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Review

Patterns of expression in the developing myocardium: towards a morphologically integrated transcriptional model

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1. Introduction

The heart is the first embryonic organ to function. Early in development, the heart shows autorhythmycity and peristaltoid contraction waves [1,2]. Contraction requires the expression of a specific set of proteins that form the contractile apparatus, i.e. the sarcomere. The contraction–relaxation cycle of the sarcomeric apparatus is mediated by changing local concentrations of free calcium. This function is achieved by another set of specific proteins, located in the sarcoplasmic reticulum and in the sarcolemma.

Fascinating questions that are still poorly understood are how the cardiogenic lineage becomes established to form the peristaltoid contracting tube without valves and how this tube becomes transformed into the synchronous-contracting four-chambered heart with unidirectional valves. It is well documented that the expression of the different isoforms of contractile proteins changes considerably during these stages (for a review see [3]). However, a detailed analysis of the changes in the patterns of gene expression in relation to cardiac morphogenesis is lacking. In the present review we try to fill this gap. We have centred our attention on gene products (mRNA and protein) expressed in the working myocardium of mammals and birds. No distinction has been made when mRNA and protein display the same pattern of expression, however we have highlighted those cases where the pattern of expression differs between mRNA and protein. The development and expression pattern of genes of the conduction system of the heart merits an independent review [4] (Moorman et al., Circ. Res., in press). Data referring to other experimental models as *Drosophila*, *Xenopus* or zebrafish (*Danio rerio*) are included only if they are helpful for our general understanding. Often only gene expression in the presumptive atria and/or presumptive ventricles is mentioned, whereas understanding the functional significance of the patterns of gene expression requires knowledge of the entire pattern including the inflow tract, atrioventricular canal and outflow tract. In those cases we have included complementary data from our laboratory, if available. The expression patterns of gene products in other tissues as the mesenchymal cells and cardiac neural crest are not covered in this review. Excellent reviews exist on these topics [5–7].

Patterns of expression have been described in three prototypical developmental stages (see for staging [8]): (i) *tubular heart (Fig. 1A)*, Carnegie stage ~9 (Human: embryonic day (E) 20; Mouse: E8; Rat: E10; Chicken: stage 10 (Hamburger and Hamilton (H/H) [9]), (ii) *looped, segmented heart (Fig. 1B)*, Carnegie stage ~012 (Human: E28; Mouse: E10.5; Rat: E12; Chicken: stage 18 (H/H)), and (iii) *septated, fetal heart (Fig. 1C)*, Carnegie stage ~23 (Human: E60; Mouse: E16; Rat: E17.5; Chicken: stage 38 (H/H)). Table 1 presents the different mRNAs/proteins included in this review and the species in which the expression patterns have been studied. Secondly, we have compiled the recent data on transgene expression during heart development as far as available (see Table 2). The analysis of such patterns of expression and their relationship with the expression of cardiac-muscle specific transcription factors have been summarised in a model of developmentally regulated cardiac gene expression.

2. Morphogenesis of the heart

The heart originates from the splanchnic mesoderm. Two primordia of epithelial cells (precardiac mesoderm or

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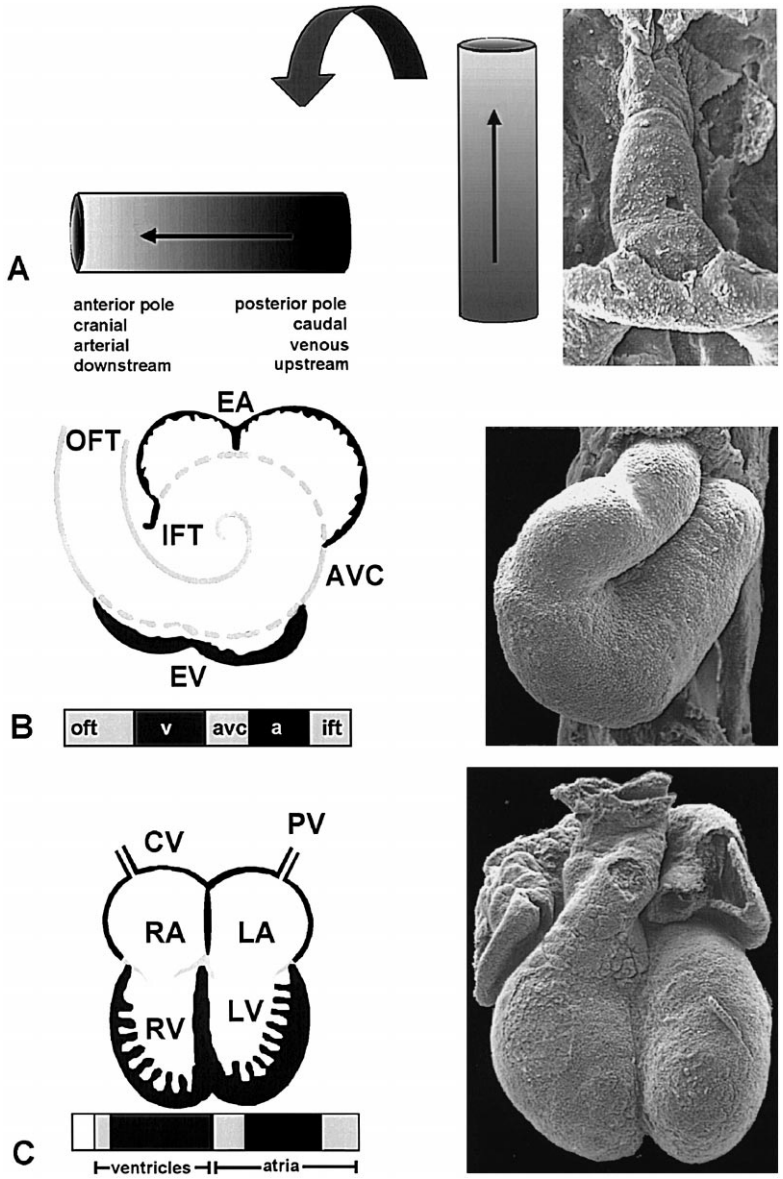


Fig. 1. Cardiac morphogenesis. Schematic representation of three different prototypic stages of cardiac development, i.e. tubular heart stage (A), segmented (embryonic) stage (B) and septated (fetal) stage (C) corresponding to approximately a similar stage of the scanning electron microscopical illustrations of the chicken (A,B) and human heart (C). No morphological boundaries can be distinguished in the tubular stage. Five morphologically identifiable segments can be delimited in the segmented heart, i.e. inflow tract (IFT), embryonic atria (EA), atrioventricular canal (AVC), embryonic ventricles (EV) and outflow tract (OFT). The atria and ventricles are septated into right and left components in the fetal stage. The outflow tract partially disappears and partially becomes incorporated into the ventricular chambers. The atrioventricular canal and the inflow tract become incorporated into the atrial chambers. Thus, the flanking segments are no longer identifiable morphologically in the fetal heart. RA, right atrium; LA, left atrium; RV, right ventricle; LV, left ventricle; CV, caval veins; PV, pulmonary veins.

cardiac crescent) are observed at both sides of the antero-posterior axis of the embryo that eventually will fuse in the midline of the body. As the tubular heart is formed, two epithelial layers are observed, the myocardium and the endocardium, which are separated by an acellular matrix, the cardiac jelly (this stage is illustrated in Fig. 1A). This initial layer of myocardium has been dubbed 'primary myocardium' [10].

With further development, the heart loops towards the right side. Subsequently the atrial and ventricular segments become recognisable. Five different segments can be distinguished: two fast-contracting segments, the atria and the ventricles, and three slow-contracting segments, the outflow tract, the atrioventricular canal and the inflow tract [10]. The region of the myocardium that is located upstream of the atria has been dubbed inflow tract. Thus, inflow tract refers to the sinus horns in the embryonic heart and, later in development, to those structures derived from it and those newly formed in the venous pole of the heart. At this stage, the ventricles are characterized by a

Table 1
List of proteins/mRNAs per species with distinctive patterns of expression in the separate cardiac compartments. R, rat; M, mouse; C, chicken; H, human; Mq, macaque.

Gene product	mRNA				Protein				
	R	M	C	H	R	M	C	H	Mq
αMHC	[a]	[3,23]	[19,20,27]		[11,22]	[a]	[17,18,25]	[24]	
βMHC	[a]	[3,23]	[20,21,27]		[11,22]	[a]	[17,18,25]	[24]	
MLC1a		[3,23]							
MLC1v	[41,42]	[3,23,41]							
MLC2a	[41]	[36,41]							
MLC2v	[39,41,42]	[3,38,41]							
MLC3f		[32,33]							
skeletal α-actin	[129]	[49,129]	[48]	[a]					
cardiac α-actin	[129]	[49,129]	[48]	[a]					
α-SMA	[129]	[129]	[48]		[11]				
tropomyosin			[57]						
tropomodulin			[55]		[54]				
slow TnI	[67]								
cardiac TnI	[66,67]	[a]			[67]				
fast TnI		[68]							
Calponin		[74,75]			[11]				
SERCA2	[91,92]								
PLB	[92]		[99]						
RyR2	[105]								
RyR3	[105]								
NCX	[a]								
NaK-ATPase α1		[110]							
NaK-ATPase α2		[110]							
NaK-ATPase α3		[110]							
Cx40	[113,115]	[112]			[113,115]				
Cx43	[113,115]	[114]			[115,116]	[114]			
MCK	[a]	[124]	[124]		[125]		[126]	[127]	
BCK		[124]	[124]		[125]		[126]	[127]	
Desmin	[129]	[129]			[11]	[130]			
α-5 integrin						[140]			
α-6 integrin		[138]					[141]		[139]
β-1 integrin	[136]								[139]
N-cadherin		[147]	[144,146]						
ANF	[186]	[187]			[189]				
AChE	[196]				[192,193]		[191]		
Nkx2.5		[37,242]							
cNkx2.8			[175,176]						
GATA-4		[167,170]	[165]						
GATA-5		[177]	[165]						
GATA-6		[171]	[165]						
MEF2A		[173]							
MEF2C		[173]							
MEF2D		[173]							
dHAND		[169,181]	[155]						
eHAND		[169,179–181]	[155]						
CARP		[183]							
SRF			[185]						
pCMF1			[184]						

[a] Data presented in this review.

trabeculated morphology (this stage is illustrated in Fig. 1B).

With further development, septation of the atria and the ventricles proceeds, and right and left components can be recognised in the atrial and ventricular segments. At the same time, the ventricles acquire a compact myocardial morphology, starting in the interventricular septum, followed by the left ventricle and subsequently the right ventricle [11]. The latest event concerning septation of the heart occurs in the outflow tract region where two endocardial ridges fuse and each ventricle, right and left, acquires an independent outlet connection (this stage is illustrated in Fig. 1C). At the end of the septation process, part of the outflow tract myocardium disappears and part becomes

Table 2
Summary of the genes and their regulatory sequences studied by means of transgenic mice during embryonic cardiac development (see Fig. 8 for illustrations).The reporter gene (R.gene), the sequence tested in vivo and the overall expression in the fetal stages is depicted. If published, candidate *cis*-acting elements and their binding proteins directing gene expression in the heart are also included. A, atria; V, ventricles; RA, right atrium; RV, right ventricle; LV, left ventricle; AVC, atrioventricular canal; OFT, outflow tract; IFT, inflow tract; CAT, chloramphenicol aminotransferase; *nlacZ*, β-galactosidase with nuclear localisation signal; *lacZ*, β-galactosidase

Gene	R.gene	Sequence	Expression	References
αMHC	CAT	5.5 kb upstream	A + V	[198]
αMHC[a]	*lacZ*	5.5 kb upstream	A + V	[199]
βMHC	CAT	2.5 kb upstream	A + V	[200,201]
MLC3f	*nlacZ*	2 kb upstream + 3′ enhancer	RA + LV	[32]
MLC3f	*nlacZ*	9 kb upstream	A + AVC + C	[13]
MLC2v	*lacZ*	280 bp upstream	OFT + RV	[118]
MLC2v	*lacZ*	28 bp (HF1a/HF1b/MEF-2)	OFT + RV	[118]
cardiac α-actin	*nlacZ*	3 kb upstream	A + V	[117]
cardiac α-actin	*nlacZ*	12 kb upstream	RA + LV	[117]
slow TnI	CAT	4.2 kb upstream	IFT + V	[68]
Desmin	*lacZ*	1 kb upstream	OFT + RV	[119]
SM22α	*lacZ*	2 kb upstream	OFT + RV	[213,214]
α B-crystallin	*lacZ*	4 kb upstream	OFT + RV	[215]
dystrophin	*lacZ*	900 bp upstream	OFT + RV	[216]

[a] The expression pattern of α-MHC-lacZ transgene is confined to the atrial myocardium in the embryonic and fetal stages and becomes up-regulated in the ventricular myocardium postnatally.

incorporated into the right ventricular infundibulum [12,13]. The atrioventricular canal and the inflow tract become incorporated into the atrial cavities.

3. Patterns of gene expression

The differential distribution of those genes whose patterns of expression are documented in the literature are schematically represented in Fig. 2, in three different stages of heart development.

3.1. Contractile proteins

3.1.1. Myosin heavy chains (MHCs)

In the mammalian heart, two myosin heavy chain isoforms are expressed during development, α-MHC and β-MHC, each encoded by a single gene [14,15] (see for review [16]). In chicken, at least three different MHC isoforms have been isolated, two of them are expressed in the atrial myocardium [17–20] and the third is mostly restricted to the ventricular myocardium [17,18,21].

In the early tubular stages of development, α-MHC and β-MHC are co-expressed along the myocardium in mice, rats, chicken and human [3,17,22–24]. α-MHC is higher in the atria/inflow tract and progressively decreases towards the ventricle/outflow tract region. β-MHC is highest expressed in the outflow tract/ventricle and decreases towards the atria/inflow tract. In rodents, β-MHC protein is almost homogeneously distributed whereas α-MHC protein shows a clear gradient of expression [22].

In the looped heart, α-MHC becomes restricted to the atria/inflow tract and β-MHC to the ventricles/outflow tract in rodents [3,23] (our own observations). Co-expression of both proteins is observed in the myocardium of the atrioventricular canal [17,22,24]. The IVS is depleted of α-MHC, whereas the trabeculated ventricular component still shows a moderate intensity of expression. On the other hand, β-MHC expression is mostly restricted to the ventricular/outflow tract compartments with just detectable levels in the atrial myocardium (Fig. 3A). No differences between IVS and trabeculated ventricles can be observed. Interestingly, α-MHC expression is transiently maintained in the outflow tract myocardium of chicken, rat and human [11,17,24]. α-MHC protein remains moderately expressed in the trabeculated component compared to the developing compact myocardial layers (i.e. ventricular free walls) during the late embryonic and fetal stages in rodent, avian and human embryos [11,17,22,24].

In the fetal stages, α-MHC is expressed in the atrial myocardium, caval veins, coronary sinus, interatrial septum and in the myocardium of the pulmonary veins. In rat and mouse, the trabeculated part of the developing ventricular myocardium still expresses α-MHC protein. β-MHC becomes restricted to the outflow tract and ventricles with no difference between the compact myocardium and the trabeculated component.

A more complex situation is observed in chicken where several atrial MHC isoforms, but only one ventricular isoform, are expressed [25,26]. Current data support the notion that a distinct MHC isoform is mostly confined to the presumptive atrial myocardium [21] whereas another atrial isoform displays a gradient along the cardiac tube [17]. Indeed, two atrial MHC mRNA isoforms have been identified, AMHC1 restricted to the venous pole of the heart [20] and CCSV2 highly expressed in the atrial myocardium and at lower levels in the ventricles [19]. This

fact explains the reports on atrial MHC gradients by some authors [17] and atrial-restricted MHC expression by other groups [20,21,25,27].

In summary, the expression of MHCs (mRNA/protein) in mammals shows a prototypical pattern of expression with anteroposterior gradients of gene expression in the tubular stage. With development, MHC isoforms become confined to atrial or ventricular myocardium, and co-expression is found transiently in the flanking segments (i.e. atrioventricular canal myocardium and outflow tract). In the late embryonic and fetal stages, α-MHC expression remains higher in the ventricular trabeculations than in the compact myocardium during similar stages of development in different species. This phenotype confers an 'atrial'-like characteristic to the trabeculations that may result in a faster contraction capacity than the compact myocardium.

3.1.2. Myosin light chains (MLCs)

The myosin light chains are classified in two groups, alkaline (or essential) and regulatory (or phosphorylatable) [28]. Five major alkali MLCs have been cloned in striated muscle. Two fast isoforms (MLC1f and MLC3f) are encoded by a single gene and result from alternative splicing [29–31]. MLC3f mRNA but not MLC1f mRNA is expressed in the developing mouse heart [32,33]. There are two slow essential MLC isoforms, MLC1v and MLC1sa. MLC1v is expressed in adult slow skeletal and cardiac muscle and is confined principally to the ventricular myocardium. MLC1sa is expressed in adult slow skeletal muscle, smooth muscle and non-muscle tissues [34]. Another isoform, MLC1a (also dubbed embryonic) is expressed in embryonic skeletal muscle and in the heart, mostly restricted to the atrial myocardium [23]. Three isoforms of the regulatory myosin light chain have been reported to be expressed in striated muscle. MLC2a is confined to the atrial myocardium, MLC2v (slow) is expressed in the ventricular myocardium and slow-twitch fibres, and MLC2f is expressed in fast skeletal muscle and transiently in the embryonic heart [35].

During the tubular stages of development, MLC1a, MLC1v and MLC2a mRNAs are expressed along the myocardium of the entire heart tube [3,23,36]. MLC2v mRNA has been reported to be exclusively expressed in the presumptive ventricular myocardium in mice [37,38] and is detectable from E8.5 [37] in mouse and E10 in rat [39]. With development, the different MLC isoforms become confined to a distinct compartment in mouse and rat [3]. In the looped heart MLC1a and MLC2a mRNAs are higher expressed in the atrial/inflow tract area, whereas MLC1v and MLC2v mRNA are predominant in the ventricular/outflow tract regions [3,23,40]. Interestingly, the regulatory MLC2a and MLC2v remain expressed, albeit in lower levels, in the slow-conducting, flanking segments, i.e. inflow tract, atrioventricular canal and outflow tract (Fig. 3B) [39,42]. The expression of MLC2f mRNA is transiently detectable at E9.5 in the mouse, however no description of its expression pattern is reported [35]. MLC3f mRNA is expressed in the atrial/inflow tract, in the ventricles and, at lower levels in the atrioventricular canal, but not in the outflow tract. It also shows higher level of expression in the atrial than in the ventricular myocardium [32]. MLC1a, MLC2a and MLC3f mRNAs show higher expression in the trabeculations than in the IVS and in the compact layer of the ventricular free wall in rodents (Fig. 4A, C; Kelly R, Zammit P, Buckingham M, in preparation). On the other hand, MLC1v and MLC2v mRNAs show a low expression in the trabecular component of the ventricles (Fig. 4B, D) [41].

In the fetal stages, MLC1a and MLC2a mRNAs show a similar pattern to that observed for the α-MHC; that is, mostly restricted to the atrial/inflow tract domains, i.e. the atrial myocardium, the interatrial septum, the coronary sinus and the myocardium surrounding the caval and pulmonary veins. MLC2a mRNA remains expressed in the areas derived from the embryonic outflow tract [42]. MLC1v mRNA is expressed exclusively in the ventricles and in the outflow tract-derived myocardium, whereas MLC2v mRNA displays high expression in the ventricular myocardium and low levels of expression in the outflow tract-derived myocardium [42], in the lower rim of the atria and in the interatrial septum [39]. Remarkably, the 'fast/atrial'-MLC isoforms (MLC1a, MLC2a and MLC3f) are higher expressed in the trabeculations than in the compact myocardium and conversely, the 'slow/ventricular'-MLC isoforms (MLC1v and MLC2v) are higher expressed in the compact myocardium than in the trabeculations (Fig. 4).

In summary, MLC2a shows a gradient in expression in the early stages of heart development, whereas MLC1a and MLC1v are homogeneously expressed. No data are available about the precise topographical distribution of MLC2v and MLC3f mRNAs in the tubular heart. In the looped heart, MLC2a and MLV2v mRNAs become confined preferentially to the atria and ventricles, respectively. Co-expression of both mRNAs is observed in the flanking segments, i.e. inflow tract, atrioventricular canal and outflow tract. Thus, the flanking, 'primary myocardium' represents a transcriptional domain that is distinct from the atrial and ventricular transcriptional domains.

3.1.3. Myosin-binding proteins (MyBPs)

Several myosin binding proteins have been described, namely, C-protein, X-protein and M-protein, each type localized in a distinct region of the sarcomere [43,44]. At present, cardiac MyBP-C mRNA has been found in adult cardiac muscle but not in skeletal muscle. Its developmental pattern of expression is unknown.

3.1.4. Actins

Three different actin isoforms are expressed through the development of the heart, each encoded by a single gene

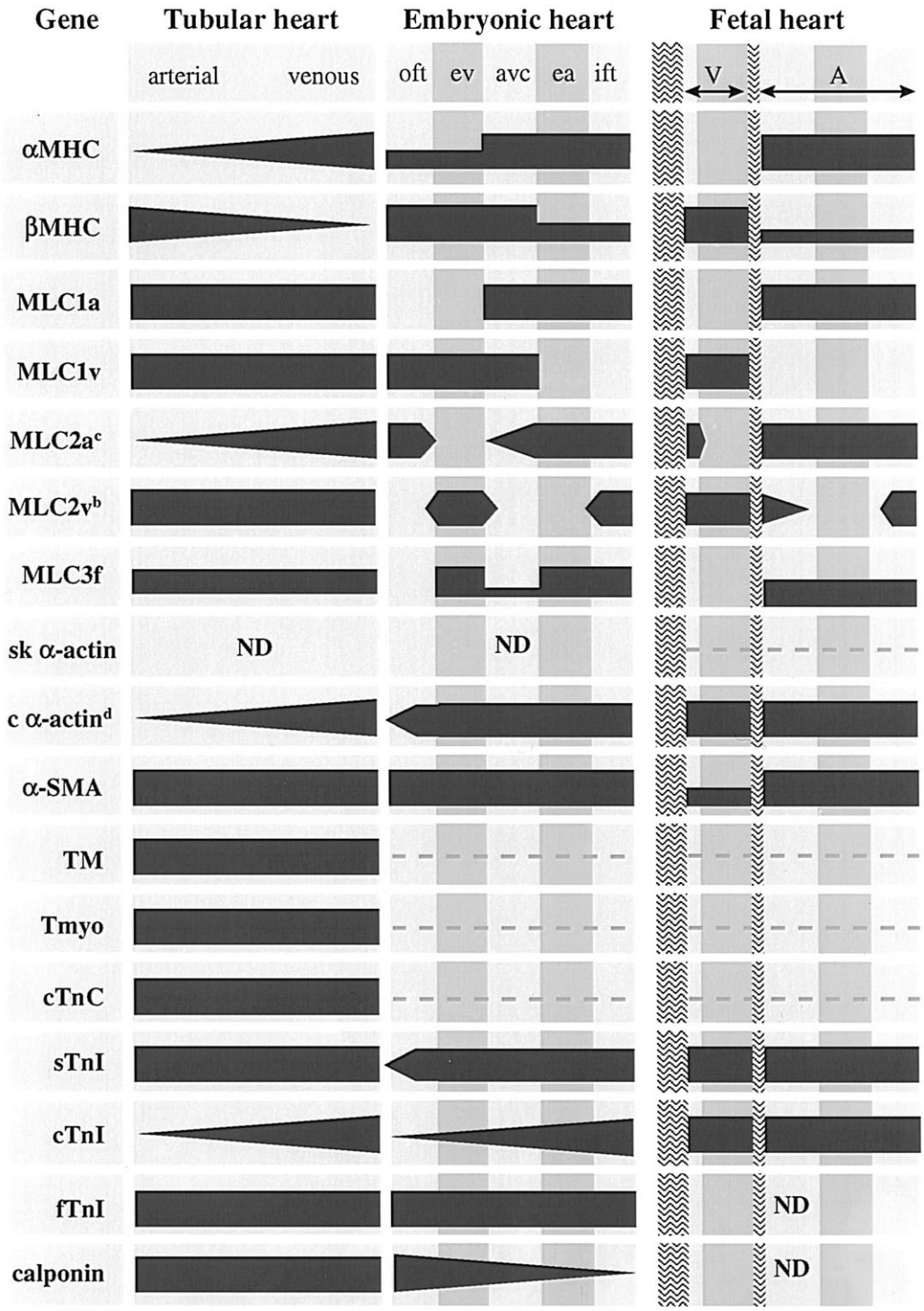


Fig. 2. Gene expression in the developing heart. The patterns of expression of the genes discussed in this review are schematically represented in the three prototypic stages of cardiac development (see Figure 1). Note that the levels of expression of a certain gene are only comparable within one developmental stage. Below the levels of detection is depicted as a dashed line. (a) MCK, Cx43 and Cx40 show differences in the expression pattern of the mRNA and its protein. Furthermore, MCK shows species-specific differences (see e.g. [125,127]). (b) MLC2v mRNA has been described to be confined to the ventricular myocardium prior to its morphological identification in the tubular heart [37,38]. We think that MLC2v mRNA is expressed along the entire cardiac tube that will, however, contribute mostly to the ventricles, outflow tract and atrioventricular canal [12]. Consequently, MLC2v mRNA is mostly expressed in the ventricular myocardium but it also extends towards the outflow tract, atrioventricular canal and part of the inflow tract (i.e. the dorsal roof of the atria) at the embryonic heart stage (see Figure 3). (c) Gene products as MCK, Cx40, MLC2a and ANF show a differential transient expression within the right and left ventricular compartments. (d) Our interpretation of the illustrations shown by Sassoon et al. [49] is that cardiac α-actin mRNA shows a postero-anterior gradient of gene expression. The authors noted a heterogeneous expression but did not describe a gradient. (e) The expression of ANF mRNA in the ventricles is almost confined to the trabeculations. In the fetal heart, ANF mRNA expression is low in the ventricles, the caval veins, and pulmonary veins and high in the atrial myocardium. ND; not determined.

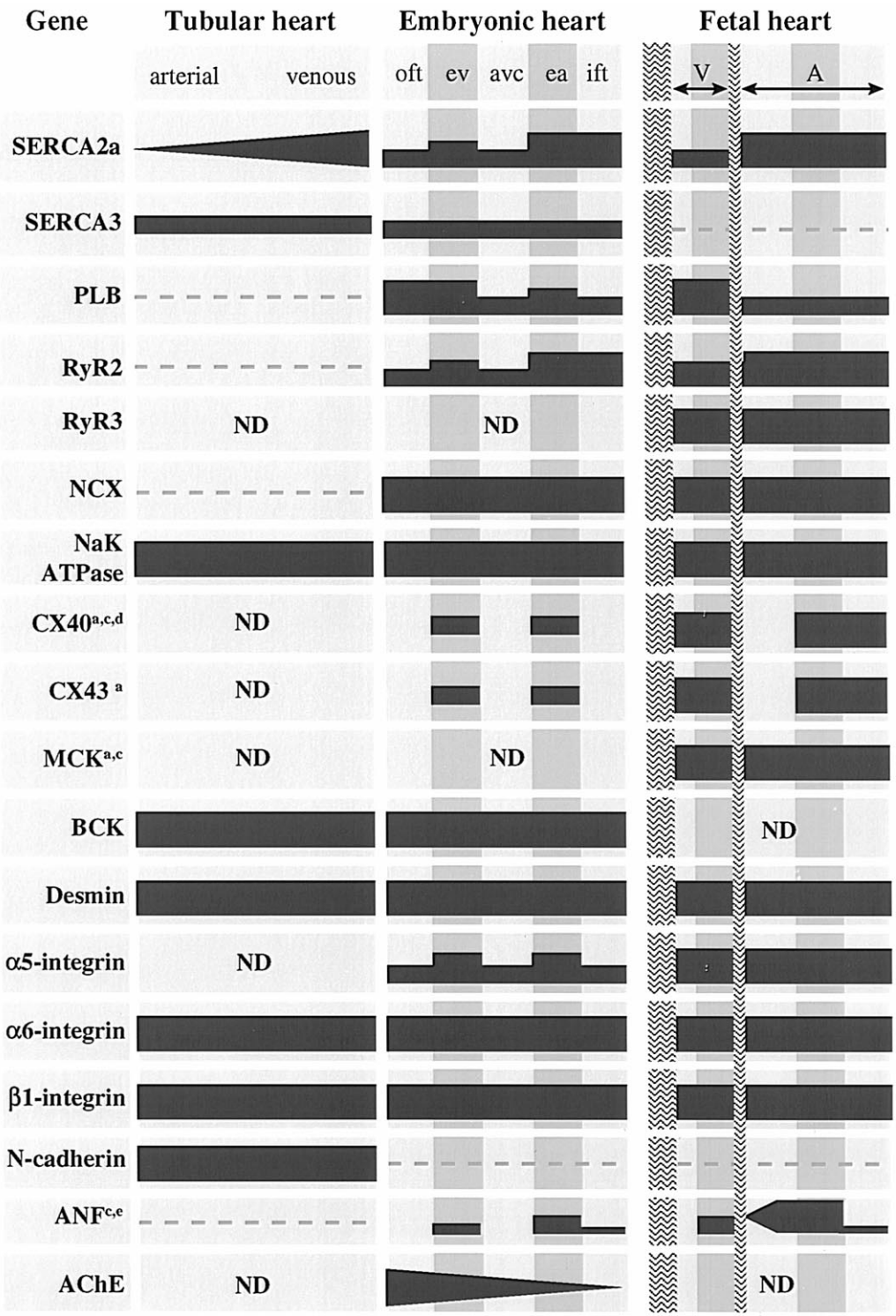


Fig. 2 (continued).

[45–47]. These comprise the two sarcomeric actins, α-skeletal actin and α-cardiac actin, and the smooth-muscle α-actin (α-SMA). The expression patterns of the actin isoforms have been studied in detail during early embryogenesis of mouse and chicken [48–50]. Sassoon et al. [49] demonstrated that cardiac α-actin is expressed from the cardiogenic plate stage onward in mice. Skeletal α-actin was detectable later in development only. Cardiac α-actin shows a gradient of expression from the inflow (venous pole, higher expression) to the outflow tract (arterial pole, lower expression). This gradient remained unnoticed in the literature owing to a mislabelling of the outflow tract as

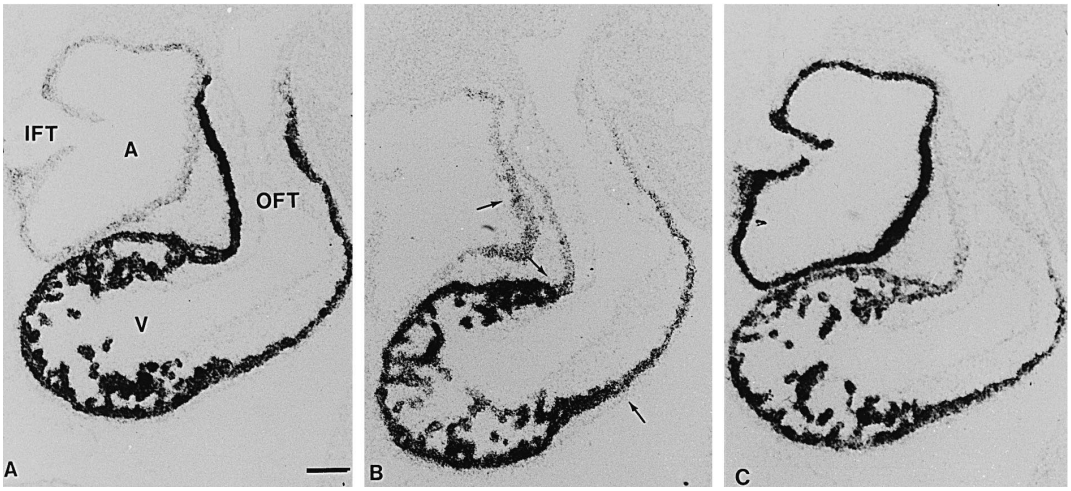


Fig. 3. Serial sections of an E12 rat heart (embryonic stage) hybridized with cRNA probes against β-MHC mRNA (A), MLC2v mRNA (B) and cardiac troponin I mRNA (C). β-MHC mRNA expression is mostly confined to the ventricular/outflow tract myocardium (V) but displays detectable levels in the atrioventricular canal (not in the photograph), the atrial (A) and inflow tract (IFT) myocardium. MLC2v mRNA is mostly confined to the ventricular myocardium but it also expressed at low levels in the outflow tract and the atrial myocardium with a steep boundary (arrows). Cardiac troponin I mRNA shows a postero-anterior gradient of gene expression, with higher expression in the inflow tract and lower levels of expression in the proximal outflow tract myocardium. No expression is observed in the distal outflow tract myocardium, which is in line with the findings of Gorza et al. [67]. Hybridization conditions were performed according to Moorman et al. [205] with slight modifications (see [13]). The cDNAs probes used are those published in Boheler et al. [240] (β-MHC), O'Brien et al. [38] (MLC2v) and Ausoni et al. [66] (cardiac TnI), respectively. Bar = 100 μm.

the embryonic atrium [49]. Ruzicka and Schwartz [48] have shown that in early stages of avian cardiac development, α-SMA is first expressed in the myocardium, followed by the expression of cardiac α-actin. Low levels of skeletal α-actin were also detected. With further development, α-SMA starts to disappear gradually from the myocardium (H/H 12) and is replaced by the sarcomeric isoforms. α-SMA remains exclusively expressed in the outflow and inflow tract in chickens [48,50]. During the late embryonic stages of development, cardiac α-actin is expressed in all cardiac compartments, with a slightly lower staining intensity in the trabeculations than in the compact myocardium in rats. No expression of skeletal α-actin was observed during these stages (D. Franco, unpublished data).

In the septated heart, a difference in staining intensity in the trabeculations can be observed when compared to the compact myocardium. α-SMA protein remains highly expressed in the trabecular component of the embryonic ventricular compartment resembling, at this time-point, the atrial myocardium phenotype in rodents [11]. In man, expression of skeletal α-actin mRNA is only observed in the fetal heart (Carnegie stage 23) and is restricted to the papillary muscle whereas cardiac α-actin is homogeneous (Fig. 5). [51].

In summary, a switch of actin isoform expression is observed during mammalian and avian heart morphogenesis. At comparable developmental stages, α-SMA becomes replaced by cardiac α-actin. α-SMA is initially expressed in the early myocardial cells and progressively becomes confined to both cardiac poles, when new myocardium is still added to the cardiac tube. In the fetal stages of development, α-SMA protein is higher expressed in the trabeculations than in the compact myocardium. A complementary pattern is observed for cardiac α-actin mRNA.

3.1.5. Tropomodulin (TM)

Most of the studies of tropomodulin have been devoted to its localization within the sarcomere [52–54]. Its expression pattern in the heart has been confined during early stages of mouse embryogenesis only, where it is homogeneously expressed along the entire tubular heart [55]. At present, data from later developmental stages are insufficient to allow conclusions.

3.1.6. Tropomyosin (Tmyo)

Three different tropomyosins isoforms have been identified, slow α and fast α and β [56]. Prados et al. [57] have partially described the pattern of expression of α-tropomyosin protein in the developing chicken heart. The earliest expression is seen in the atrial and the ventricular myocardium of the segmented heart (Carnegie stage 12, stage 18 H/H). However, these authors do not describe the expression in the flanking segments. In fetal heart, tropomyosin expression increases; the trabeculations show higher expression than the compact myocardium [57]. Further topographical analysis of tropomyosin expression in the early tubular heart and the embryonic stage is desirable to understand the functional significance of tropomyosin expression during cardiac development.

3.1.7. Troponins

The troponin complex is involved in the regulation of striated muscle contraction by their interaction with Ca^{2+}

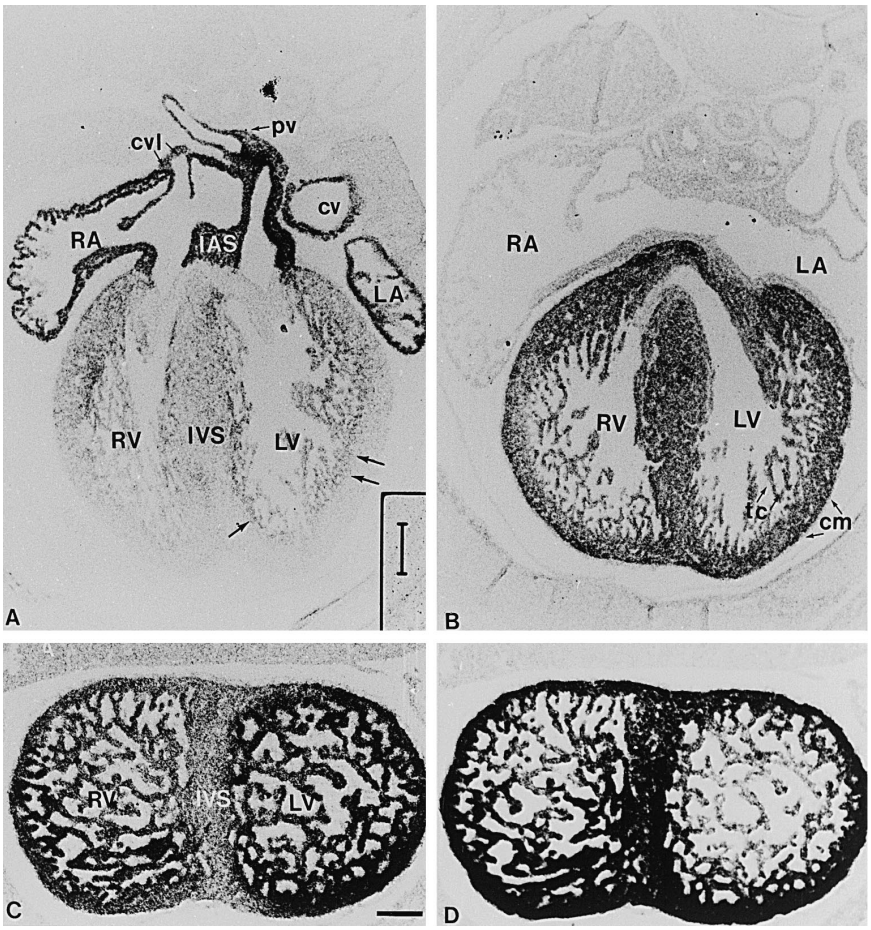


Fig. 4. Serial sections in a four-chambered view of an E14 mouse heart hybridized with cRNA probes against MLC1a mRNA (A) and MLC1v mRNA (B). Expression of MLC1a is confined to the right atrium (RA), left atrium (LA), interatrial septum (IAS), pulmonary veins (pv), left caval vein (cv) and right caval veins leaflet (cvl). Within the ventricular myocardium, MLC1a remains predominantly expressed in the trabeculated portion (arrows) whereas almost no expression is observed in the compact layer. MLC1v mRNA is confined to the ventricular compartment being lower in the trabeculated component that in the compact myocardium. (C, D) Serial transverse sections of an E14 rat heart hybridized with cRNA probes against MLC2a and MLC2v mRNA, respectively. Observe that MLC2a mRNA is expressed highly in the trabeculations and lower in the compact myocardium and interventricular septum (IVS). MLC2v mRNA is predominantly expressed in the compact myocardium and interventricular septum and lower in the trabeculated myocardium. Hybridization conditions were performed according to Moorman et al. [205] with slight modifications (see [13]). The cDNAs probes used are those published in Barton et al. [40] (MLC1a), Lyons et al. [23] (MLC1v), Kubalak et al. [36] (MLC2a) and O'Brien et al. [38] (MLC2v), respectively. Bars = 300 μm (A, B); 220 μm (C, D).

ions. It consists of three different subunits, troponin C (TnC), troponin I (TnI) and troponin T (TnT) (see for a review [58,59]).

In striated muscle two different TnC isoforms are expressed, a fast isoform and a slow/cardiac isoform, each encoded by a single gene [60–62]. The slow/cardiac TnC isoform is expressed in the heart [63] but no data are available about the topographic expression. There is no evidence of isoform switching during cardiac development [64]. Cardiac TnC protein is first detected during chicken development in H/H stage 10 along the cardiac tube and remains homogeneously expressed until hatching [65].

In striated muscle three different genes code for the different isoforms, i.e. fast TnI, slow TnI and cardiac TnI. In cardiac muscle all three isoforms are expressed transiently during development [66–68]. In the adult heart only cardiac TnI mRNA is expressed [64,67]. During early development (Carnegie stage ~9, rat E10), no TnI isoforms can be detected in the tubular rat heart. The first expression is observed at E11 along the myocardium [66]. With further development, both atrial/inflow tract and ventricular myocardium show weak expression of slow TnI and high expression of cardiac TnI. Our own studies on the expression of TnI mRNAs show that cardiac TnI mRNA displays a postero-anterior gradient of gene expression, expression decreasing from the venous pole toward the arterial pole of the heart (Fig. 3C). Slow skeletal troponin I mRNA, in contrast, is almost homogeneously expressed in the developing heart with significantly lower expression in the outflow tract myocardium (D. Franco, unpublished data). These data are in agreement with those reported by Gorza et al. [67] with the exception that there

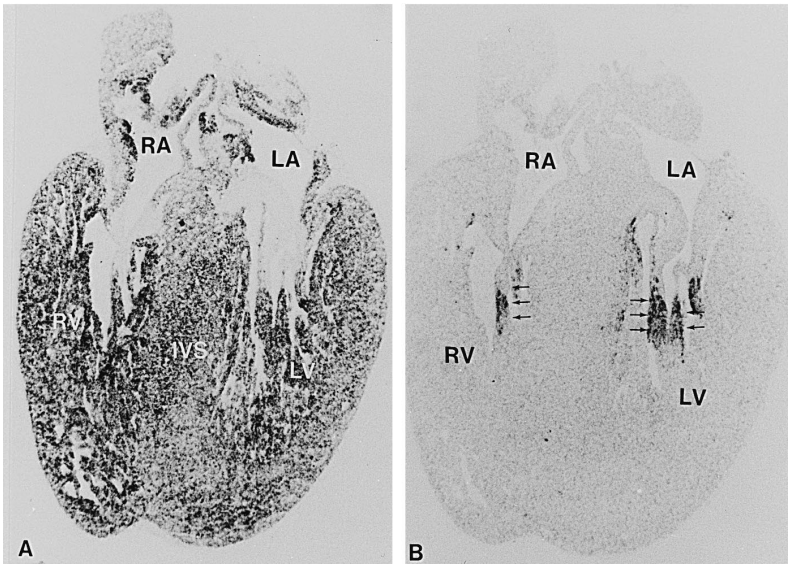


Fig. 5. Four-chamber view of a 9 week-old human embryonic heart (fetal stage). Sections are hybridized with cRNA probes against cardiac α-actin mRNA (A) and skeletal α-actin mRNA (B). Cardiac α-actin mRNA is observed in all myocardial cells; right atrium (RA), left atrium (LA), right ventricle (RV) and left ventricle (LV), whereas skeletal α-actin is confined to the myocytes of the papillary muscles of the tricuspid and mitral valves (arrows). Human fetuses were obtained after legal abortions at the Academic Medical Center (AMC, University of Amsterdam) and at the Postgraduate Medical School in Budapest. The present study was approved by the respective local medical-ethical committees. Hybridization conditions were performed according to Moorman et al. [205] with slight modifications (see [13]). The cDNAs probes against human cardiac α-actin and skeletal α-actin were kindly provided by Dr. Ketty Schwartz (Paris). IVS, interventricular septum. Bars = 300 μm.

is not a sharp boundary between outflow tract and right ventricular myocardium for cardiac TnI expression but just a gradient. Recently, a transient expression of fast TnI mRNA has been reported with a homogeneous distribution in all embryonic cardiac chambers at just detectable levels [68]. As the heart acquires a compact and a trabeculated component in the developing myocardium both, cardiac TnI and slow TnI mRNAs, are higher expressed in the compact myocardium than in the trabeculations (own observations).

Gorza et al. [67] have observed a delay between cardiac TnI mRNA and protein expression. The mRNA was detected in the atrial and the ventricular myocardium from E11 onward, whereas the protein was observed only in atrial myocardium from E18. In contrast, the expression of the slow TnI protein parallels the mRNA expression. These results illustrate that the different TnI isoforms display different programs of gene expression. Furthermore, they illustrate that cardiac TnI is differently regulated at the posttranscriptional level in different regions of the developing myocardium [67]. In contrast with mammals, cardiac TnI mRNA is already detectable in the precardiac mesoderm in *Xenopus* and its expression remains exclusively in the developing myocardium [69].

In summary, the separate TnI isoforms are expressed differently in the distinct cardiac compartments. The cardiac TnI gene is an example of a gene that is initially expressed in a postero-anterior gradient along the cardiac tube and becomes then homogeneously expressed. It also illustrates that distinct post-transcriptional regulation occurs in the separate cardiac compartments. A different pattern of gene expression is observed at the same developmental stage between different vertebrates which can probably be attributed to a heterochrony in cardiac development.

Three genes code for TnT in striated muscle; fast TnT, slow TnT and cardiac TnT, each of them generating a variety of transcripts by alternative splicing [70–73]. The only TnT gene known to be expressed within the heart is the cardiac TnT. As holds for the TnI isoforms, a shift from the embryonic TnT isoform to the adult isoform goes concomitantly with a regionalisation of the transcripts. Sabry and Dhoot [73] reported that expression of cardiac TnT was higher in the embryonic atrial myocardium than in the ventricular myocardium during rat development. Further studies are required to link the expression of cardiac TnT in relation to cardiac morphology during embryogenesis.

3.1.8. Calponin

Calponin is exclusively found in the adult smooth muscle and it is also expressed in the myocardium during early stages of development [74,75]. Three different genes have been described based on their nucleotide sequences [76–78]. So far, only calponin-h1 mRNA (also referred to as smooth muscle calponin) has been extensively studied during cardiac development in mouse [74,75]. The first expression of calponin mRNA is observed at E9.5 with a higher expression in the outflow tract myocardium and lower expression in the rest of the myocardial compart-

ments. Significant expression was also observed in the developing smooth muscle cells of the dorsal aorta. At E14, calponin mRNA has decreased in all cardiac chambers and becomes almost undetectable at E18 [74,75]. Ya et al. [11] have reported calponin protein expression during cardiac development with a similar spatio-temporal distribution of its mRNA in rats. The fact that calponin mRNA/protein is transiently expressed during early stages of heart development, similar to other smooth muscle proteins, e.g. α-SMA, SM22α [11,79] may reflect the developmental history of cardiac muscle from the visceral mesoderm. Other smooth muscle proteins are not expressed, e.g. smooth muscle-MHC, caldesmon [11,80], which makes the functional role of an early transient expression of smooth muscle proteins in the developing heart puzzling.

3.1.9. Other sarcomeric proteins

Other sarcomeric proteins, not forming an intrinsic part of the thick and thin filaments, have been identified, such as titin, α-actinin and myomesin, although there is little information about their topographical distribution in the developing heart [81,82]. Different isoforms of titin are expressed in cardiac and skeletal muscle [83] which are likely to be derived from alternative splicing of the same gene [84,85]. Titin mRNA is already expressed in the cardiac plate mesoderm and remains expressed in both atrial and ventricular myocardium with no apparent regional differences [85]. No data are available about the distribution pattern of myomesin [87] or α-actinin [86].

3.2. Calcium handling

3.2.1. Sarcoplasmic reticulum calcium ATPase (SERCA)

In mammals, three different SERCA genes have been identified; SERCA1 is expressed in fast skeletal muscle only [88], SERCA2 gives rise to two different mRNAs by alternative splicing which are expressed in cardiac, slow skeletal muscle and some smooth muscle (SERCA2a), and in smooth muscle and non-muscle cells (SERCA2b) [89,90]. SERCA3 mRNA is more ubiquitous but a detailed topographical distribution is still lacking [91].

Within the heart two isoforms are known at present to be expressed, SERCA2a and SERCA3 [91]. Their distribution patterns have been investigated during rat cardiac development [91,92]. The first expression of SERCA2a mRNA is observed as early as the precardiac mesoderm is formed [92]. At the tubular looping stage, SERCA2a mRNA shows a caudocranial gradient of expression, high in the venous pole and lower in the arterial pole. As the heart acquires five different segments, expression of SERCA2a mRNA remains lower in the ventricles than in the atria, with a drop in expression in the flanking segments, inflow tract, outflow tract and in particular in the atrioventricular canal [92]. At E13, the compact myocardium shows higher expression of SERCA2a mRNA than the trabeculations [92]. In the fetal stages a different level of expression remains between the atrial and the ventricular myocardium. The expression pattern of SERCA3 mRNA has not been studied in detail. Expression appears in the tubular heart (Carnegie stage ~9, rat E10) and disappears in the septated heart (Carnegie stage 23, rat E17.5) [91].

In conclusion, the calcium pump encoded by the SERCA2a gene shows a postero-anterior gradient of gene expression and displays with further development a segmented pattern of expression. This regionalised distribution of SERCA2a mRNA is in agreement with the functional properties of fast-contracting segments: short contraction duration in the upstream segments and increasing towards the arterial pole. The flanking segments have the longest contraction duration as they function as sphincter to substitute the valves. Differences in the phenotype are also observed within the ventricular segment. Expression is lower in the trabecular portion than in the compact myocardium. A functional interpretation of these differences may be that relaxation starts in the outer compact myocardium, where expression levels are the highest. Further analysis is, however required to assess the functional differences in phenotype between trabeculations and compact myocardium as the calcium pump function is mediated by phospholamban, the action of which depends upon its phosphorylation.

3.2.2. Phospholamban (PLB)

The sarcoplasmic calcium ATPase activity is regulated by PLB. PLB is expressed in cardiac, slow-twitch and smooth muscle but not in fast-twitch muscle tissue [93,94]. PLB is encoded by a single gene directing the synthesis of the same protein in cardiac and slow-twitch muscle [95,96]. Binding of PLB to the SERCA2 protein inhibits its activity, whereas phosphorylation of PLB prevents binding to the calcium pump [97,98].

The spatio-temporal distribution of PLB mRNA expression has been studied during embryonic heart development in rat [92] and chicken [99]. In rats, the first expression of PLB mRNA was detected in the segmented heart (Carnegie stage 12, rat E12). PLB mRNA expression shows a craniocaudal gradient of gene expression opposite to that of SERCA [92]. Similar expression is detectable during embryonic chicken development (Carnegie stage 12, stage 14 H/H) [99]. In the septated heart, the atrial/inflow tract shows lower mRNA expression than the ventricular/outflow tract region. As the trabeculations and compact myocardium start to develop, regional differences are observed, the compact myocardium being more heavily stained that the inner trabeculations [92].

The expression of PLB (and of SERCA, see above) supports the differences in cardiac function, that is fast relaxation in the upstream parts of the heart tube owing to high levels of expression of SERCA2 and low levels of PLB, and long contraction duration downstream owing to

slow calcium clearance due to low levels of SERCA2 and high levels of PLB. It also supports the notion of a quicker relaxation of the compact myocardium than in the trabeculations. Nevertheless, although in general transcriptional regulation is the predominant regulatory mechanism, post-transcriptional control has to be taken in account.

3.2.3. Ryanodine receptor (RyR)

The extrusion of calcium from the sarcoplasmic reticulum into the cytosol is mediated by the RyR [100,101]. At present three different forms of RyR have been identified [101–104]. RyR1 is expressed predominantly in skeletal muscle, RyR2 in cardiac muscle and RyR3 in the brain (see for a recent review [101]). The pattern of expression of the three different RyR isoforms has been studied during rat heart development [105] (our own data).

In the tubular heart, the RyR2 mRNA expression is evenly detectable along the embryonic cardiac tube, whereas RyR1 and RyR3 transcripts are not detectable.

In the embryonic heart, as in the previous stage, only RyR2 transcript is detectable in the myocardium [105]. RyR2 mRNA is slightly higher in the atrial and ventricular segments, beautifully demonstrating the development of fast contracting segments of working myocardium.

In the fetal stage, RyR2 is expressed in all myocardiocytes with no clear differences in spatial distribution. The atrial myocardium tends slightly to have a higher expression. At this stage, expression of RyR3 transcripts is observed for the first time in both atrial and ventricular cardiomyocytes. Interestingly, RyR2 mRNA is significantly lower expressed in the trabeculations than in the compact myocardium (Fig. 6A). Both transcripts, RyR2 and RyR3, remain expressed in the myocardium until adulthood. No expression of RyR1 mRNA is observed in any stage of development [105].

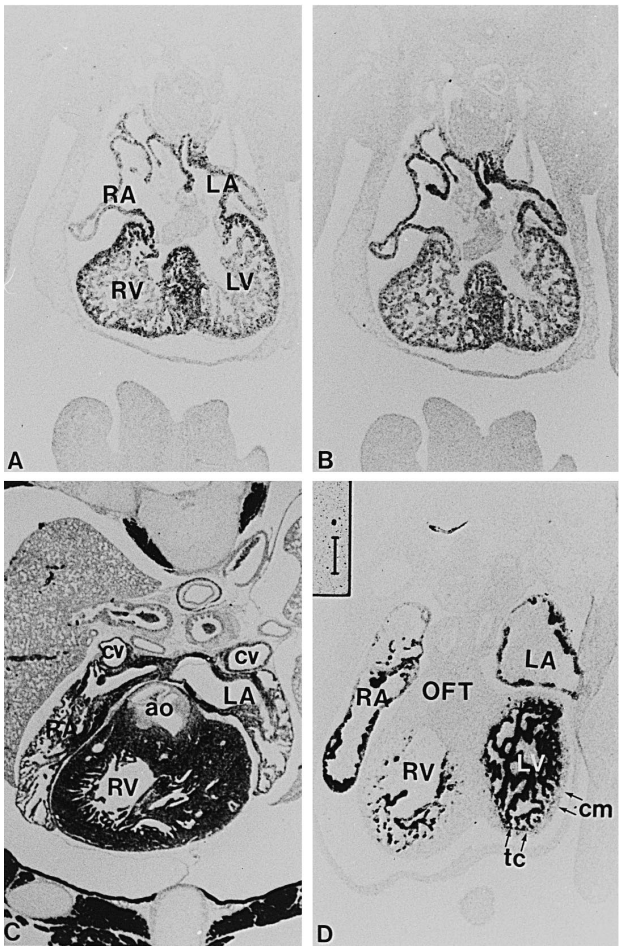


Fig. 6. Serial sections in four-chamber view of an E14 rat heart (fetal stage) hybridized with cRNA probes against cardiac ryanodine receptor mRNA (A) and sodium–calcium exchanger mRNA (B). Expression is observed in all cardiac chambers with a relative low expression in the trabeculated portion of the ventricular myocardium. MCK mRNA expression in an E16 rat heart section (C) is observed in the right atrium (RA), left atrium (LA), right ventricle (RV), left ventricle (LV) and caval veins (cv) but not in the pulmonary veins. ANF mRNA expression in an E12 rat heart (D) is observed in the right atrium (RA) and left atrium (LA) and in the trabeculated portion (tc) of the right (RV) and left ventricle (LV) but neither in the compact myocardium (cm) nor in the outflow tract (OFT). Hybridization conditions were performed according to Moorman et al. [205] with slight modifications (see e.g. [13]). The cDNAs probes used are those published in Buskin et al. [241] (MCK), and Seidman et al. [188] (ANF). The cDNAs probes against ryanodine receptor 2 isoform and the cardiac NCX were kindly provided by Dr. Kenneth Boheler (USA). Bar = 250 μm (A, B, D); 300 μm (C).

3.2.4. Sodium–calcium exchanger (NCX)

The exchange of calcium ions through the sarcolemma is mediated by the NCX. Different isoforms of the NCX are expressed in different tissues which are derived from a single gene by alternative splicing [106,107].

Within the heart, only one spliced variant has been detected at present [107] and its topographical expression has been recently studied during rat development ([107]; our own data). At the tubular stage, NCX mRNA is homogeneously expressed and remains so during further development when the different cardiac segments are formed. Only differences between the trabeculations and the compact myocardium were observed in the embryonic and fetal stage (Carnegie stage 12–23, rat E12–17.5), resembling the expression pattern of the cardiac RyR and SERCA2 mRNAs (Fig. 6B). Thus the NCX seems not to contribute to the segmental differences in calcium clearance. In the late fetal period and the adult rat heart a homogeneous expression is observed along the myocardium.

3.2.5. Sodium–potassium ATPase (NaK-ATPase)

The sodium–potassium ATPase (NaK-ATPase) is a membrane-bound enzyme responsible for the regulation of the electrochemical gradient. Molecular characterization of the NaK-ATPase has identified three α-subunits (α-1, α-2 and α-3) and, at present, two β-subunits (β-1 and β-2), in mammals. The tissue-specific distribution of the α and β isoforms has been documented by Northern blotting and RNAse protection assay analyses during rat development

[108,109], but only the topographical pattern of gene expression of the α-isoforms in mice has been reported [110].

In the tubular stage, α-1 transcript is expressed in all embryonic and extraembryonic tissues but with higher intensity in the heart. The α-2 transcript is observed also along all embryonic and extraembryonic tissues but at lower levels than the α-1 isoform. Both isoforms, α-1 and α-2, are homogeneously expressed within the myocardium. The α-3 transcript is not detectable in most of the embryonic tissue, including the heart, but positive in the neural system.

In the segmented heart, the expression of the α-3 transcript is first detectable within the atrial and the ventricular myocardium [110]. No differences in expression have been observed within the distinct cardiac compartments. The expression of α-2 isoform is, in this stage, higher than the level of expression of the α-3 isoform.

In the fetal heart, expression of α-3 increases whereas α-2 expression decreases. Interestingly, Herrera et al. [110] have observed that the apical ventricular myocytes display lower expression levels of α-1 transcript than the myocardial cells located at the base of the heart, and that α-1 mRNA is higher expressed in the trabeculations than in the compact myocardium, whereas α-3 mRNA shows a reciprocal expression, high expression in the compact myocardium and low expression in the trabeculations. It is proposed by the authors that this pattern of expression contributes to the coordinated myocardial contraction. This notion can also account for the differences in expression between trabeculations and compact myocardium.

3.3. Impulse conduction

3.3.1. Connexins

Propagation of the impulse in the heart is mediated by intercellular channels that aggregate into gap junctions between cardiomyocytes. These channels are formed by transmembrane proteins called connexins (Cxs). Fourteen different connexins have been identified in mammals (see for a recent review [111]). Among them, Cx40, Cx43 and Cx45 have been shown to be associated with the myocytes of the mammalian heart.

The expression patterns of Cx40 and Cx43 have been studied at the mRNA [112,113] and protein level in rat [114–116]. Cx40 protein expression has been studied immunohistochemically during mouse heart development as well [112]. Cx45 mRNA has been studied by Northern blot analysis only [112]. The first expression of Cx40 and Cx43 mRNAs is observed in the atria and the ventricles, but not in the flanking segments, i.e. inflow tract, atrioventricular canal and outflow tract in the looped heart in rats. Interestingly, the expression pattern between the trabeculations and the compact myocardium in the fetal stage of development (Carnegie stage 20, rat E16) is different for each Cx. Cx43 mRNA is strongly positive in the outer compact myocardium whereas the trabeculations show a lower staining intensity. On the other hand, Cx40 mRNA shows a higher expression in the trabeculations than in the outer compact myocardium. With further development, Cx40 mRNA disappears first from the right ventricle and later from the left ventricular myocardium, showing a temporal right/left difference in expression [113]. Van Kempen et al. [113] have suggested that the temporally separate disappearance of Cx40 mRNA from the fetal ventricles implies that right and left ventricles mature independently. This notion is in agreement with recent data from transgenic mice carrying distinct regulatory sequences of genes expressed in cardiac muscle (see e.g. [32,117–119]), which demonstrate different transcriptional specificities in the right and left ventricles.

None of these two Cx mRNAs are detectable in the sinoatrial node, atrioventricular node and proximal parts of the bundle branches during development of the rat heart. In early stages of development, Cx40 protein shows a similar expression pattern as its mRNA. Species-specific differences are observed in the fetal stage where Cx40 protein is expressed predominantly in the atrial myocardium and in the ventricular conduction system in the mouse [112].

Cx43 protein and its mRNA have been detected during rat heart development from E13 (Carnegie stage 14) onward, in both atria and ventricles but not in the inflow tract, atrioventricular canal and outflow tract [116]. Gourdie et al. [115] reported expression of the protein three days earlier (Carnegie stage 9, rat E10) possibly due to the use of fluorescent antibodies which give a higher sensitivity of detection. As soon as the ventricles become trabeculated, a higher Cx43 protein expression is observed in the trabeculations compared with the compact myocardium [115]. In this stage, the subepicardial layer of the ventricles and the interventricular septum, that is, the compact myocardium, do not react with the antibody against Cx43 protein. Thus, the expression of Cx43 is exclusively confined to the trabeculations, whereas the mRNA is not [116]. In the fetal stage the levels of Cx43 protein increase both in the trabeculations and in the interventricular septum and compact ventricular layers. In the venous pole of the heart, the myocardium surrounding the caval veins and the pulmonary veins remains negative as well as the sinoatrial node. In neonates, Cx43 protein becomes equally distributed among the ventricular myocardium and its expression is observed in the caval veins and pulmonary veins but not in the sinoatrial node [113].

Cxs display a segmented pattern of expression matching the differences in function of the distinct segments [10,92,120]. A yet unsolved issue is the fact that Cx43 mRNA is highly expressed in the ventricular compact myocardium and at just detectable levels in the trabeculations, whereas Cx43 protein is highly expressed in the trabeculations and is less abundant in the compact myocardium. The distribution of Cx43 protein is compatible with a faster conduction via the trabeculations [120].

3.4. Energy metabolism

3.4.1. Creatine kinases

In mammals and chicken, two different but related cytosolic creatine kinases (CK), called MCK (muscle-specific) and BCK (brain-specific), and one unrelated mitochondrial gene product (named 'Mi') are found (for a review see [121–123]).

Both MCK and BCK mRNAs have been studied during embryonic mouse and chicken development by in situ hybridization [124]. The first expression of BCK mRNA is observed in the tubular heart (Carnegie stage ~9, mouse E8) whereas MCK mRNA is first observed in the late segmented heart (Carnegie stage 16, mouse E12.5). As MCK becomes expressed in the myocardium, the expression of BCK decreases to undetectable levels in the septated heart [124]. BCK mRNA is homogeneously expressed within the cardiac muscle but MCK mRNA expression starts exclusively in the right ventricle [3] and becomes homogeneously distributed one day later. In rat, such a right–left different patterning is also transiently observed later in development (D. Franco, unpublished data) and becomes homogeneously expressed in the ventricular myocardium in the septated heart (Fig. 6C).

The first expression of MCK protein is observed around the same developmental cardiac stage, the segmented heart (Carnegie stage 14–15), in chicken [126], rat [125] and in man [127]. Regional differences in protein expression were observed among the different species. In chicken, the outflow tract, the ventricular trabeculations, the roof of the atrial cavities and the interventricular septum stain strongly with a polyclonal antibody against MCK. The possibility was considered that this pattern could be related to the conduction system [126]. In rats, MCK protein initially is observed in the outflow tract and the trabecules of the right ventricle (E12–14), and subsequently in the venous valves, interatrial septum and sinoatrial node. At E18 homogeneous expression was observed in the entire myocardium [125]. In man, the compact myocardium displays a weak expression compared to the trabeculations. Regional differences were observed at the venous pole of the heart. Those areas derived from the primitive sinus venosus, e.g. the junction of the vena cava superior and the right atrium, the left aspect of the right venous valve and the proximal part of the left sinus horns were almost negative whereas elsewhere the atrial myocardium was positive.

In essence, the expression of creatine kinase isoforms during development shows a shift in expression from BCK to MCK in different species. Interestingly, in mice and rats a transient regionalisation of the expression to the right ventricle/outflow tract is observed at mRNA and protein level, illustrating that this constitutes a separate transcriptional domain. Heterogeneity was also observed at the venous pole of the heart in different species, suggesting a different origin of the participating components. Further analysis is required to understand the different cues directing expression to each region of the venous pole of the heart.

3.5. Intermediate filaments

3.5.1. Desmin

Desmin is a muscle cytoskeletal protein belonging to the family of the intermediate filament proteins. Desmin is derived from a single gene. No data are available about the spatio-temporal expression of desmin mRNA during embryogenesis [128].

Desmin protein is one of the first muscle-specific proteins detected during embryogenesis and it is expressed before titin, sarcomeric actin and myosin heavy chain [83,129]. At mouse E8.5, desmin is detected in the cardiac primordia; its expression increases with development [130]. In the developing rat heart, desmin is homogeneously expressed during the tubular stage [11]. As the compact myocardial layer is formed, desmin expression becomes very high in the trabeculations whereas the compact myocardium and the interventricular septum display low expression levels. At fetal stages, desmin starts to disappear from the ventricular myocardium but expression remains high in the ventricular conduction system and in the atrial myocardium [11]. A very similar developmental profile is observed during mouse embryonic and fetal development (J. Ya and D. Franco, unpublished data). The high expression of desmin in the trabeculations versus the compact myocardium confers a 'primitive' phenotype to the trabeculations, the functional significance of which is not clear yet.

3.6. Cell–cell and cell–matrix contact molecules

3.6.1. Integrins

Integrins are heterodimer receptor molecules formed by two subunits, α and β. At present 16 α subunits and 8 β subunits have been described that result in 22 functional molecules (for a review see e.g. [131–134]). Cardiac myocytes express several isoforms of integrins, among which the predominant types are α-1, α-5, α-6 and β-1 [135–142].

α-1 integrin is expressed during embryonic chicken development (Carnegie stage 9–16, stages H/H 12–29), however no data are available about its distribution in the developing heart [137]. The expression pattern of α-5 integrin has been studied in two different mammalian species, macaque and rat [139,140]. Pow and Hendrickx [139] reported a transient weak expression of α-5 integrin protein in all myocardial cells during the early stages of heart development (tubular stage) in the macaque. In rat, similar levels of expression of α-5 integrin are observed in the atrial and ventricular myocardium whereas the outflow tract and atrioventricular canal show a lower staining intensity. Expression is not exclusively restricted to the myocardium as the endocardial lining of the atrioventricular cushions and of the outflow tract cushions is also

positive. With further development α-5 integrin is higher expressed in the trabeculations than in the compact myocardium [139]. α-6 integrin mRNA is expressed homogeneously during the early stages of development. With further development a transmural gradient of expression in the ventricular myocardium is observed at the mRNA level in mice [138] and at the protein level in chicken [141]. In both cases a higher expression is observed in the trabeculations than in the compact myocardium. Hierck et al. [138] have reported that α-6 integrin mRNA expression is weak in the interventricular septum (i.e. the first structure with a compact myocardial morphology) in the segmented heart. In the fetal stage, low expression is observed in the ventricular compact layers and no expression in the interventricular septum. The expression of β-1 integrin has been studied during late embryonic/fetal stages in rats. Carver et al. [136] reported that β-1 integrin (protein and mRNA) is more prominent in areas with intense remodelling, such as the trabeculations (E11.5). With further development (E15) a homogeneous expression in the ventricular myocardium is observed. β-1 integrin is not only expressed in the myocardium but also in the mesenchymal cells of the developing valves.

Little insight is yet available into the function of integrins which hinders the interpretation of their role during cardiac morphogenesis. Integrin expression is regionalized within the heart suggesting that they may play an important role in the distinct functioning of each cardiac segment.

3.6.2. Cadherins

Cadherins are a family of cell surface glycoproteins that mediate calcium-dependent cell adhesion. They are located in the adherens junctions where they interact with the actin cytoskeleton. They have a highly conserved cytoplasmic domain that acts as binding domain for cytoplasmic proteins called catenins. Cadherins play an important role in different morphogenetic processes such as gastrulation [143,144], neurulation [145] and cardiogenesis [146–148]. At present, only N-cadherin (A-CAM) has been reported to be expressed in the myocardium [146,148–151], in chicken. In the tubular heart, N-cadherin seems to be homogenous expressed throughout the myocardium [144]. No data are available on the expression of N-cadherin in the segmented and fetal heart.

3.7. Cardiac-specific transcription factors

During the last couple of years increasing information has become available regarding transcription factors that play essential roles during heart morphogenesis (see e.g. [152]), such as members of the transcription factor families of the homeobox genes (NK family, see for review [153]), GATA genes (see for review [154]) and MADS genes (MEF-2 and bHLH; see for review [155]). The role of such transcription factors seems highly conserved during evolution, as homologous genes are involved in cardiogenesis of different species ranging from *Drosophila* [156–158], zebrafish [159,160], *Xenopus* [161–164], chicken [165] and mouse [37,166–174].

3.7.1. Homeobox genes

Several homeobox genes of the NK2 subfamily are expressed within the precardiac mesoderm and, with further development, in the myocardial component of the heart [153]. Nkx2.5 transcript is first observed in the cardiac crescent; in the tubular heart stage, it is homogeneously distributed along the entire heart in both the atrial and ventricular myocardiocytes and remains expressed during fetal and adult stages [242].

Recently, a new member of the NK family has been cloned from chicken embryos (cNkx2.8) which shows a regionalised expression within the myocardium [175,176]. First expression of cNkx2.8 transcript is observed in the precardiac mesoderm. It is homogeneously distributed in the myocardium at the tubular heart (Carnegie stage ~9; mouse E8.5). In the embryonic heart (Carnegie stage 12; mouse E10.5) expression becomes regionalised to the cranial and caudal pole of the heart and its expression extends beyond the myocardium, i.e. in the endoderm. In the cranial pole of the heart cNkx2.8 transcripts extend from the distal outflow tract to the aortic sac and pharyngeal pouches 2 and 3. In the caudal pole of the heart, expression of cNkx2.8 mRNA is observed in the sinus venosus and extends into the septum transversum [175,176]. In later stages, expression in the myocardium is no longer observed [175,176].

3.7.2. GATA family

At present three members of the GATA family have been described to be expressed in the myocardium (see for a review [154]). GATA-4 transcript can first be detected in the precardiac mesoderm and is evenly expressed within the myocardial component in later stages, as well as in other embryonic structures [170].

More recently, GATA-5 mRNA has been demonstrated to have a dynamic pattern of expression during mouse embryogenesis. First expression of GATA-5 transcript is observed in the precardiac mesoderm. In the embryonic, segmented heart (Carnegie stage ~9; mouse E8.5), GATA-5 is expressed throughout the myocardium with higher expression in the outflow tract and inflow tract regions than in the atrial and ventricular segments. In the septated heart (Carnegie stage 23, mouse E14.5), GATA-5 is no longer observed in the myocardium but remains expressed in the atrial endocardium [177].

The developmental pattern of expression of GATA-6 mRNA mostly follows that observed for GATA-4 mRNA. No differences in expression have been reported within the myocardial component. In the fetal stage, in addition to the expression in the myocardium, GATA-6 mRNA becomes expressed in the developing aorta and the right and left caval veins as well [171].

3.7.3. Myocyte enhancer factor (MEF)-2 family

Three different members (MEF2A, 2C, 2D) of the MEF family are expressed in the developing heart, however no differential expression is observed within the different cardiac compartments [173]. MEF2C can first be detected in the precardiac mesoderm at approximately E7.5 (Carnegie stage ~9) in mice. The expression of MEF2A and MEF2D has been observed for the first time one day later within the developing myocardium as well as in a number of other embryonic tissues.

3.7.4. Basic helix–loop–helix (bHLH) family

The bHLH family of transcription factors includes skeletal muscle-specific genes as MyoD, Myf-5, Mrf-4 and myogenin, which play an essential role in skeletal-muscle specific sarcomeric gene expression, but none of them are expressed in the developing myocardium (see for a review [178]).

Recently, two novel bHLH members, now named dHAND and eHAND, were found to be expressed in the developing heart [155,179,180]. In chicken, both transcripts are firstly expressed in the precardiac mesoderm. In the tubular heart stage, dHAND and eHAND are evenly expressed within the myocardium [155].

In contrast to the avian condition, the homologous dHAND and eHAND transcripts in mouse show a very dynamic pattern of expression during heart morphogenesis [169,179–181]. dHAND and eHAND transcripts are firstly observed in the precardial mesoderm, in mice. Both HAND genes are expressed in the early tubular heart (Carnegie stage ~9; mouse E8.5) but dHAND shows an anteroposterior gradient of expression whereas eHAND is homogeneously expressed. In the embryonic heart, expression of eHAND is no longer detectable in the presumptive right ventricle. On the other hand, dHAND becomes confined to the presumptive right ventricle and the outflow tract [169,181]. In the fetal stage, expression of eHAND remains detectable at low levels in the myocardium. dHAND mRNA is not detectable in the fetal stage in the myocardium and its expression becomes confined to neural crest derivates [169,181].

3.7.5. Other transcription factors

Transcription factors as the serum response factor (SRF), the cardiac ankyrin repeat protein (CARP) and a novel gene named pCMF1, are also involved in cardiac muscle-specific gene expression in vitro [182–184].

The SRF seems to have a homogeneous expression within the myocardium in the tubular stage (Carnegie stage ~9, chicken H/H 11). No data are available from later stages of development [185]. CARP expression has been studied during mouse embryogenesis. CARP mRNA is first expressed evenly in the tubular heart (Carnegie stage ~9; mouse E8.5) and, with further development (Carnegie stage 12; mouse E10.5), expression decreases in the ventricle but remains higher in the outflow tract and atria. Expression of pCMF1 is firstly observed throughout the myocardium in the fusing cardiac plates (Carnegie stage ~9; chicken H/H 9) and remains evenly expressed in the myocardium until the embryonic stage (Carnegie stage 12; chicken (H/H20) [184].

3.8. Miscellanea

Atrial natriuretic factor (ANF) is a peptide hormone with natriuretic and vasoactive properties which is expressed in the developing and adult mammalian heart [186,187]. ANF is encoded by a single gene [188]. The first expression is observed in the tubular stage (Carnegie stage ~9, mouse E8) in the developing mouse heart with a postero-anterior gradient. At E9 a regionalised expression was observed in the looped heart, the presumptive left ventricle and right atrium showed consistently higher expression of ANF mRNA than the presumptive right ventricle [187]. The flanking segments, that is outflow tract, atrioventricular canal and inflow tract do not show ANF expression (D. Franco, unpublished data). With further development, as the heart has become septated (Carnegie stage 16, rat E14), the trabeculated portion of the left ventricle expresses similar amounts of ANF transcript as the atrial myocardium [187]. In contrast, the right and left ventricular free walls (compact myocardium) and the interventricular septum show almost no expression of ANF mRNA (Fig. 6D). At the protein level, no differences in expression are observed between right and left components in the fetal rat ventricles but more stained cells were located in the trabeculations than in the compact layer [189]. Moreover, heterogeneous expression is observed in the venous pole of the heart, structures such as the caval veins, interatrial septum and coronary sinus do not express ANF whereas the atrial myocardium does express it (own observations).

Acetylcholinesterase is a key enzyme in the cholinergic signal transduction pathway encoded by a single gene [190]. Acetylcholinesterase activity has been demonstrated in the developing chicken [191] and rat heart [192,193], particularly in the developing outflow tract myocardium and the ventricular trabeculations. Its expression declines in the fetal period and disappears shortly after birth. AChE activity was initially hypothesised to constitute an early marker for the development of the conduction system [192] but recently it was shown that AChE did not colocalise with the HNK-1 epitope [193] which has been correlated with the ventricular conduction system [194,195]. More recently, it has been shown that neither AChE, nor choline-acetyltransferase mRNAs (the enzyme that synthesises acetylcholine) are observed in the developing rat heart [196] leaving up an open question of how AChE activity can be present in the ventricular trabeculations and outflow tract myocardium.

4. Patterns of transgene expression

The objective of studies on the regulatory cues of gene expression is to understand how a certain gene becomes expressed in a distinct spatio-temporal fashion. The identification of *cis*-acting regulatory DNA elements in flanking sequences of the transcription initiation site by transgenic mice (in vivo assay) has increased our understanding about the molecular mechanisms of gene regulation. Within the heart, certain genes are expressed along the entire cardiac tube (e.g. actins) whereas others become restricted to certain segments (e.g. MHCs and MLCs). In the present section we summarise the current knowledge of the different patterns of expression in transgenic mice. We do not go into the different transcriptional activators (see for a recent review [197]) unless they are relevant for the understanding of the regionalised expression of the transgenes. Table 2 summarizes the genes reviewed and Fig. 7 shows the different expression patterns reported until now, in schematic cartoons of a heart corresponding to approximately mouse E10.5 (Carnegie stage 12).

4.1. Contractile proteins

4.1.1. Myosin heavy chains

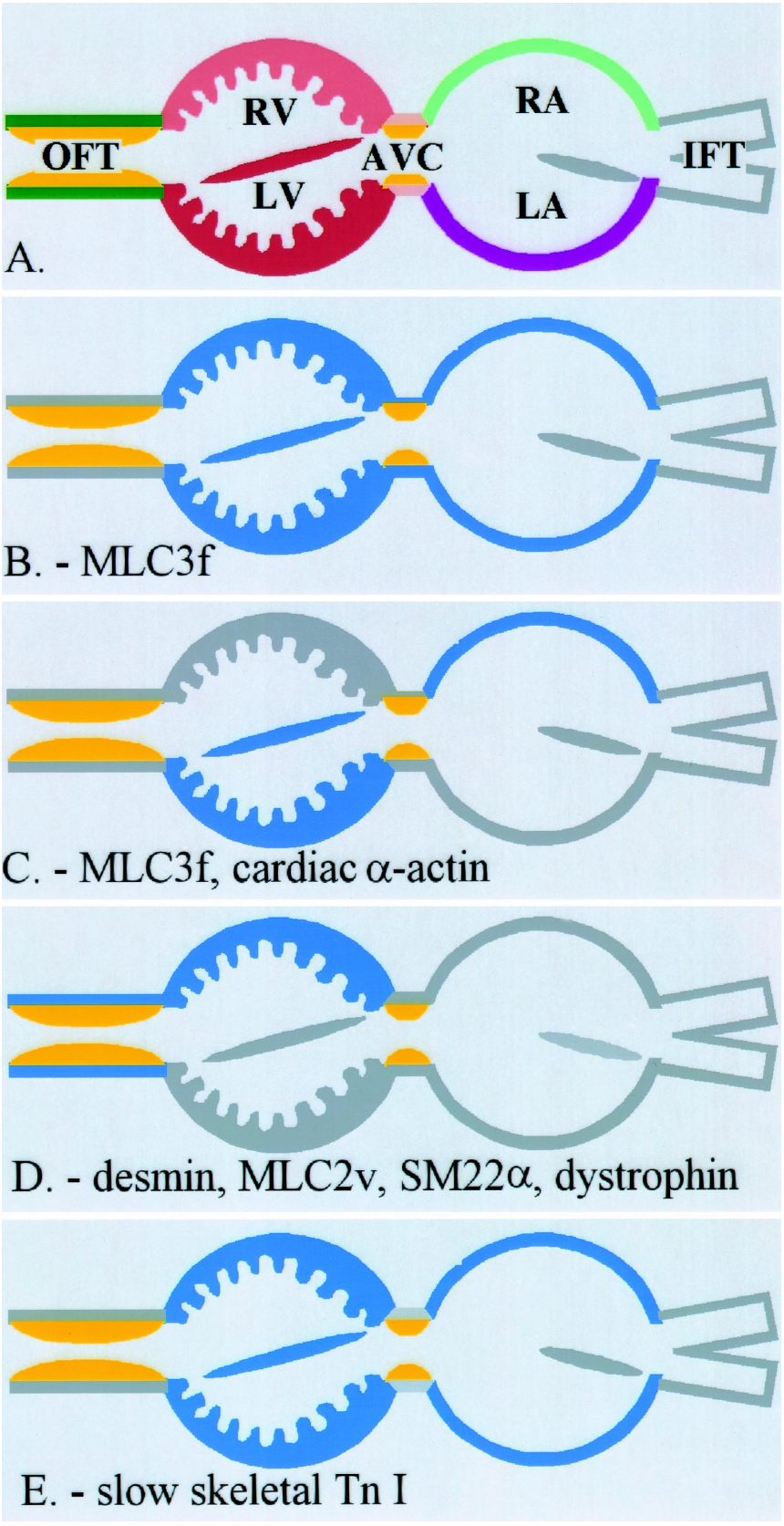
Subramanian et al. [198] have reported that a sequence of 5.5 kilobases (kb) upstream of the transcription starting site of the α-MHC gene is capable of conferring expression of the chloramphenicol acetyltransferase (CAT) reporter gene to the adult atrial and ventricular myocardium. No differences in expression between atrial and ventricular myocardium have been observed. These data are in agreement with the adult expression pattern of the α-MHC endogenous mRNA. Palermo et al. [199] using a 5.5 kb α-MHC promoter transgene coupled to the lacZ reporter gene demonstrated that expression of the transgene becomes confined to the atrial myocardium in the segmented heart (Carnegie stage 12; mouse E10.5) and remains expressed in the atrial myocardium as well as it becomes up-regulated in the ventricular myocardium postnatally.

Rindt et al. [200] have shown that 5kb of the upstream sequences of the β-MHC gene are able to confer cardiac-specific expression but 2.5 kb or less upstream sequences failed to confer transgene expression in the adult heart. Knotts et al. [201] have shown that the onset of expression of the β-MHC transgene (with 5.5 kb upstream sequence) is similar to the endogenous gene and restricted to the heart. Expression of the transgene is not restricted to the ventricular myocardium at E12 and extended into the atria. Knotts et al. [201] suggest therefore that additional elements, not present in the 5.5 kb upstream sequence, are required for the down-regulation of the β-MHC gene in the atrial part of the developing heart. We note, however, that low levels of β-MHC mRNA are normally observed in the atrial myocardium in embryonic and fetal stages (Fig. 3A). Hence, the pattern of transgene expression described by Knotts et al. [201] may reflect precisely the expression of the endogenous gene.

4.1.2. Myosin light chains

The regulatory elements of two myosin light chain genes have been studied in detail regarding the regulation of gene expression, i.e. the mouse MLC3f and the rat MLC2v genes. Recently, Franco et al. [13] have shown that 9 kb upstream sequences of the MLC3f gene confer expression to both atria and ventricles in adult hearts except in right ventricular outlet, i.e. the former outflow tract. The analysis of the early stages of development showed expression in the atrial and ventricular myocardium but neither in the outflow tract nor in the inflow tract myocardium. Kelly et al. [32] reported that 2 kb upstream of the transcription start site of the MLC1f/3f gene coupled to the MLC1f/3f 3′ muscle-specific enhancer, is sufficient to confer expression of the β-galactosidase reporter gene to the adult right atrium and left ventricle. Analysis of early stages of development indicates that initially the entire cardiogenic mesoderm expresses the transgene and that regionalised expression of the transgene was first observed in the tubular heart (Carnegie stage ∼9, mouse E8.5). A comparative analysis of transgene expression in the tubular heart stage in both MLC3f transgenic mice revealed a symmetrical expression in the early looping heart after which regionalised expression becomes apparent [13]. McGrew et al. [33] have reported that 628 bp promoter sequence of the rat MLC3f gene confers higher CAT reporter gene expression in atrial myocardium than in the ventricular myocardium. They do not mention whether differences between right and left components are observed. Two putative *cis*-acting elements, a MEF-2 site and a GATA-4 site were found to be important for MLC3f gene expression in vitro.

The rat MLC2v gene has been extensively studied in vitro and in vivo using different reporter genes [202–204,206]. Lee et al. [204] showed that transgenic mice carrying a 250 bp fragment of the MLC2v promoter, display expression in the ventricular myocardium. The authors reported that the left ventricle expressed 10-fold higher luciferase activity than the right ventricle. More recently, Ross et al. [118] have reported that the same 250 bp MLC2v promoter sequence driving the β-galactosidase gene as reporter gene confers expression to the right ventricle and outflow tract but not to the left ventricular and atrial myocardium. The difference with the study of Lee et al. [204] remains unaccounted for. Expression of β-galactosidase activity was first observed in the precardiac mesoderm. With further development expression is homogeneous in the tubular heart and the first heterogeneous expression arises in the looped heart, when the right and left ventricular portion become morphologically different. Chimeric sequences of a 28 bp fragment containing



binding sites of HF-1a/HF-1b/MEF-2 factors directed expression of the transgene in a similar fashion than the 250 bp MLC2v endogenous promoter [118]. The use of the two-hybrid system with the 28 bp MLC2V minimal promoter has led to the identification of a novel cardiac-specific transcription factor, the cardiac ankyrin repeat protein (CARP). Although the CARP transcript shows a regionalised pattern of expression during heart morphogenesis, no role in right–left specification has been established [183].

The study of the regulatory sequences of the MLC genes demonstrates that distinct transcriptional specificities exist not only for the right and left components of atrial and ventricular segments but also for the flanking segments. Moreover, the right–left differences in the heart develop far beyond the time that bilateral symmetry develops in the body and develop according to the antero-posterior axis of the heart.

4.1.3. Actins

At present, only cardiac α-actin gene has been studied in transgenic mice. The combinatorial effect of two distinct enhancers (distal enhancer and proximal enhancer) and the promoter were studied regarding the ability to confer cardiac expression. Biben et al. [117] have shown that the cardiac α-actin promoter alone or in combination with a distal enhancer is not sufficient to confer cardiac expression. However, the combination of promoter and proximal enhancer gave expression in the atria and the ventricles with the exception of the outflow tract. The combination of both enhancers (distal and proximal) plus the promoter gives rise to a regionalised expression pattern in the left ventricle and right atria similar to that described by Kelly et al. [32].

From the analysis of the regulation of actin genes it can be deduced that different mechanisms are operative for the cardiac and skeletal gene expression. Most importantly, these transgenic mice illustrate that different transcriptional specificity is present in the distinct cardiac compartments that should be the result from the interaction of discrete regulatory elements of the flanking sequences and available transcription factors.

4.1.4. Troponins

The regulation of the human slow troponin I gene has been studied using transgenic mice. The 4.2 kb upstream sequence of the transcription initiation site is sufficient to confer CAT transgene expression to the cardiac muscle during development. However, an aberrant topographic CAT expression was observed as compared with the endogenous gene. Expression of CAT reporter gene was very low in the outflow tract and ventricles, whereas it was high in the inflow tract [68]. Levitt et al. [207] have shown that the promoter of the human slow skeletal TnI gene is not sufficient to confer cardiac expression of the reporter gene in adult mice. However, no data are given regarding the development of the heart, thus it might be that expression is present in the early stages of development. Similar studies using regulatory sequences of the rat slow skeletal TnI gene [208] and the fast skeletal TnI gene [209] failed to confer expression of the reporter gene to the heart. However, no data are available about transgene expression in the developing heart.

4.2. Intermediate filaments

The regulatory sequences conferring skeletal expression of the human desmin gene have been analyzed in transgenic mice carrying β-galactosidase as reporter gene. The 1 kb upstream sequence of the transcription start site of the human desmin gene confers expression to skeletal muscle but not to the atrial or ventricular working myocardium. Li et al. [210] suggested that transgene expression was confined, topographically, to the same area that Virágh and Challice [211] reported as the early ventricular conduction system using electron microscopy. These data are derived from whole-mount histochemical analysis in which the conduction system is not recognisable. Thus, further analyses are required to verify this assumption. Kuisk et al. [119] have shown that 1 kb upstream sequence of the mouse desmin gene is able to drive expression of the reporter gene to the working myocardium in the developing heart. The earliest expression was observed at mouse embryonic day 8 (cardiac crescent), and becomes restricted to the right ventricle and outflow tract myocardium in the looped heart (Carnegie stage 12). Point mutation analysis of MEF-2 and/or E-box consensus of the desmin promoter region sequences revealed in transgenic mice that β-galactosidase expression is dependent on MEF-2 binding but is not affected by E-box sites. Thus, it could be that members of the MEF-2 family of transcription factors are involved in the regionalised specification of gene expression in the developing heart. However, MEF-2 (MEF2A, MEF2C and MEF2D) mRNAs have been reported to be

Fig. 7. Transgene expression of distinct promoter/enhancers constructs during embryonic cardiac development allows to distinguish seven transcriptional domains (myocardium in different colours: OFT, outflow tract; RV, right ventricle; LV, left ventricle; AVC, atrioventricular canal; RA, right atrium; LA, left atrium; IFT, inflow tract) as illustrated in panel A. The yellow colouring indicates the endocardial cushions. Expression is conferred either to: (B) RA, LA, RV, LV and AVC (e.g. MLC3f, [13]); (C) the LV, AVC and RA (MLC3f, cardiac α-actin [32,117]); (D) the RV and outflow tract (OFT) (e.g. desmin, MLC2v, SM22α, dystrophin [79,118,119,216]); (E) the RV, LV, RA, LA but not in the OFT, AVC nor inflow tract (IFT) (e.g. slow skeletal troponin I; [207]). Note that none of the transgenes are expressed in the inflow tract myocardium and atrial septum.

homogeneously expressed along the cardiac tube [173] arguing against a primary role in the regionalisation of gene expression, although deletion of the MEF2C gene leads to aberrant heart morphogenesis mainly caused by the absence of the right ventricular chamber formation [168]. Further analysis at the protein level will be crucial to our understanding of transcriptional specificity during cardiac development.

4.3. Other genes

The regulation of ANF and MCK has been studied during cardiac development by transgenic mice. However, no data are available as to their expression during cardiac development [212]. Transgenic mice carrying regulatory sequences of the SM22α, a smooth-muscle protein also expressed in the heart [79], show transgene expression in the developing heart [213]. Expression of the β-galactosidase reporter gene is restricted to right ventricle and outflow tract myocardium. The earliest expression is observed in the cardiac crescent [213,214]. A similar pattern of expression has been reported in transgenic mice carrying 4 kb of the flanking sequence of the α-B crystalline/small heat shock protein coupled to β-galactosidase [215]. The expression of the *lacZ* reporter gene in transgenic mice carrying a 900 bp promoter region of the mouse dystrophin gene reveals expression confined to the right ventricle and the outflow tract. First expression of the reporter gene is observed in the looped heart confined to the right ventricle and outflow tract (Carnegie stage ~9; mouse E8.5) and remains unchanged until adulthood [216].

In summary, the analysis of transgenic mice has revealed that cardiac and skeletal gene expression programs are directed by separate *cis*-acting elements. Furthermore, they illustrate that more than merely atrial and ventricular myocardial transcriptional domains are present within the heart, as the embryonic flanking segments display a unique transcriptional specificity (see Fig. 7) that is maintained in the adult heart.

5. Cardiac function during development

Differential expression has been illustrated for many genes within the atrial and the ventricular compartments during cardiac embryogenesis. In many cases, the expression in the flanking segments, i.e. outflow tract, atrioventricular canal and inflow tract, has been overlooked. Comparison of Table 1 and Fig. 2 convincingly shows that these segments have a unique pattern of expression. The flanking segments often have retained the molecular phenotype of the original 'primary myocardium' [10,120,217]. We have pointed out recently that in the segmented heart, the slow-conducting flanking segments function as valves [10]. This notion was based on electrophysiological measurements and patterns of gene expression [10]. We now will elaborate on the development of the trabeculations and compact myocardium in the ventricular component.

The distinct molecular phenotype of the trabeculations versus the compact myocardial component of the ventricular compartment is seen in different species from very early in development onward. The analysis is based on a wide set of proteins/mRNAs involved in a variety of cardiac functions and is summarised in Fig. 8. The distinct phenotype is no longer detectable in the late fetal period, suggesting that the trabeculations play a critical function during early cardiac development only, which subsequently is taken over by the compact myocardial component.

The molecular phenotype of the ventricular compartments suggests that the trabeculations have a faster contraction, based upon α-MHC expression (see e.g. [22]),

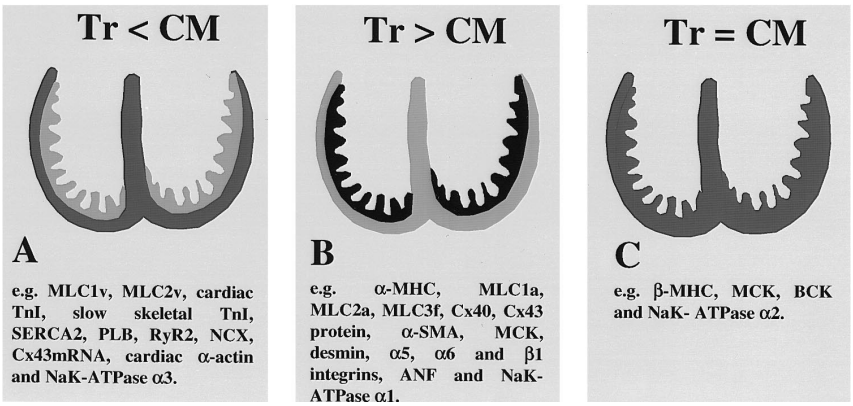


Fig. 8. Gene expression in the developing ventricular myocardium. Many genes show an transient heterogeneous expression in the ventricular myocardium; three main patterns can be distinguished: (A) Lower expression in the trabeculations and higher in the developing compact myocardium (e.g. MLC1v, MLC2v, cardiac TnI, slow skeletal TnI, SERCA2, PLB, RyR2, NCX, Cx43 mRNA, cardiac α-actin and NaK-ATPase α3). (B) Other genes/proteins show the opposite pattern, i.e. lower expression in the compact myocardium than in the trabeculations (e.g. α-MHC, MLC1a, MLC2a, MLC3f, Cx40, Cx43 protein, α-SMA, MCK, desmin, α5, α6 and β1 integrins, ANF and NaK-ATPase α1). (C) Only few genes are homogeneously expressed (e.g. β-MHC, MCK, BCK and NaK-ATPase α2). Tr, trabeculations; CM, compact layer.

and faster impulse conduction, based upon connexin43 protein expression [116] and cell–cell interaction molecules (see e.g. [140,141]), than the surrounding compact myocardium during the embryonic and fetal period. Furthermore, a higher expression of MCK is observed in the trabeculations as compared to the surrounding compact myocardium [125]. The high expression of creatine kinase during the late embryonic and fetal stages of cardiac development in the trabeculations is consistent with a higher energetic requirement, reminiscent to fast-twitch fibres as reported for skeletal muscle [218–220].

The distinct pattern of expression within the trabeculations versus the compact myocardium is reflected at both the protein and at the mRNA level for most of the gene products analyzed, e.g. myosin isoforms. In some cases, the same level of mRNA expression within the entire ventricular compartment is observed whereas an increased expression of protein is found in the trabeculations, e.g. MCK. This difference is apparently a consequence of post-transcriptional regulation. Remarkably, connexin 43 mRNA is highly expressed in the compact myocardium whereas its protein is almost absent [113,116]. The reason of this peculiar distribution (connexin 43 mRNA/protein) is enigmatic. However, the distribution of the protein is compatible with its function and electrophysiological observations [120,221], suggesting that the impulse is conducted via the interiorly located trabecular component and to the compact myocardium.

Based on the patterns of expression of mRNA encoding for proteins involved in calcium handling, one might speculate that the highest calcium pump activity is present in the exteriorly localised compact myocardium. However, calcium pump activity relies on the state of phosphorylation of phospholamban [222,223]. Therefore additional analyses are required using appropriate antibodies to assess the distribution of the different phosphorylation forms of phospholamban.

It is noteworthy that transmural gradients of expression between the inner and outer side of the adult ventricles have been reported in literature also [224]. It is well documented that in the adult heart differences between the electrophysiological characteristics of the subendocardial and subepicardial myocytes exist [97,225–228]. The existence of a regional heterogeneity in the pattern of expression of the proteins involved in contraction–relaxation coupling is indicative of the fact that such discrete electrophysiological behaviour might also occur in the embryonic heart.

The analysis of other contractile proteins as α-SMA and intermediate filaments such as desmin, suggest that the trabeculations retain some characteristics of the ‘primary myocardium’. On the other hand, the spatio-temporal expression of α5 integrin and α-SMA [140] suggests that the trabeculations are more differentiated than the ventricular compartment. Similar differences in integrin expression have been reported for other isoforms [136,138,141]. The heterogeneity in cell–cell and cell–matrix interactions support the notion that the trabeculations display a more efficient intracellular coupling (viz. faster impulse conduction and contraction) than the compact ventricular myocardium.

Our hypothesis on the function of the trabecular component is underscored by two independent studies in transgenic mice with a targeted loss-of-function mutation in the retinoid X receptor α (RXRα) and the neuregulin signal-transduction pathway. RXRα mutant mice show hypoplastic ventricular development with thinned compact ventricular walls and aberrant expression of MLC2a mRNA [229–231]. Homozygous embryos die between E13 and E16. Cardiac failure has been demonstrated as the responsible cause of death [229]. More recently, it has been reported that mutant mice lacking neuregulin show a primary cardiac malformation characterized by an arrest of the formation of the ventricular trabeculations [232]. Homozygous embryos die in utero around E11 showing an anomalous cardiac contraction pattern. Furthermore, the generation of targeted mutant mice lacking the neuregulin cell-surface receptors, erbB2 and erbB4, showed similar cardiac malformations, i.e. the absence of ventricular trabeculations at E11 [233,234]. These data show that the trabeculations exert a pivotal role in the early stages of cardiac function, as the embryos lacking trabeculations die in utero at E11. The compact myocardium becomes vital to cardiac function in the early fetal stage, since embryos lacking a thickening of the ventricular free walls die between E13 and 16.

6. Transcriptional regulation in the developing heart

In this section we will try to summarize the data in a simplistic model of patterns of transcription factors along the cardiac tube. We do realize that almost no distribution patterns of transcription factors have been reported yet. Of necessity, the model will be therefore speculative. Taking together the different patterns of expression of the endogenous genes, and reporter genes in transgenic mice, one can conclude that in the early tubular heart (or even before, in the cardiac crescent), certain genes are expressed homogeneously along the antero-posterior axis of the cardiac tube, whereas others show gradients of expression along this axis. At present several different *cis*-acting elements and their DNA-binding proteins (transcription factors) have been identified in the mammalian heart (see for recent reviews, [197,235]). MEF-2 and GATA-4 transcription factor families have been revealed to play a critical role in cardiac expression both in vitro and in vivo. However, none of these transcription factors have been demonstrated to show a regionalised pattern of expression in the developing heart. The available data indicate that they are equally expressed in all cardiac segments. The patterns of gene expression in opposite gradients along the anteropos-

terior axis of the tubular heart imply, however, that transcription factors controlling gene expression should be expressed in gradients also. This hypothesis is underscored by recent reports on the expression of MEF-2 family members in gradients during the early stages of cardiac morphogenesis in *Xenopus* [236]. It has been recently shown that dHAND, a member of the bHLH transcription factor family, is expressed in a cranio-caudal gradient along the tubular heart [181]. Thus we propose that in the straight cardiac tube, opposite gradients of transcription factors are present (Fig. 9A).

In the looped heart (Carnegie stage 12), five functionally different segments can be distinguished, i.e. the inflow tract, the atria, the atrioventricular canal, the ventricles and the outflow tract. At this stage, many contractile genes become restricted to either the atrial/inflow tract or the ventricular/outflow tract myocardium, having the border line at the transition between the ventricle and the atrioventricular canal. Interestingly, some genes maintain their original pattern of expression. Thus, atrial-specific genes, as α-MHC and MLC2a remain expressed in the arterial pole of the heart, whereas ventricular-specific genes, as MLC2v mRNA/protein are observed in the venous pole of the heart. The atrioventricular canal shows co-expression of both isoforms. In the ventricle, the atrial isoforms are no longer expressed (or at low levels); in the atrium, ventricular isoforms are no longer expressed (or at low levels). These observations may imply that in each of the domains (i.e. atrial domain and ventricular domain) a negative (repressor) domain-specific transcription factor is present (see Fig. 9B). This hypothetical model of transcription provides a simple explanation for the single atrial/ventricular isoforms expression in the atria/ventricles, and the co-expression of both isoforms in the flanking segments, i.e. inflow tract, atrioventricular canal and outflow tract (Fig. 9C). This hypothesis is underscored by the expression pattern of several transcription factors. GATA-5 mRNA is first homogeneously expressed along the tubular heart but becomes down-regulated in the ventricular myocardium with development [177]. More interestingly, eHAND and dHAND, members of the bHLH transcription factor family, show almost a complementary pattern of expression in the ventricular region of the developing heart [169,181]. dHAND is restricted to the outflow tract and the right ventricular free wall myocardium whereas eHAND is expressed mostly in the left ventricular free wall myocardium and transiently in the distal region of the outflow tract, where the expression expands into the aortic sac and branchial arches. Deletion of dHAND gene causes cardiac arrest soon after looping because of the apparent absence of a right ventricular chamber [169].

In the third stage of development, the heart becomes septated and, hence, distinct morphological right and left atrial and ventricular components can be recognised. It has been shown in transgenic mice that the right and left components are also transcriptionally different (see e.g. [32,117,118]). The combination of the studies of MLC3f and MLC2v transgenic mice suggest that the transcriptional specification of the right and left ventricles is not acquired before these components are morphologically identifiable. This is far later than the establishment of

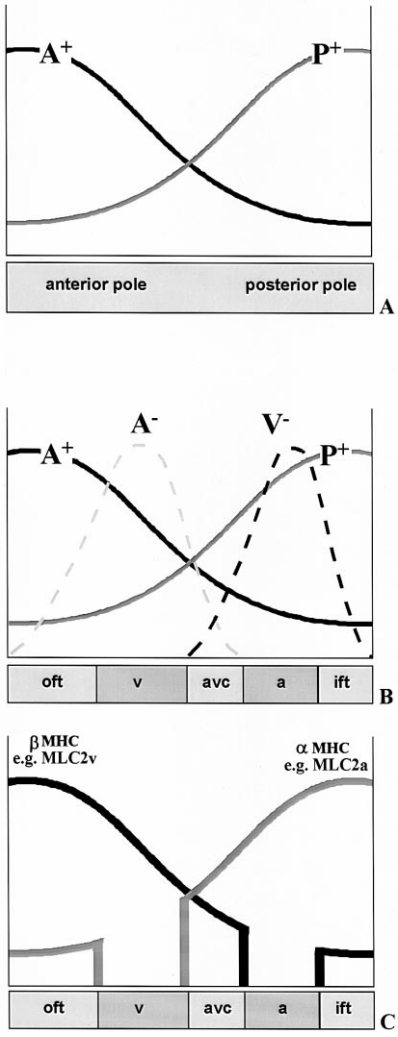


Fig. 9. A model of cardiac transcription. Panel A illustrates the hypothetical distribution of transcription factors (A^+, anterior; P^+, posterior) in anteroposterior (A^+) or posteroanterior (P^+) gradients of expression along the tubular heart. Both transcription factors are supposed to activate gene transcription resulting in gradients of expression of cardiac-specific genes (e.g. α-MHC, SERCA2a, cardiac TnI, cardiac α-actin). With the acquirement of five different cardiac segments, we think that at least two new transcription factors should become expressed (panel B) that would act as compartment-specific repressors (A^- and V^-), to account for the selective down-regulation of 'ventricular'-specific genes in the atria (V^-) or 'atrial'-specific genes in the ventricles (A^-). Examples that support this notion are the developmental expression of α-MHC and β-MHC mRNA that eventually become restricted to the atrial or ventricular domains [3,23]. This hypothesis predicts co-expression of α and β MHC in the outflow tract, atrioventricular canal and inflow tract, which indeed is observed. This pattern of expression is in fact also observed at the embryonic stage for the regulatory myosin light chains (depicted in panel C).

left–right sidedness in the embryo. These data support the notion that right–left myocardial specification does not depend on the right–left body axis but on the formation of the cardiac segments along the antero-posterior axis of the heart. Most interestingly these data beautifully demonstrate the presence of flanking segments as separate transcriptional domains as functionally demonstrated by De Jong et al. [120]. A possible model of right–left transcriptional specification could be reminiscent to the segmentation in *Drosophila* [237,238]. Combinatorial distribution of atrial/ventricular-'specific' activators and repressors could delimit seven transcriptional components, namely, inflow tract, right atrium, left atrium, atrioventricular canal, left ventricle, right ventricle and outflow tract. At present experimental evidence to support this model is rudimentary.

7. Perspectives

We have presented an overview of the complex of the patterns of cardiac gene expression during embryonic development. We have proposed a model to explain how the expression of different genes becomes restricted to morphologically identifiable compartments of the heart, reminiscent to the development of segments in *Drosophila*. As stated before, few patterns of expression of factors (i.e. transcription factors) have been described to support this model. At present, members of the bHLH family of transcription factors have been reported to become transiently expressed in distinct morphologically identifiable cardiac segments, allowing the possibility of a 'compartment-specific' regulation of gene expression (see [169,181,239]). Further search to find new transcription factors with a regionalised pattern of gene expression along the myocardium will provide insight into the understanding of regulation of gene expression within the developing heart.

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References

[1] Kamino K. Optical approaches to ontogeny of electrical activity and related functional organization during early heart development. Physiol Rev 1991;71:53–91.
[2] Van Mierop LHS. Localization of pacemaker in chick embryo heart at the time of initiation of heart beat. Am J Physiol 1967;212:407–415.
[3] Lyons GE. In situ analysis of the cardiac muscle gene program during embryogenesis. Trends Cardiovasc Med 1994;4:70–77.
[4] Moorman AFM, De Jong F, Lamers WH. Development of the conduction system of the heart. Pace 1997;20:2087–2092.
[5] Eisenberg LM, Markwald RR. Molecular regulation of atrioventricular valvuloseptal morphogenesis. Circ Res 1995;77:1–6.
[6] Kirby ML. Cellular and molecular contributions of the cardiac neural crest to cardiovascular development. Trends Cardiovasc Med 1993;3:18–23.
[7] Noden DM, Poelmann RE, Gittenberger-de Groot AC. Cell origins and tissue boundaries during outflow tract development. Trends Cardiovasc Med 1995;5:69–75.
[8] Butler H, Juurlink BHJ. An atlas for staging mammalian and chick embryos. Boca Raton, Florida: CRC Press, 1987.
[9] Hamburger V, Hamilton JL. A series of normal stages in the development of the chick embryo. J Morphol 1951;88:49–92.
[10] Moorman AFM, Lamers WH. Molecular anatomy of the developing heart. Trends Cardiovasc Med 1994;4:257–264.
[11] Ya J, Markman MWM, Wagenaar GTM, Blommaart PJE, Moorman AFM, Lamers, WH. Expression of the smooth-muscle proteins alpha smooth-muscle actin and calponin, and of the intermediate filament protein desmin are parameters of cardiomyocyte maturation in the prenatal rat heart. Anat Rec 1997;249:495–505.
[12] De la Cruz MV, Sánchez-Gómez C, Palomino M. The primitive cardiac regions in the straight tube heart (stage 9) and their anatomical expression in the mature heart: an experimental study in the chick embryo. J Anat 1989;165:121–131.
[13] Franco D, Kelly R, Moorman AFM, Buckingham M. Regionalised transcriptional domains of myosin light chain 3F transgenes in the embryonic mouse heart: morphogenetic implications. Dev Biol 1997;188:17–33.
[14] Lompré AM, Nadal-Ginard B, Mahdavi V. Expression of the cardiac ventricular α- and β-myosin heavy chain genes is developmentally and hormonally regulated. J Biol Chem 1984;259:6437–6446.
[15] Mahdavi V, Izumo S, Nadal-Ginard B. Developmental and hormonal regulation of sarcomeric myosin heavy chain gene family. Circ Res 1987;60:804–814.
[16] Emerson CPJ, Berstein SI. Molecular genetics of myosin. Ann Rev Biochem 1987;56:695–726.
[17] De Jong F, Geerts WJC, Lamers WH, Los JA, Moorman AFM. Isomyosin expression patterns in tubular stages of chicken heart development: a 3-D immunohistochemical analysis. Anat Embryol 1987;177:81–90.
[18] De Jong F, Geerts WJC, Lamers WH, Los JA, Moorman AFM. Isomyosin expression pattern during formation of the tubular chicken heart: a 3D immunohistochemical analysis. Anat Rec 1990;226:213–227.
[19] Oana S, Matsuoka R, Nakajima H, et al. Molecular characterization of a novel atrial-specific myosin heavy-chain gene expressed in the chick embryo. Eur J Cell Biol 1995;67:42–49.

[20] Yutzey KE, Rhee JT, Bader D. Expression of the atrial-specific myosin heavy chain AMHC1 and the establishment of anteroposterior polarity in the developing chicken heart. Development 1994;120:871–883.
[21] Bisaha JG, Bader D. Identification and characterization of a ventricular-specific avian myosin heavy chain, VMHC1: Expression in differentiating cardiac and skeletal muscle. Dev Biol 1991;148:355–364.
[22] De Groot IJM, Lamers WH, Moorman AFM. Isomyosin expression pattern during rat heart morphogenesis: an immunohistochemical study. Anat Rec 1989;224:365–373.
[23] Lyons GE, Schiaffino S, Sassoon D, Barton P, Buckingham ME. Developmental regulation of myosin expression in mouse cardiac muscle. J Cell Biol 1990;111:2427–2437.
[24] Wessels A, Vermeulen JLM, Virágh S, Kálmán F, Lamers WH, Moorman AFM. Spatial distribution of ''tissue specific'' antigens in the developing human heart and skeletal muscle: II. An immunohistochemical analysis of myosin heavy chain isoform expression patterns in the embryonic heart. Anat Rec 1991;229:355–368.
[25] Evans D, Miller JB, Stockdale FE. Developmental patterns of expression and coexpression of myosin heavy chain in atria and ventricles of the avian heart. Dev Biol 1988;127:376–383.
[26] De Jong F, De Groot IJM, Geerts WJC, et al. Immunohistochemical evidence for two differentially expressed atrial myosin heavy chain isoforms during avian cardiogenesis, In: Carraro U, editor. Sarcomeric and non-sarcomeric muscles: basic and applied research prospects for the 90s. Padova: Unipress, 1988:299–304.
[27] Yutzey KE, Bader D. Diversification of cardiomyocytes cell lineages during early heart development. Circ Res 1995;77:216–219.
[28] Barton PJR, Buckingham ME. The myosin alkali light chain proteins and their genes. Biochem J 1985;231:249–261.
[29] Nabeshima YI, Fujii-Kuriyama Y, Muramatsu M, Ogata K. Alternative transcription and two modes of splicing result in two myosin light chains from one gene. Nature 1984;308:333–338.
[30] Periasamy M, Strehler EE, Garfinkel LI, Gubits RM, Ruiz-Opazo N, Nadal-Ginard B. Fast skeletal muscle myosin light chains 1 and 3 are produced from a single gene by a combined process of differential RNA transcription and splicing. J Biol Chem 1984;259:13595–13604.
[31] Robert B, Daubas P, Akimenko MA, et al. A single locus in the mouse encodes both myosin light chains 1 and 3, a second locus corresponds to a related pseudogene. Cell 1984;39:129–140.
[32] Kelly R, Alonso S, Tajbaksh S, Cossu G, Buckingham M. Myosin light chain 3F regulatory sequences confer regionalised cardiac and skeletal muscle expression in transgenic mice. J Cell Biol 1995;129:383–396.
[33] McGrew MJ, Bogdanova N, Hasegawa K, Hughes SH, Kitsis RN, Rosenthal N. Distinct gene expression patterns in skeletal and cardiac muscle are dependent on common regulatory sequences in the MLC1/3 locus. Mol Cell Biol 1996;16:4524–4534.
[34] Hailstones DL, Gunning PW. Characterization of human myosin light chains 1sa and 3nm: Implications for isoform evolution and function. Mol Cell Biol 1990;10:1095–1104.
[35] Faerman A, Shani M. The expression of the regulatory myosin light chain 2 gene during mouse embryogenesis. Development 1993;118:919–929.
[36] Kubalak SW, Miller-Hance WC, O'Brien TX, Dyson E, Chien KR. Chamber specification of atrial myosin light chain-2 expression precedes septation during murine cardiogenesis. J Biol Chem 1994;269:16961–16970.
[37] Lyons I, Parsons LM, Hartley L, et al. Myogenic and morphogenetic defects in the heart tubes of murine embryos lacking the homeo box gene Nkx2-5. Genes Dev 1995;9:1654–1666.
[38] O'Brien TX, Lee KJ, Chien KR. Positional specification of ventricular myosin light chain 2 expression in the primitive murine heart tube. Proc Natl Acad Sci USA 1993;90:5157–5161.
[39] Wagenaar GTM, Markman MWM, Lamers WH, Moorman AFM. Myosin light chain 2 V gene expression does not demarcate the anatomical boundaries of the rat embryonic ventricle. J Mol Cell Cardiol 1995;27:A79.
[40] Barton PJR, Robert B, Cohen A, et al. Structure and sequence of the myosin alkali light chain gene expressed in adult cardiac atria and fetal striated muscle. J Biol Chem 1988;263:12669–12676.
[41] Franco D, Jing Y, Wagenaar GTM, Lamers WH, Moorman, AFM. The trabecular component of the embryonic ventricle, In: Ost'ádal B, Nagano M, Takeda N, Dhalla NS, editors. The developing heart. New York: Lippincott Raven, 1996:51–60.
[42] Franco D, Wagenaar GTM, Jing Y, Moorman AFM, Lamers WH. Contribution of the embryonic outflow tract myocardium to the adult rat heart. Eur Heart J 1996;17:40.
[43] Bennett P, Craig R, Starr R, Offer G. The ultrastructural location of C-protein, X-protein and H-protein in rabbit muscle. J Muscle Res Cell Motil 1986;7:550–567.
[44] Fürst DO, Vinkemeier U, Weber K. Mammalian skeletal muscle C-protein: purification from bovine muscle, binding to titin and the characterization of a full-length human cDNA. J Cell Sci 1992;102:769–778.
[45] Chang KS, Zimmer WE, Bergsma DJ, Dodgson JB, Schwartz RJ. Isolation and characterization of six different chicken actin genes. Mol Cell Biol 1984;4:2498–2508.
[46] Czosnek H, Nudel U, Mayer Y, et al. The genes coding for the cardiac muscle actin, the skeletal muscle actin and the cytoplasmic β-actin are located on three different mouse chromosomes. EMBO J 1983;2:1977–1979.
[47] Vandekerckhove J, Weber K. The complete amino acid sequence of actins from bovine aorta, bovine heart, bovine fast skeletal muscle and rabbit slow skeletal muscle. Differentiation 1979;14:123–133.
[48] Ruzicka DL, Schwartz RJ. Sequential activation during avian cardiogenesis: vascular smooth muscle α-actin gene transcripts mark the onset of cardiac differentiation. J Cell Biol 1988;107:2575–2585.
[49] Sassoon DA, Garner I, Buckingham ME. Transcripts of α-cardiac and α-skeletal actins are early markers for myogenesis in the mouse. Development 1988;104:155–164.
[50] Sugi Y, Lough J. Onset of expression and regional deposition of alpha-smooth and sarcomeric actin during avian heart development. Dev Dyn 1992;193:116–124.
[51] Boheler KR, Moorman AFM. Molecular aspects of myocardial differentiation. Eur Heart J 1995;16:3–7.
[52] Gregorio CC, Fowler VM. Tropomodulin function and thin filament assembly in cardiac myocytes. Trends Cardiovasc Med 1996;6:136–141.
[53] Gregorio CC, Weber A, Bondad M, Pennise CR, Fowler VM. Requirement of pointed-end capping by tropomodulin to maintain actin filament length in embryonic chick cardiac myocytes. Nature 1995;377:83–86.
[54] Sussman MA, Sakhi S, Barrientos P, Ito M, Kedes L. Tropomodulin in rat cardiac muscle. Localization of protein is independent of messenger RNA distribution during myofibrillar development. Circ Res 1994;75:221–232.
[55] Ito M, Swanson B, Sussman MA, Kedes L, Lyons G. Cloning of tropomodulin cDNA and localization of gene transcripts during mouse embryogenesis. Dev Biol 1995;167:317–328.
[56] Muthuchamy M, Pajak L, Howles P, Doetschman T, Wieczorek DF. Developmental analysis of tropomyosin gene expression in embryonic stem cells and mouse embryos. Mol Cell Biol 1993;13:3311–3323.
[57] Prados J, Fernández JE, Garrido F, et al. Expression of α-tropomyosin during cardiac development in the chick embryo. Anat Rec 1992;234:301–309.
[58] Collins JH. Myosin light chains and troponin C: structural and

evolutionary relationships revealed by amino acid sequence comparisons. J Muscle Res Cell Motil 1991;12:3–25.
[59] Nakayama S, Kretsinger RH. Evolution of the EF-hand family of proteins. Annu Rev Biophys Biomol Struct 1994;23:473–507.
[60] Collins JH, Greaser ML, Potter JD, Horn MJ. Determination of the amino acid sequence of troponin C from rabbit skeletal muscle. J Biol Chem 1977;252:6356–6362.
[61] Dhoot GK, Frearson N, Perry SV. Polymorphic forms of troponin T and troponin C and their localization in striated muscle cell types. Exp Cell Res 1979;122:339–350.
[62] Wilkinson JM. Troponin C from rabbit slow skeletal and cardiac muscle is the product of a single gene. Eur J Biochem 1980;103:179–188.
[63] Parmacek MS, Leiden JM. Structure and expression of the murine slow/cardiac troponin C gene. J Biol Chem 1989;264:13217–13225.
[64] Schiaffino S, Gorza L, Ausoni S. Troponin isoform switching in the developing heart and its functional consequences. Trends Cardiovasc Med 1993;3:12–17.
[65] Toyota N, Shimada Y. Differentiation of troponin in cardiac and skeletal muscles in chicken embryos as studied by immunofluorescence microscopy. J Cell Biol 1981;91:497–504.
[66] Ausoni S, De Nardi C, Moretti P, Gorza L, Schiaffino S. Developmental expression of rat cardiac troponin I mRNA. Development 1991;112:1041–1051.
[67] Gorza L, Ausoni S, Mercial N, Hastings KEM, Schiaffino S. Regional differences in troponin I isoform switching during rat heart development. Dev Biol 1993;156:253–264.
[68] Zhu L, Lyons GE, Juhasz O, Joya JE, Hardeman EC, Wade R. Developmental regulation of troponin I isoform genes in striated muscles of transgenic mice. Dev Biol 1995;169:487–503.
[69] Drysdale TA, Tonissen KF, Patterson KD, Crawford MJ, Krieg PA. Cardiac troponin I is a heart-specific marker in the *Xenopus* embryo expression during abnormal heart morphogenesis. Dev Biol 1994;165:432–441.
[70] Breitbart RE, Nguyen HT, Medford RM, Destree AT, Mahdavi V, Nadal-Ginard B. Intricate combinatorial patterns of exon splicing generate multiple regulated troponin T isoforms from a single gene. Cell 1985;41:67–82.
[71] Cooper TA, Ordahl CP. A single cardiac troponin T gene generates embryonic and adult isoforms via developmentally regulated alternate splicing. J Biol Chem 1985;260:11140–11148.
[72] Sabry MA, Dhoot GK. Identification of and pattern of transitions of cardiac, adult slow and slow skeletal muscle-like embryonic isoforms of troponin T in developing rat and human skeletal muscles. J Muscle Res Cell Motil 1991;12:262–270.
[73] Sabry MA, Dhoot GK. Identification of and changes in the expression of troponin T isoforms in developing avian and mammalian heart. J Mol Cell Cardiol 1989;21:85–91.
[74] Miano JM, Olson EN. Expression of the smooth muscle cell calponin gene marks the early cardiac and smooth muscle cell lineages during mouse embryogenesis. J Biol Chem 1996;271:7095–7103.
[75] Samaha FF, Ip HS, Morrisey EE, et al. Developmental pattern of expression and genomic organization of the calponin-h1 gene. J Biol Chem 1996;271:395–403.
[76] Duband JL, Gimona M, Scatena M, Sartore S, Small JV. Calponin and SM 22 as differentiation markers of smooth muscle: spatiotemporal distribution during avian embryonic development. Differentiation 1993;55:1–11.
[77] Gimona M, Herzog M, Vandekerckhove J, Small JV. Smooth muscle specific expression of calponin. FEBS Lett 1990;274:159–162.
[78] Takahashi K, Nadal-Ginard B. Molecular cloning and sequence analysis of smooth muscle calponin. J Biol Chem 1991;266:13284–13288.
[79] Li L, Miano JM, Cserjesi P, Olson EN. SM22α, a marker of adult smooth muscle, is expressed in multiple myogenic lineages during embryogenesis. Circ Res 1996;78:188–195.
[80] Miano JM, Cserjesi P, Ligon KL, Periasamy M, Olson EN. Smooth muscle myosin heavy chain exclusively marks the smooth muscle lineage during mouse embryogenesis. Circ Res 1994;75:803–812.
[81] Kurzban GP, Wang K. Giant polypeptides of skeletal muscle titin: sedimentation equilibrium in guanidine hydrochloride. Biochem Biophys Res Commun 1988;150:1155–1161.
[82] Maruyama K, Hu DH, Suzuki T, Kimura S. Binding of actin filaments to connectin. J Biochem 1987;101:1339–1346.
[83] Hill CS, Duran S, Lin Z, Weber K, Holtzer H. Titin and myosin, but not desmin, are linked during myofibrillogenesis in postmitotic mononucleated myoblasts. J Cell Biol 1986;103:2185–2196.
[84] Fritz JD, Wolff JA, Greaser ML. Partial titin cDNA sequence isolated from rabbit cardiac muscle RNA. J Muscle Res Cell Motil 1993;14:347–350.
[85] Kolmerer B, Olivieri N, Witt CC, Herrmann BG, Labeit S. Genomic organization of M line titin and its tissue-specific expression in two distinct isoforms. J Mol Biol 1996;256:556–563.
[86] Schachat FH, Canine AC, Briggs MM, Reedy MC. The presence of two skeletal muscle α-actinins correlates with troponin-tropomyosin expression and Z-line width. J Cell Biol 1985;101:1001–1008.
[87] Bantle S, Keller S, Haussmann I, et al. Tissue-specific isoforms of chicken myomesin are generated by alternative splicing. J Biol Chem 1996;271:19042–19052.
[88] Korczak B, Zarain-Herzberg A, Brandl CJ, Ingles CJ, Green NM, MacLennan DH. Structure of the rabbit fast-twitch skeletal muscle Ca^{2+}-ATPase gene. J Biol Chem 1988;263:4813–4819.
[89] Lompré AM, De la Bastie D, Boheler KR, Schwartz K. Characterization and expression of the rat heart sarcoplasmic reticulum Ca^{2+}-ATPase mRNA. FEBS Lett 1989;249:35–41.
[90] Lytton J, MacLennan DH. Molecular cloning of cDNAs from human kidney coding for two alternatively spliced products of the cardiac Ca^{2+}-ATPase gene. J Biol Chem 1988;263:15024–15031.
[91] Anger M, Samuel JL, Marotte F, Wuytack F, Rappaport L, Lompré AM. In situ mRNA distribution of sarco(endo)plasmic reticulum Ca^{2+}-ATPase isoforms during ontogeny in the rat. J Mol Cell Cardiol 1994;26:539–550.
[92] Moorman AFM, Vermeulen JLM, Koban MU, Schwartz K, Lamers WH, Boheler KR. Patterns of expression of sarcoplasmic reticulum Ca^{2+} ATPase and phospholamban mRNAs during rat heart development. Circ Res 1995;76:616–625.
[93] Eggermont JA, Wuytack F, Verbist J, Casteels R. Expression of endoplasmic-reticulum Ca^{2+}-pump isoforms and of phospholamban in pig smooth-muscle tissues. Biochem J 1990;271:649–653.
[94] Jorgensen AO, Jones LR. Localization of phospholamban in slow but not fast canine skeletal muscle fibers. An immunocytochemical and biochemical study. J Biol Chem 1986;261:3775–3781.
[95] Fujii J, Ueno A, Kitano K, Tanaka S, Kadoma M, Tada M. Complete complementary DNA-derived amino acid sequence of canine cardiac phospholamban. J Clin Invest 1987;79:301–304.
[96] Fujii J, Lytton J, Tada M, MacLennan DH. Rabbit cardiac and slow-twitch muscle express the same phospholamban gene. FEBS Lett 1988;227:51–55.
[97] Raeymaekers L, Hofmann F, Casteels R. Cyclic, GMP-dependent protein kinase phosphorylates phospholamban in isolated sarcoplasmic reticulum from cardiac and smooth muscle. Biochem J 1988;252:269–273.
[98] Simmerman HKB, Collins JH, Theibert JL, Wegener AD, Jones LR. Sequence analysis of phospholamban. Identification of phosphorylation sites and two major structural domains. J Biol Chem 1986;261:13333–13341.
[99] Toyofuku T, Doyle DD, Zak R, Kordylewski L. Expression of phospholamban mRNA during early avian muscle morphogenesis is distinct from that of α-actin. Dev Dyn 1993;196:103–113.
[100] Langer GA. Myocardial calcium compartmentation. Trends Cardiovasc Med 1994;4:103–109.

[101] Marks AR. Expression and regulation of ryanodine receptor/calcium release channels. Trends Cardiovasc Med 1996;6:130–135.
[102] Marks AR, Tempst P, Hwang KS, et al. Molecular cloning and characterization of the ryanodine receptor/junctional channel complex cDNA from skeletal muscle sarcoplasmic reticulum. Proc Natl Acad Sci USA 1989;86:8683–8687.
[103] Takeshima H, Nishimura S, Matsumoto T, et al. Primary structure and expression from complementary DNA of skeletal muscle ryanodine receptor. Nature 1989;339:439–445.
[104] Zorzato F, Fujii J, Otsu K, et al. Molecular cloning of cDNA encoding human and rabbit forms of the Ca^{2+} release channel (ryanodine receptor) of skeletal muscle. J Biol Chem 1990;265:2244–2256.
[105] Gorza L, Vettore S, Tessaro A, Sorrentino V, Vitadello M. Regional and age-related differences in mRNA composition of intracellular Ca^{2+}-release channels of rat cardiac myocytes. J Mol Cell Cardiol 1997;29:1023–1036.
[106] Lee S-L, Yu ASL, Lytton J. Tissue-specific expression of Na^+–Ca^{2+} exchanger isoforms. J Biol Chem 1994;269:14849–14852.
[107] Quednau BD, Nicoll DA, Philipson KD. Tissue specificity and alternative splicing of the Na^+/Ca^{2+} exchanger isoforms NCX1, NCX2 and NCX3 in rat. Am J Physiol 1997;272:C1250–C1261.
[108] Schneider JW, Mercer RW, Gilmore-Herbert M, et al. Tissue specificity, localization in brain, and cell-free translation of mRNA encoding the A3 isoform of Na^+ K^+-ATPase. Proc Natl Acad Sci USA 1988;85:284–288.
[109] Orlowski J, Lingrel JB. Tissue-specific and developmental regulation of rat Na, K-ATPase catalytic α isoform and β subunit mRNAs. J Biol Chem 1988;263:10436–10442.
[110] Herrera VLM, Cova T, Sassoon D, Ruiz-Opazo N. Developmental cell-specific regulation of Na^+–K^+-ATPase α_1-, α_2-, and α_3-isoform gene expression. Am J Physiol 1994;266:C1301–C1312.
[111] Gros DB, Jongsma HJ. Connexins in mammalian heart function. Bioessays 1996;18:719–730.
[112] Delorme B, Dahl E, Jarry-Guichard T, et al. Developmental regulation of connexin40 gene expression in mouse heart correlates with the differentiation of the conduction system. Dev Dyn 1995;204:358–371.
[113] Van Kempen MJA, Vermeulen JLM, Moorman AFM, Gros DB, Paul DL, Lamers WH. Developmental changes of connexin40 and connexin43 mRNA-distribution patterns in the rat heart. Cardiovasc Res 1996;32:886–900.
[114] Fromaget C, El Aoumari A, Gros D. Distribution pattern of connexin43, a gap junctional protein, during the differentiation of mouse heart myocytes. Differentiation 1992;51:9–20.
[115] Gourdie RG, Green RC, Severs NJ, Thompson RP. Immunolabeling patterns of gap junction connexins in the developing and mature rat heart. Anat Embryol 1992;185:363–378.
[116] Van Kempen MJA, Fromaget C, Gros D, Moorman AFM, Lamers WH. Spatial distribution of connexin-43, the major cardiac gap junction protein, in the developing and adult rat heart. Circ Res 1991;68:1638–1651.
[117] Biben C, Hadchouel J, Tajbakhsh S, Buckingham M. Developmental and tissue-specific regulation of the murine cardiac actin gene in vivo depends on distinct skeletal and cardiac muscle-specific enhancer elements in addition to the proximal promoter. Dev Biol 1996;173:200–212.
[118] Ross RS, Navankasattusas S, Harvey RP, Chien KR. An HF-1a/HF-1b/MEF-2 combinatorial element confers cardiac ventricular specificity and established an anterior-posterior gradient of expression. Development 1996;122:1799–1809.
[119] Kuisk IR, Li H, Tran D, Capetanaki Y. A single mEF2 site governs desmin transcription in both heart and skeletal muscle during mouse embryogenesis. Dev Biol 1996;174:1–13.
[120] de Jong F, Opthof T, Wilde AAM, et al. Persisting zones of slow impulse conduction in developing chicken hearts. Circ Res 1992;71:240–250.
[121] Kammermeier H. High energy phosphate of the myocardium: concentration versus free energy change. Basic Res Cardiol 1987;82:31–36. Suppl. 2.
[122] Kammermeier H. Why do cells need phosphocreatine and a phosphocreatine shuttle?. J Mol Cell Cardiol 1987;19:115–118.
[123] Babbit PC, Kenyon GL, Kuntz LD, et al. Comparison of nine creatine kinase primary structures: implications for structure–activity relationships. J Protein Chem 1986;5:1–14.
[124] Lyons GE, Mühlebach S, Moser A, et al. Developmental regulation of creatine kinase gene expresion by myogenic factors in embryonic mouse and chick skeletal muscle. Development 1991;113:1017–1029.
[125] Hasselbaink HDJ, Labruyère WT, Moorman AFM, Lamers WH. Creatine kinase isozyme expression in prenatal rat heart. Anat Embryol 1990;182:195–203.
[126] Lamers WH, Geerts WJC, Moorman AFM, Dottin RP. Creatine kinase isozyme expression in embryonic chicken heart. Anat Embryol 1989;179:387–393.
[127] Wessels A, Vermeulen JLM, Virágh S, et al. Spatial distribution of ''tissue-specific'' antigens in the developing human heart and skeletal muscle. I. An immunohistochemical analysis of creatine kinase isoenzyme expression patterns. Anat Rec 1990;228:163–176.
[128] van Groningen JJM, Bloemers HPJ, Swart GWM. Rat desmin gene structure and expression. Biochim Biophys Acta 1994;1217:107–109.
[129] Babai F, Musevi-Aghdam J, Schurch W, Royal A, Gabbiani G. Coexpression of α-sarcomeric actin, α-smooth muscle actin and desmin during myogenesis in rat and mouse embryos I. Skeletal muscle. Differentiation 1990;44:132–142.
[130] Schaart G, Viebahn C, Langmann W, Ramaekers FCS. Desmin and titin expression in early postimplantation mouse embryos. Development 1989;107:585–596.
[131] Huttenlocher A, Sandborg RR, Horwitz AF. Adhesion in cell migration. Curr Opin Cell Biol 1995;7:697–706.
[132] Mould AP. Getting integrins into shape: recent insights into how integrin activity is regulated by conformational changes. J Cell Sci 1996;109:2613–2618.
[133] Ruoslathi E, Engvall E. Perspectives series: cell adhesion in vascular biology. J Clin Invest 1997;99:1149–1152.
[134] Horwitz AF. Integrins and health. Sci Am 1997:46–53.
[135] Buck CA, Baldwin HS, DeLisser H, et al. Cell adhesion receptors and early mammalian heart development: an overview. CR Acad Sci Paris 1993;316:849–859.
[136] Carver W, Price RL, Raso DS, Terracio L, Borg TK. Distribution of β-1 integrin in the developing rat heart. J Histochem Cytochem 1994;42:167–175.
[137] Duband JL, Belkin AM, Syfrig J, Thiery JP, Koteliansky V. Expression of α1 integrin, a laminin-collagen receptor, during myogenesis and neurogenesis in the avian embryo. Development 1992;116:585–600.
[138] Hierck BP, Gittenberger-de Groot AC, Van Iperen L, Brouwer A, Poelmann RE. Expression of the β4 integrin subunit in the mouse heart during embryonic development: retinoic acid advances β4 expression. Dev Dyn 1996;207:89–103.
[139] Pow CST, Hendrickx AG. Integrin distribution during heart development in the long-tailed macaque (*M. fascicularis*). Anat Rec 1995;243:241–253.
[140] Roman J, McDonald JA. Expression of fibronectin, the integrin α5, and α-smooth muscle actin in heart and lung development. Am J Respir Cell Mol Biol 1992;6:472–480.
[141] Collo G, Domanico SZ, Klier G, Quaranta V. Gradient of integrin alpha 6A distribution in the myocardium during early heart development. Cell Adhes Commun 1995;3:101–113.
[142] Stepp MA, Urry LA, Hynes RO. Expression of alpha 4 integrin

mRNA and protein and fibronectin in the early chicken embryo. Cell Adhes Commun 1994;2:359–375.
[143] Hatta K, Takeichi M. Expression of N-cadherin adhesion molecules associated with early morphogenetic events in chick development. Nature 1986;320:447–449.
[144] Hatta K, Takagi S, Fujisawa H, Takeichi M. Spatial and temporal expression pattern of N-cadherin cell adhesion molecules correlated with morphogenetic processes of chicken embryos. Dev Biol 1987;120:215–227.
[145] Inoue T, Chisaka O, Matsunami H, Takeichi M. Cadherin-6 expression transiently delineates specific rhombomeres, other neural tube subdivisions, and neural crest subpopulations in mouse embryos. Dev Biol 1997;183:183–194.
[146] Duband JL, Volberg T, Sabanay I, Thiery JP, Geiger B. Spatial and temporal distribution of the adherens-junction-associated adhesion molecule A-CAM during avian embryogenesis. Development 1988;103:325–344.
[147] Radice GL, Rayburn H, Matsunami H, Knudsen KA, Takeichi M, Hynes RO. Developmental defects in mouse embryos lacking N-cadherin. Dev Biol 1997;181:64–78.
[148] Linask KK, Knudsen KA, Gui YH. N-cadherin–catenin interaction: necessary component of cardiac cell compartmentalization during early vertebrate heart development. Dev Biol 1997;185:148–164.
[149] Shiraishi I, Takamatsu T, Fujita S. 3-D observation of N-cadherin expression during cardiac myofibrillogenesis of the chick embryo using a confocal laser scanning microscope. Anat Embryol 1993;102:115–120.
[150] Linask KK, Lash JW. Early heart development: Dynamics of endocardial cell sorting suggests a common origin with cardiomyocytes. Dev Dyn 1993;195:62–69.
[151] Linask KK. N-cadherin localization in early heart development and polar expression of Na^+, K^+-ATPase, and integrin during pericardial coelom formation and epithelialization of the differentiating myocardium. Dev Biol 1992;151:213–224.
[152] Olson EN, Srivastava D. Molecular pathways controlling heart development. Science 1996;272:671–676.
[153] Harvey RP. *NK-2* homeobox genes and heart development. Dev Biol 1996;178:203–216.
[154] Evans T. Regulation of cardiac gene expression by GATA-4/5/6. Trends Cardiovasc Med 1997;7:75–83.
[155] Srivastava D, Cserjesi P, Olson EN. A subclass of bHLH proteins required for cardiac morphogenesis. Science 1995;270:1995–1999.
[156] Bour BA, O'Brien MA, Lockwood WL, et al. *Drosophila* MEF2, a transcription factor that is essential for myogenesis. Genes Dev 1995;9:730–741.
[157] Lilly B, Galewsky S, Firulli AB, Schulz RA, Olson EN. D-MEF2: a MADS box transcription factor expressed in differentiating mesoderm and muscle cell lineages during *Drosophila* embryogenesis. Proc Natl Acad Sci USA 1994;91:5662–5666.
[158] Gajewski K, Kim Y, Lee YM, Olson EN, Schulz RA. *D-mef2* is a target for Tinman activation during *Drosophila* heart development. EMBO J 1997;16:515–522.
[159] Lee KH, Xu Q, Breitbart RE. A new *tinman*-related gene, *nkx2.7*, anticipates the expression of *nkx2.5* and *nkx2.3* in zebrafish heart and pharyngeal endoderm. Dev Biol 1996;180:722–731.
[160] Ticho BS, Stainier DYR, Fishman MC, Breitbart RE. Three zebrafish MEF2 genes delineate somitic and cardiac muscle development in wild-type and mutant embryos. Mech Dev 1996;59:205–218.
[161] Kelley C, Blumberg H, Zon LI, Evans T. GATA-4 is a novel transcription factor expressed in endocardium of the developing heart. Development 1993;118:817–827.
[162] Jiang Y, Evans T. The *Xenopus* GATA-4/5/6 genes are associated with cardiac specification and can regulate cardiac-specific transcription during embryogenesis. Dev Biol 1996;174:258–270.
[163] Cleaver OB, Patterson KD, Krieg PA. Overexpression of the *tinman*-related genes *XNkx-2.5* and *XNkx-2.3* in *Xenopus* embryos results in myocardial hyperplasia. Development 1996;122:3549–3556.
[164] Gove C, Walmsley M, Nijjar S, et al. Over-expression of GATA-6 in *Xenopus* embryos blocks differentiation of heart precursors. EMBO J 1997;16:355–368.
[165] Laverriere AC, MacNeill C, Mueller C, Poelmann RE, Burch JBE, Evans T. GATA-4/5/6, a subfamily of three transcription factors transcribed in developing heart and gut. J Biol Chem 1994;269:23177–23184.
[166] Molkentin JD, Lin Q, Duncan SA, Olson EN. Requirement of the transcription factor GATA4 for heart tube formation and ventral morphogenesis. Genes Dev 1997;11:1061–1072.
[167] Kuo CT, Morrisey EE, Anandappa R, et al. GATA4 transcription factor is required for ventral morphogenesis and heart tube formation. Genes Dev 1997;11:1048–1060.
[168] Lin Q, Schwarz J, Bucana C, Olson EN. Control of mouse cardiac morphogenesis and myogenesis by transcription factor MEF2C. Science 1997;276:1404–1407.
[169] Srivastava D, Thomas T, Lin Q, Kirby ML, Brown D, Olson EN. Regulation of cardiac mesodermal and neural crest development by the bHLH transcription factor, dHAND. Nature Genet 1997;16:154–160.
[170] Heikinheimo M, Scandrett JM, Wilson DB. Localization of transcription factor GATA-4 to regions of the mouse embryo involved in cardiac development. Dev Biol 1994;164:361–373.
[171] Morrisey EE, Ip HS, Lu MM, Parmacek MS. GATA-6: a zinc finger transcription factor that is expressed in multiple cell lineages derived from lateral mesoderm. Dev Biol 1996;177:309–322.
[172] Komuro I, Izumo S. Csx: A murine homeobox-containing gene specifically expressed in the developing heart. Proc Natl Acad Sci USA 1993;90:8145–8149.
[173] Edmondson DG, Lyons GE, Martin JF, Olson EN. *Mef2* gene expression marks the cardiac and skeletal muscle lineages during mouse embryogenesis. Development 1994;120:1251–1263.
[174] Grépin C, Robitaille L, Antakly T, Nemer M. Inhibition of transcription factor GATA-4 expression blocks in vitro cardiac muscle differentiation. Mol Cell Biol 1995;15:4095–4102.
[175] Boettger T, Stein S, Kessel M. The chicken *NKX2.8* homeobox gene: a novel member of the NK-2 gene family. Dev Genes Evol 1997;207:65–70.
[176] Reecy JM, Yamada M, Cummings K, et al. Chicken Nkx-2.8: a novel homeobox gene expressed in early heart progenitor cells and pharyngeal pouch-2 and -3 endoderm. Dev Biol 1997;188:295–311.
[177] Morrisey EE, Ip HS, Tang Z, Lu MM, Parmacek MS. GATA-5: A transcriptional activator expressed in a novel temporally and spatially-restricted pattern during embryonic development. Dev Biol 1997;183:21–36.
[178] Kern MJ, Argao EA, Potter SS. Homeobox genes and heart development. Trends Cardiovasc Med 1995;5:47–54.
[179] Cserjesi P, Brown D, Lyons GE, Olson EN. Expression of the novel basic Helix–loop–Helix gene *eHAND* in neural crest derivates and extraembryonic membranes during mouse development. Dev Biol 1995;170:664–678.
[180] Hollenberg SM, Sternglanz R, Cheng PF, Weintraub H. Identification of a new family of tissue-specific basic helix–loop–helix proteins with a two-hybrid system. Mol Cell Biol 1995;15:3813–3822.
[181] Biben C, Harvey RP. Homeodomain factor Nkx2-5 controls left/right asymmetric expression of bHLH gene *eHand* during murine heart development. Genes Dev 1997;11:1357–1369.
[182] Catala F, Wanner R, Barton P, Cohen A, Wright W, Buckingham M. A skeletal muscle-specific enhancer regulated by factors binding to E and CArG boxes is present in the promoter of the mouse myosin light-chain 1A gene. Mol Cell Biol 1995;15:4585–4596.
[183] Zou Y, Evans S, Chen J, Kuo HC, Harvey RP, Chien KR. CARP, a

cardiac ankyrin repeat protein, is downstream in the *Nkx2-5* homeobox gene pathway. Development 1997;124:793–804.
[184] Wei Y, Bader D, Litvin J. Identification of a novel cardiac-specific transcript critical for cardiac myocyte differentiation. Development 1996;122:2779–2789.
[185] Croissant JD, Kim JH, Eichele G, et al. Avian serum response factor expression restricted primarily to muscle cell lineages is required for α-actin gene transcription. Dev Biol 1996;177:250–264.
[186] Nemer M, Lavigne JP, Drouin J, Thibault G, Gannon M, Antakly T. Expression of atrial natriuretic factor gene in heart ventricular tissue. Peptides 1986;7:1147–1152.
[187] Zeller R, Bloch KD, Williams BS, Arceci RJ, Seidman CE. Localized expression of the atrial natriuretic factor gene during cardiac embryogenesis. Genes Dev 1987;1:693–698.
[188] Seidman CE, Bloch KD, Klein KA, Smith JA, Seidman JG. Nucleotide sequences of the human and mouse atrial natriuretic factor genes. Science 1984;226:1206–1209.
[189] Scott JN, Jennes L. Distribution of atrial nutriuretic factor in fetal rat atria and ventricles. Cell Tissue Res 1987;248:479–481.
[190] Soreq H, Ben-Aziz R, Prody CA, et al. Molecular cloning and construction of the coding region for human acetylcholinesterase reveals a G+C-rich attenuating structure. Proc Natl Acad Sci USA 1990;87:9688–9692.
[191] Lamers WH, Geerts WJC, Moorman AFM. Distribution pattern of acetylcholinesterase in early embryonic chicken hearts. Anat Rec 1990;228:297–305.
[192] Lamers WH, te Kortschot A, Moorman AFM, Los JA. Acetylcholinesterase in prenatal rat heart: a marker for the early development of the cardiac conductive tissue?. Anat Rec 1987;217:361–370.
[193] Nakamura T, Ikeda T, Shimokawa I, et al. Distribution of acetylcholinesterase activity in the rat embryonic heart with reference to HNK-1 immunoreactivity in the conduction tissue. Anat Embryol 1994;190:367–373.
[194] Ito H, Iwasaki K, Ikeda T, Sakai H, Shimokawa I, Matsuo T. HNK-1 expression pattern in normal and bis-diamine induced malformed developing rat heart: three dimensional reconstruction analysis using computer graphics. Anat Embryol 1992;186:327–334.
[195] Nakagawa M, Thompson RP, Terracio L, Borg TK. Developmental anatomy of HNK-1 immunoreactivity in the embryonic rat heart: co-distribution with early conduction tissue. Anat Embryol 1993;187:445–460.
[196] Franco D, Moorman AFM, Lamers WH. Expression of the cholinergic signal-transduction pathway components during embryonic rat heart development. Anat Rec 1997;248:110–120.
[197] Robbins J. Regulation of cardiac gene expression during development. Cardiovasc Res 1996;31:E2–E16.
[198] Subramaniam A, Jones WK, Gulick J, Wert SE, Neumann J, Robbins J. Tissue-specific regulation of the α-myosin heavy chain gene promoter in transgenic mice. J Biol Chem 1991;266:24613–24620.
[199] Palermo J, Gulick J, Colbert M, Fewell J, Robbins J. Transgenic remodeling of the contractile apparatus in the mammalian heart. Circ Res 1996;78:504–509.
[200] Rindt H, Gulick J, Knotts S, Neumann JC, Robbins J. In vivo analysis of the murine β-myosin heavy chain gene promoter. J Biol Chem 1993;268:5332–5338.
[201] Knotts S, Sánchez A, Rindt H, Robbins J. Developmental modulation of a β myosin heavy chain promoter-driven transgene. Dev Dyn 1996;206:182–192.
[202] Franz WM, Brem G, Katus HA, Klingel K, Hofschneider PH, Kandolf R. Characterization of a cardiac-selective and developmentally upregulated promoter in transgenic mice. Cardioscience 1994;5:235–243.
[203] Lee KJ, Hickey R, Zhu H, Chien KR. Positive regulatory elements (HF-1a and HF-1b) and a novel negative regulatory element (HF-3) mediate ventricular muscle-specific expression of myosin light-chain 2-luciferase fusion genes in transgenic mice. Mol Cell Biol 1994;14:1220–1229.
[204] Lee KJ, Ross RS, Rockman HA, et al. Myosin light chain-2 luciferase transgenic mice reveal distinct regulatory programs for cardiac and skeletal muscle-specific expression of a single contractile protein gene. J Biol Chem 1992;267:15875–15885.
[205] Moorman AFM, De Boer PAJ, Vermeulen JLM, Lamers WH. Practical aspects of radio-isotopic in situ hybridization on RNA. Histochem J 1993;25:251–266.
[206] Zou Y, Chien KR. EFI_A/YB-1 is a component of cardiac HF-1A binding activity and positively regulates transcription of the myosin light-chain 2v gene. Mol Cell Biol 1995;15:2972–2982.
[207] Levitt LK, O'Mahoney JV, Brennan KJ, et al. The human troponin I slow promoter directs slow fiber-specific expression in transgenic mice. DNA Cell Biol 1995;14:599–607.
[208] Banerjee-Basu S, Buonanno A. *Cis*-acting sequences of the rat troponin I slow gene confer tissue- and development-specific transcription in cultured muscle cells as well as fiber type specificity in transgenic mice. Mol Cell Biol 1993;13:7019–7028.
[209] Hallauer PL, Bradshaw HL, Hastings KEM. Complex fiber-type-specific expression of fast skeletal muscle troponin I gene constructs in transgenic mice. Development 1993;119:691–701.
[210] Li Z, Marchand P, Humbert J, Babinet C, Paulin D. Desmin sequence elements regulating skeletal muscle-specific expression in transgenic mice. Development 1993;117:947–959.
[211] Virágh S, Challice CE. The development of the conduction system in the mouse embryo heart. IV. Differentiation of the atrioventricular conduction system. Dev Biol 1982;89:25–40.
[212] Shield MA, Haugen HS, Clegg CH, Hauschka SD. E-box sites and a proximal regulatory region of the muscle creatine kinase gene differentially regulate expression in diverse skeletal muscles and cardiac muscle of transgenic mice. Mol Cell Biol 1996;16:5058–5068.
[213] Li L, Miano JM, Mercer B, Olson EN. Expression of the SM22alpha promotor in transgenic mice provides evidence for distinct transcriptional regulatory programs in vascular and visceral smooth muscle cells. J Cell Biol 1996;132:849–859.
[214] Moessler H, Mericskay M, Li Z, Nagl S, Paulin D, Small JV. The SM 22 promotor directs tissue-specific expression in arterial but not in venous or visceral smooth muscle cells in transgenic mice. Development 1996;122:2415–2425.
[215] Haynes JI II, Duncan MK, Piatigorsky J. Spatial and temporal activity of the αB-crystallin/small heat shock protein gene promoter in transgenic mice. Dev Dyn 1996;207:75–88.
[216] Kimura S, Abe K, Suzuki M, et al. A 900 bp genomic region from the mouse dystrophin promoter directs *lacZ* reporter expression only to the right heart of transgenic mice. Dev Growth Differ 1997;39:257–265.
[217] De Jong F, Moorman AFM, Virágh S. Cardiac development: Prospects for a morphologically integrated molecular approach. In: Sipes IG, McQueen CA, Gandolfi AJ, editors. Comprehensive Toxicology. Cambridge: Cambridge University Press, 1997:5–26.
[218] Booth FW, Thomason DB. Molecular and cellular adaptation of muscle in response to exercise: perspectives of various models. Physiol Rev 1991;71:541–585.
[219] Kushmerick MJ. Energetics studies of muscles of different types. Basic Res Cardiol 1987;82:17–30. (Suppl. 2).
[220] Rall JA. Energetic aspects of skeletal muscle contraction: Implications of fiber types. Exerc Sport Sci Rev 1985;13:33–74.
[221] Arguello C, Alanis J, Pantoja O, Valenzuela B. Electrophysiological and ultrastructural study of the atrioventricular canal during the development of the chick embryo. J Mol Cell Cardiol 1986;18:499–510.

[222] Colyer J. Control of the calcium pump of cardiac sarcoplasmic reticulum. A specific role for the pentameric structure of phospholamban?. Cardiovasc Res 1993;27:1766–1771.
[223] Tada M, Katz AM. Phosphorylation of the sarcoplasmic reticulum and sarcolemma. Ann Rev Physiol 1982;44:401–423.
[224] Antzelevitch C, Sicouri S, Lukas A, Nesterenko VV, Liu DW, Di Diego JM. Regional differences in the electrophysiology of ventricular cells: physiological and clinical implications. In: Zipes DP, Jalife J, editors. Cardiac Electrophysiology: From Cell to Bedside. Philadelphia: W.B. Sanders Co., 1994:228–245.
[225] Furukawa T, Myerburg RJ, Furukawa N, Bassett AL, Kimura S. Differences in transient outward currents of feline endocardial and epicardial myocytes. Circ Res 1990;67:1287–1291.
[226] Liu DW, Gintant GA, Antzelevitch C. Ionic bases for electrophysiological distinctions among epicardial, midmyocardial, and endocardial myocytes from the free wall of the canine left ventricle. Circ Res 1993;72:671–687.
[227] Kimura S, Bassett AL, Kohya T, Kozlovskis PL, Myerburg RJ. Simultaneous recording of action potentials from endocardium and epicardium during ischemia in the isolated cat ventricle: relation of temporal electrophysiologic heterogeneities to arrythmias. Circulation 1986;74:401–409.
[228] Maylie J, Morad M. A transient outward current related to calcium release and development of tension in elephant seal atrial fibres. J Physiol 1984;357:267–292.
[229] Dyson E, Sucov HM, Kubalak SW, et al. Atrial-like phenotype is associated with embryonic ventricular failure in retinoid X receptor $\alpha -/-$ mice. Proc Natl Acad Sci USA 1995;92:7386–7390.
[230] Sucov HM, Dyson E, Gumeringer CL, Price J, Chien C, Evans RM. RXRα-mutant mice establish a genetic basis for vitamin A signalling in heart morphogenesis. Genes Dev 1994;8:1007–1018.
[231] Gruber PJ, Kubalak SW, Pexieder T, Sucov HM, Evans RM, Chien KR. *RXR*α deficiency confers genetic susceptibility for aortic sac, conotruncal, atrioventricular cushion, and ventricular muscle defects in mice. J Clin Invest 1996;98:1332–1343.
[232] Meyer D, Birchmeier C. Multiple essential functions of neuregulin in development. Nature 1995;378:386–390.
[233] Gassmann M, Casagranda F, Orioli D, et al. Aberrant neural and cardiac development in mice lacking the ErbB4 neuregulin receptor. Nature 1995;378:390–394.
[234] Lee KF, Simon H, Chen H, Bates B, Hung MC, Hauser C. Requirement for neuregulin receptor erbB2 in neural and cardiac development. Nature 1995;378:394–398.
[235] Mably JD, Liew C-C. Factors involved in cardiogenesis and the regulation of cardiac-specific gene expression. Circ Res 1996;79:4–13.
[236] Chen JN, Fishman MC. Zebrafish *tinman* homolog demarcates the heart field and initiates myocardial differentiation. Development 1996;122:3809–3816.
[237] Driever W, Nüsslein-Volhard C. The *bicoid* protein determines position in the *Drosophila* embryo in a concentration-dependent manner. Cell 1988;54:95–104.
[238] Driever W, Nüsslein-Volhard C. A gradient of *bicoid* protein in *Drosophila* embryos. Cell 1988;54:83–93.
[239] Lyons GE. Vertebrate heart development. Curr Opin Genet Dev 1996;6:454–460.
[240] Boheler KR, Chassagne C, Martin X, Wisnewsky C, Schwartz K. Cardiac expressions of α- and β-myosin heavy chains and sarcomeric α-actins are regulated through transcriptional mechanisms. J Biol Chem 1992;267:12979–12985.
[241] Buskin JN, Jaynes JB, Chamberlain JS, Hauschka SD. The mouse muscle creatine kinase cDNA and deduced amino acid sequences: comparison to evolutionarily related enzymes. J Mol Evol 1985;22:334–341.
[242] Lints TJ, Parsons LM, Hartley L, Lyons I, Harvey RP. *Nkx-2.5:* a novel murine homeobox gene expressed in early heart progenitor cells and their myogenic descendants. Development 1993;119:419–431.