binding site 2 appeared to be most important for this transcriptional activation. The rat Cx43 proximal promoter has been extensively studied in rat primary neonatal cardiomyocytes, thoracic aorta smooth muscle and normal kidney cells [44,66,67], which all are known to express Cx43. Each of the Sp- and AP1-binding sites was shown to contribute to promoter activity and to bind the transcription factors Sp1/Sp3 or AP1, respectively. In trans-activation assays, Sp1 and Sp3 were both able to activate the rat Cx43 promoter. Within the rat Cx43 promoter, a negative regulatory element, as detected in the mouse, was not identified; however, the mouse “activator” might very well correspond with one of the Sp1/Sp3-binding elements in the rat promoter. Altogether these results indicate that rat Cx43 proximal promoter activity is determined by the transcription factors Sp1, Sp3 and AP1. Interestingly, rat proximal promoter activity could hardly be detected in mouse neuroblastoma cells, which correlated with the lack of endogenous Cx43 RNA and protein expression in these cells, suggesting some cell type-specificity. These results may be explained by the absence of Sp1, Sp3 and/or AP1 expression or the presence of a neural-specific repressor in the neuroblastoma cells.

Because of the similarities in proximal promoter regulation by ubiquitously expressed transcription factors (Sp1, Sp3, AP1) in different Cx43-expressing cell types (including cardiomyocytes), it is likely that cell type-specific expression of Cx43 depends on additional activators or repressors. Several studies have provided evidence that Nkx2.5 may serve such an additional role for Cx43 expression in the heart. As for Cx40, reduced Cx43 protein and RNA levels were noticed in mice overexpressing a putative dominant-negative mutant of Nkx2.5 in the heart, suggesting an activating role for this homeoprotein [52]. However, mice overexpressing wild type Nkx2.5 in the heart also displayed reduced Cx43 expression, suggesting that Nkx2.5 may act as a transcriptional repressor of Cx43 as well.
In agreement with the latter study, we have shown that adenoviral-mediated overexpression of Nkx2.5 in rat neonatal ventricular myocytes results in a dramatic decrease of endogenous Cx43 protein and RNA levels and a two-fold drop in rat Cx43 proximal promoter activity [67]. The drop in promoter activity could not completely account for the observed reduction in protein/RNA, suggesting the involvement of more distal regulatory regions as well. Thus, Nkx2.5 appears to be able to act as an activator as well as a repressor of Cx43 expression; the precise molecular mechanism has not been elucidated yet. transcriptional cofactors, such as members of the T-box gene family, may determine whether Nkx2.5 acts as an activator or as a repressor. Indeed, Tbx2 has been identified as a negative regulator of Cx43 expression at the transcriptional level [70–72], but the effect of other T-box family members on Cx43 expression has not been reported.

Besides knowledge on Cx43 gene structure and proximal promoter regulation insight has also been gained on signaling events affecting Cx43 transcription in cardiac and noncardiac cells. In human primary myometrial cells, Cx43 transcription and proximal promoter activity were increased upon activation of protein kinase C with the phorbol ester TPA suggesting the involvement of the protein kinase C pathway in the up-regulation of myometrial Cx43 at the onset of labor [46]. TPA was further shown to upregulate and activate c-jun and c-fos, the molecular constituents of AP1, which exert their inducing effect on Cx43 proximal promoter activity through AP1-binding site 2 (Fig. 3B). Responsiveness of Cx43 transcription and/or proximal promoter activity has also been shown to prostaglandin E₂, parathyroid hormone and 8Br-cAMP in osteoblastic cells, to the thyroid hormones T₃ and T₄ in liver cells, to the Ras-signaling pathway in fibroblasts and to the Wnt-signaling pathway in neural crest-derived cells [73–77]. The responsive element for parathyroid hormone has been mapped to the (−31, +1) region, relative to the TIS, and the responsiveness in bone cells of both endogenous Cx43 and its proximal promoter was confirmed in transgenic mice carrying a 1.8-kb Cx43 proximal promoter/reporter construct; the transcriptional effectors of parathyroid hormone induction, however, have not been reported [74]. The thyroid hormone responsive element has been characterized as the (−480, −464) region, and binding of a heterodimer of the retinoid X receptor α/thyroid hormone receptor α to this element was demonstrated [75]. In mouse fibroblasts, the (+149, +158) region has been identified as the binding site for the heat shock protein HSP90 and c-myc, which mediate the transcriptional up-regulation of Cx43 by the Ras-Raf-MAPK pathway [76]. In cardiac myocytes, activation of the Wnt-signaling pathway and dibutyryl-cAMP were shown to induce Cx43 protein and RNA expression [78,79]. These responses appear to be transcriptionally regulated, since Cx43 proximal promoter activity increases correspondingly with treatment [77,78]. Although the Cx43 proximal promoter contains evolutionary conserved cyclic AMP and TCF/LEF (the transcriptional effectors of Wnt-signaling) binding elements, the precise molecular mechanism for induction has not been elucidated.

In contrast, activation of c-jun N-terminal kinase (JNK) resulted in the downregulation of Cx43 RNA and protein, both in transgenic mouse hearts and cultured cardiomyocytes [80]. As mentioned above, AP1 is not only an activator of the Cx43 proximal promoter in several different cell types, including cardiomyocytes, but also well known as a downstream target of JNK. Further studies are evidently necessary to resolve this discrepancy. Altogether these studies illustrate that the Cx43 gene regulatory region is the target of diverse signaling events in different cell types, but that the precise molecular mechanisms and signal transduction molecules involved still have to be elucidated.

4. Myocardial connexin gene expression in perspective

This review gives an overview of our current knowledge on cardiac connexin transcriptional control. With the elucidation of gene structures and the in vitro characterization of proximal promoters, the exploration of this research field has just begun. Although the regulatory regions and transcription factors essential for Cx40 and Cx43 proximal promoter activity in cultured cardiomyocytes have now been defined, it is unlikely that these are sufficient to provide for their complex developmental and regional-specific expression patterns in the heart. It becomes increasingly clear that the expression of a particular gene in different compartments of the (developing) heart is controlled by different regulatory modules, which respond to distinct sets of transcription factors and which may be separated several kb of each other, in the gene’s complete regulatory region. To identify such modules, in vivo studies are essential in which the expression pattern of a reporter gene, brought under control of the regulatory region of the gene of interest, is compared with the endogenous pattern of the gene in mice. Comparative genomics to identify conserved noncoding sequences, such as shown in Fig. 2 for cardiac connexin genes, may aid in targeting potentially important regions. After identification of these modules, the regulatory elements and transcription factors involved need to be further characterized by both in vitro and in vivo approaches. For the Cx43 regulatory region, such in vivo studies have only been performed to map the regions essential for parathyroid hormone responsiveness to a 1.8-kb [74] and for expression in neural crest cell lineages in the mouse embryo to a 6.8-kb promoter fragment [81,82].

The level of myocardial connexin expression in particular cell types is most likely determined in more than one way. The alternative use of 5’-exons to generate different transcripts, as has been reported for the human Cx40 [42]
and mouse Cx43 genes [47] (Fig. 1), implies the presence of more than one promoter which may or may not be used in a cell type-specific manner. In rat liver cells, DNA methylation of the Cx43 proximal promoter has been correlated with the absence of Cx43 expression [83]. However, post-transcriptional control mechanisms have also been implicated in Cx43 expression. The presence of an internal ribosome entry site (IRES) in the 5′-UTR of Cx43 mRNA has been suggested to be important for the maintenance of Cx43 expression under stressful conditions [84]. Parathyroid hormone and retinoic acid have been shown to influence protein expression levels via sequences located in the 3′-UTR of Cx43 mRNA [74,85], influencing either mRNA stability or the rate of translation. Although regulation at the transcriptional level is expected to be a major determinant in myocardial connexin gene expression, other mechanisms clearly may influence the level of expression as well.

Acknowledgements

We are grateful to our colleagues in the Department of Medical Physiology for technical support and valuable discussions. The Netherlands Heart Foundation (grant 99.088) and The European Community (contract #QLG1-CT-1999-00516) have supported our studies.

References


