UP-DATE

Genetics and molecular biology in cardiology (X)

Regulation of myocardial gene expression during heart development

Diego Franco, Jorge Domínguez, María del Pilar de Castro and Amelia Aránega

Departamento de Biología Experimental. Área de Biología Celular. Facultad de Ciencias Experimentales. Universidad de Jaén.

The heart is an organ with special significance in medicine and developmental biology. The development of the heart and its vessels during embryogenesis is the result of numerous and complex processes. At present, our understanding is based on decades of meticulous anatomical studies. However, the spectacular progress of modern molecular biology and developmental biology has marked the beginning of a new era in embryology. The molecular bases for cardiogenesis are just emerging. Several families of genes with restricted expression to the heart have been identified in the last years, including genes encoding for contractile proteins, ion channels as well as transcription factors involved in tissue specific gene expression. Likewise, the analyses of regulatory elements have increased our understanding of the molecular mechanisms directing gene expression. In this review, we illustrate the different patterns of gene and transgene expression in the developing myocardium. These data demonstrate that the wide molecular heterogeneity observed in the developing myocardium is not restricted to embryogenesis but it also remains in the adulthood. Therefore, such molecular diversity should be taken into account on the design of future gene therapy approaches, having thus direct clinical implications.

Key words: *Gene expression. Cardiogenesis. Transcriptional regulation.*

Regulación de la expresión génica en el miocardio durante el desarrollo cardíaco

El corazón es un órgano con una especial significación en medicina, en biología del desarrollo y desde el punto de vista evolutivo. La formación del corazón y sus vasos durante la embriogénesis es el resultado de numerosos y complicados procesos. Nuestro conocimiento actual de cómo estos procesos se llevan a cabo está basado en décadas de minuciosos estudios anatómicos. Sin embargo, el espectacular avance de la biología molecular del desarrollo ha marcado el inicio de una nueva era en la embriología, y las bases moleculares de la cardiogénesis están comenzando a emerger. En los últimos años se han identificado varias familias de genes con un patrón específico de expresión en el corazón. Entre ellos se engloban proteínas contráctiles, canales iónicos y factores de transcripción que dictan la expresión de genes específicos de tejido. Así mismo, el análisis de elementos reguladores de la expresión génica constituye, en la actualidad, la clave para la futura aplicación de la terapia génica. Este artículo recoge los últimos datos de patrones de expresión de genes específicos de miocardio durante el desarrollo cardíaco, y también datos recientes derivados del análisis funcional de zonas de regulación génica (transgénicos). Los distintos patrones de expresión revelan una amplia heterogeneidad molecular en el miocardio, y su conocimiento abre las puertas a importantes aplicaciones clínicas en el futuro.

Palabras clave: *Expresión génica. Cardiogénesis. Regulación transcripcional.*

INTRODUCTION

In recent years there have been important advances in our knowledge of the specific expression of tissue and the molecular mechanisms that regulate it. It has been shown repeatedly that different genes (and their protein products) experience changes in their spatiotemporal distribution and level of expression during ontogenesis; these changes are especially dynamic during heart development. The formation of the heart in

Correspondence: Dr. D. Franco.
Departamento de Biología Experimental.
Facultad de Ciencias Experimentales.
Edificio B-3, 313. Universidad de Jaén. 23071 Jaén.
e-mail: dfranco@ujaen.es

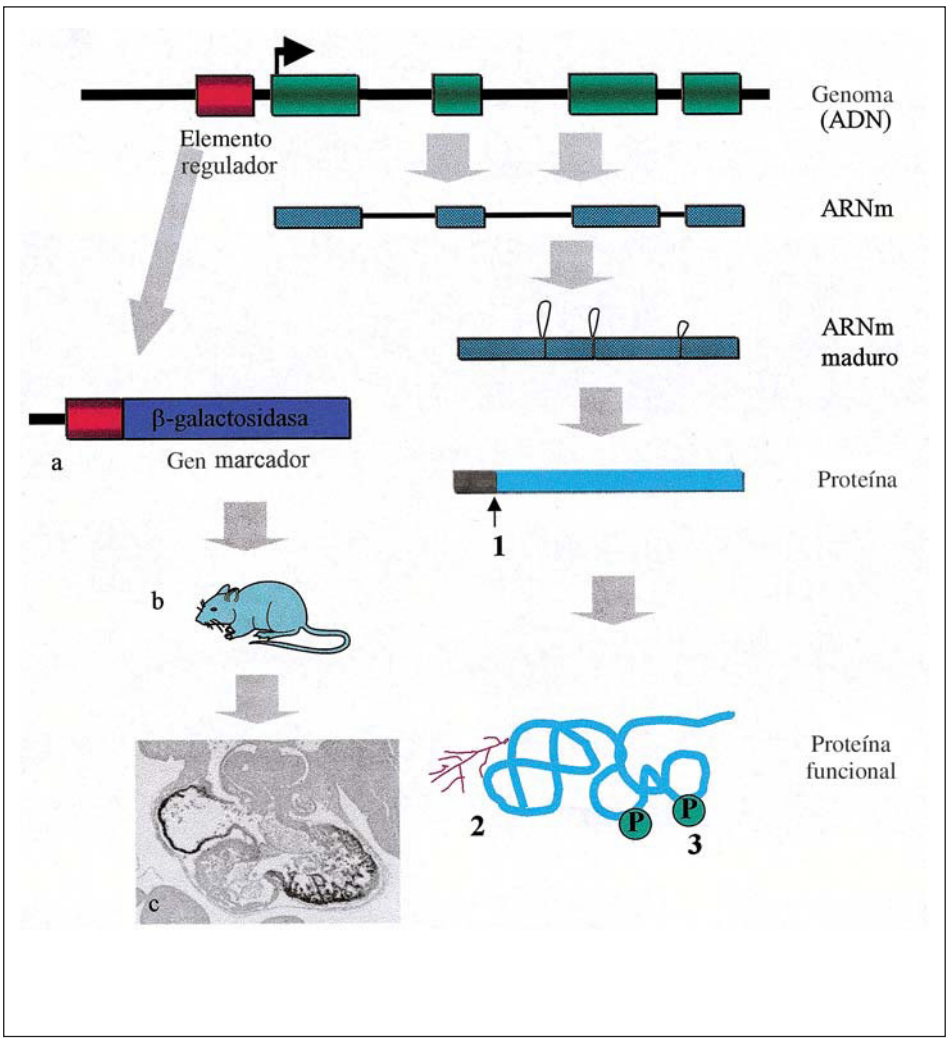

Fig. 1. Schematic representation of gene regulation processes and methods of transgene analysis. The genome is transcribed in discrete areas (genes), originating messenger RNA (transcripts) undergo different maturation processes and are translated into proteins. The study of gene expression is made by means of various techniques (PCR, RPA, Northern blot, etc.), among which hybridization in situ allows the local distribution of transcripts to be visualized in a morphological context. Similarly, different immunohistochemical techniques allow the localization of protein distribution. Nevertheless, neither the presence of messenger RNA nor of native protein ensures that the protein is functional, since diverse mechanisms of posttranscriptional control exist, such as excision of a region of the preprotein (1), glycosylation (2), or phosphorylation (3). The mechanism of regulation of gene expression is controlled by discrete elements of genomic DNA located mainly in regions anterior to the beginning of transcription. Identification of these regions allows gene construction (a) in which regulatory elements and a gene marker are inserted. This gene produces a protein that is easily detectable using histoenzymatic methods. This construct is inserted in the genome of the mouse (b) thus permitting analysis of the distribution of the gene marker (c), which constitutes an indicator of the transcription potential of these regulatory elements.

vertebrates requires the coordination of several complex processes ranging from the differentiation of the precardiac crests to the formation of an adult heart with four chambers and their corresponding valves. In this article we propose to offer an updated view of the embryology of the heart, including recent advances in molecular biology involving the complex molecular processes that control the specific expression of tissues.

MOLECULAR MECHANISMS OF GENE EXPRESSION

The mechanisms that control gene expression are determined mainly by the transcription of messenger RNA (mRNA) from genomic DNA (Figure 1). Detailed knowledge of the promoter regions of the genes, which are essential for transcription, and of the transcription factors involved in promoter activation are a prerequisite for understanding the mechanisms of gene regulation. Nevertheless, this is only the first event that takes place before this gene can originate a functional protein. The transcription of DNA into mRNA by means of RNA polymerase II entails the generation of a primary mRNA (transcriptional regulation) that later gives rise to mature mRNA by means of an exon and intron splicing process (Figure 1). Mature RNA is translated to protein by the ribosomes, and in certain cases this protein must then be modified (postranscriptional regulation) to make it functional; for example, by catalytic processing (transformation of preprotein to protein), phosphorylation (union of phosphate groups) or by glycosylation (union of sugar complexes). In most cases, the main mechanism of control is transcriptional, which means that the presence of encoding mRNA is a good indicator of the presence of this protein in a given tissue. Nevertheless, this paradigm, although generally certain, does not imply that the protein is functional. For example, growth factors such as TGF (transforming growth factor) must be activated by catalysis of the preprotein to become functional. Therefore, the presence of mRNA must be interpreted with care when making functional extrapolations. In this review, we

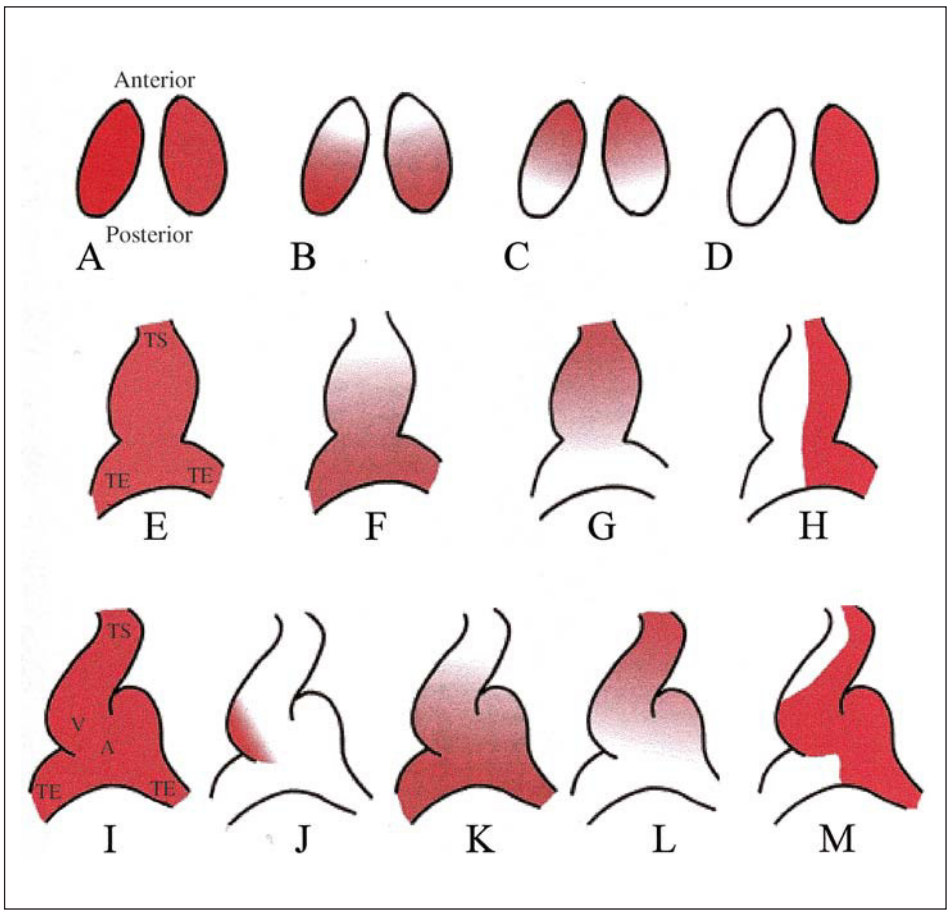

Fig. 2. Schematic representation the diverse patterns of gene expression in the stages of cardiac crests (A-D), linear cardiac tube (E-H), and cardiac loop (I-M). In all stages genes are distributed homogeneously (e.g., Nkx2.5) (panels A, E and I), in anteroposterior or posteroanterior gradients (e.g., MHC) (panels B, C, F, G, K and L) and differentially between right and left (e.g., Pitx2) (panels D and H). With cardiac looping, there is remodeling of the left/right expression (Pitx2; panels H and M) and new originate patterns that demarcate the dorsoventral differences (e.g., ANF; panel J). IT indicates inflow tract; OT, outflow tract; a, atrial primordium; V, ventricular primordium.

have centered mainly on the study of transcriptional regulation and have emphasized only cases in which evidence of postranscriptional control exists.

The aim of this review is to illustrate the different patterns of expression of the main gene families with specific myocardial expression, such as structural proteins (contractile proteins; myosins, actins and the troponin complex), ion channels (potassium and sodium channels), and transcription factors in different phases of cardiogenesis (Figures 2-4). These observations are framed in the context of the functional analysis of certain DNA regulator zones (animal models of transgenesis) (Figure 1). The data shown correspond for the most part to the mouse, because this is currently one of the most frequently used animal models in molecular biology and constitutes a solid basis for extrapolation to a human context. Next, a brief introduction to cardiac embryology will be given and then we will describe in detail the patterns of expression of endogenous genes and transgenes.

CARDIAC EMBRYOLOGY

During embryonal development, the heart evolves from a simple tubular structure to a highly complex multichamber organ. This process requires the differentiation and growth of different embryonal structures. During cardiogenesis, six prototypical phases can be distinguished. The cells destined to form the cardiac tube are symmetrically arrayed in two crests, the precardiac crests (first stage). They receive signals from the ectoderm and endoderm and form the future myocardiocytes (Figure 2). Later, the cardiac crests join in the embryonal midline to produce the primitive cardiac tube (second stage) (Figure 2). In this stage, the heart is formed by only two cell layers, the myocardium and endocardium, separated by a cell matrix called cardiac jelly. Next, the cardiac tube loops to the right, constituting the first morphological sign of corporal asymmetry in embryonal development (third stage) (Figure 2). This looping culminates with the formation of an embryonal heart in which different myocardial regions become distinguishable. The embryonal heart is formed by the inflow tract, embryonal atrium, atrioventricular canal, embryonal ventricle, and outflow tract (fourth stage) (Figure 3). Each of these myocardial regions has a differential pattern of expression, as well as different functional characteristics. The inflow tract, atrioventricular canal, and outflow tract present endocardial cushions on their inner

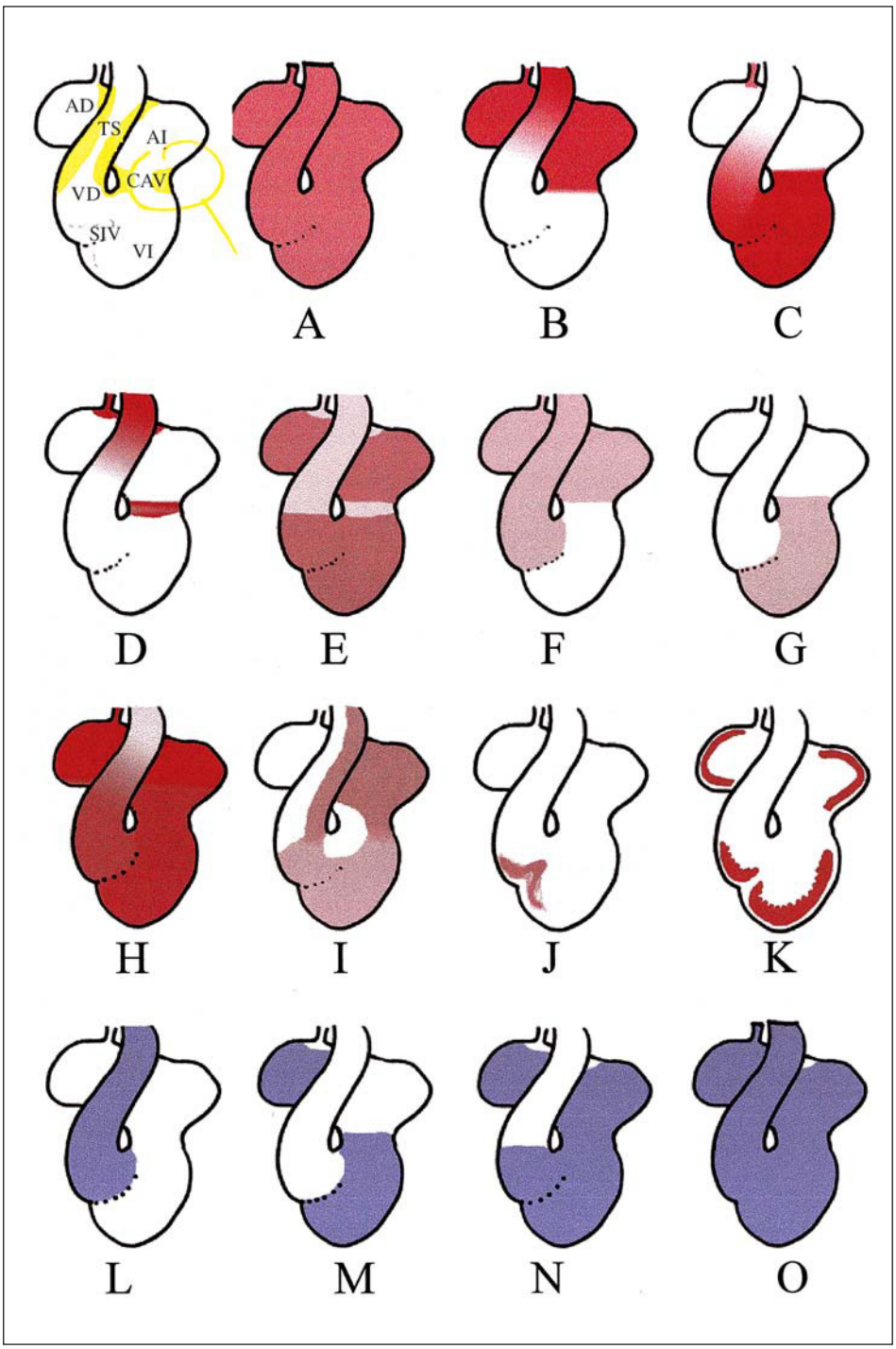

Fig. 3. Schematic representation of diverse patterns of gene (A-K) and transgene (L-O) expression in the embryonal stage. Aside from the patterns observed in earlier stages (A-C), in this stage a broad range of new expression patterns are generated (D-K). Five domains of expression are delimited (e.g., SERCA2a, PLB; panels D and E), systemic and pulmonary differences (dHAND and eHAND; panels F and G), constitutive anteroposterior gradients (cardiac troponin I; panel H), right and left differences (Pitx2, panel I), differentiation of the conduction system (Irx1 and Irx2; panel J) or the trabeculated myocardium (ANF; panel K). Panels L-O illustrate the different patterns of expression observed with diverse regulatory elements in transgenic mice. RA indicates right atrium; LA, left atrium; RV, right ventricle; LV, left ventricle; IVS, interventricular septum; OT, outflow tract; AVC, atrioventricular canal.

surface, whereas the atrial and ventricular chambers are trabeculated and lack mesenchymal structures.

During the fetal stage these 5 structures have to form a septum to obtain a heart with a double circuit, systemic (oxygenated blood) and pulmonary (venous blood) (Figure 4). Septation of the primitive ventricle generates the right and left ventricles by development of the interventricular septum. The primitive atrium is divided into left and right atria by the formation of the complex of primary and secondary interatrial septa (fifth stage). It is interesting to note that the separation of the inflow and outflow tracts, and atrioventricular canal, takes place by the fusion of the cardiac cushion mesenchyme and its later replacement by myocardiocytes through a process denominated «myocardialization.» In addition, restructuring of the different embryonal fields takes place to originate two atrial and two ventricular chambers, all with their own inflow

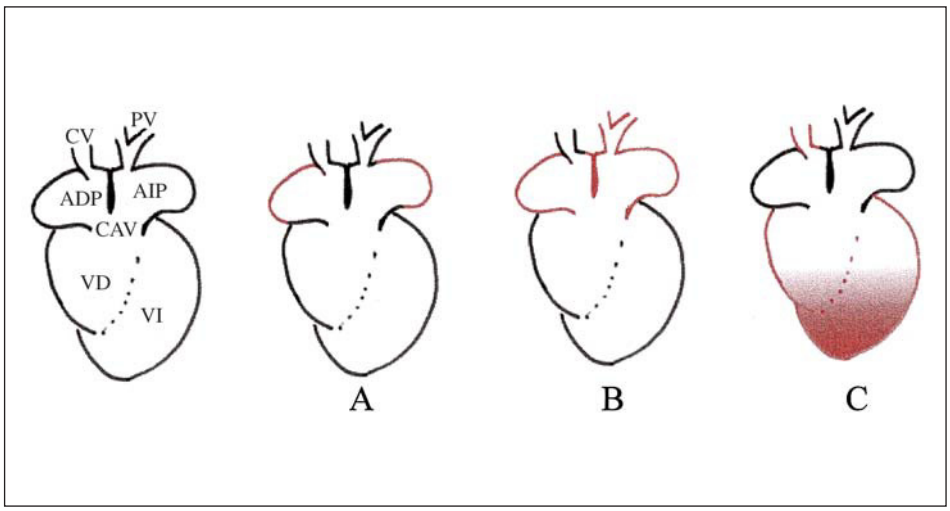

Fig. 4. Schematic representation of patterns of gene expression in the fetal stage. The expression of different genes establishes that atria with pectineal muscles have an exclusive pattern of expression, for example, ANF (panel A). MLC3F expression indicates that the muscle of the caval veins is different from those of the pulmonary veins (including the interatrial septum) (panel B), whereas MLC2v establishes that the caval veins are different from the rest of the atrial myocardium (panel C). RAP indicates right atrium with pectineal muscles; LAP, left atrium with pectineal muscles; RV, right ventricle; LV, left ventricle; CV, caval veins; PV, pulmonary veins; IAS, interatrial septum.

and outflow tracts, in the adult heart. Basically this is the same configuration as in the adult heart, with the separation of the four chambers being completed (sixth stage).

PATTERNS OF GENE EXPRESSION IN THE CARDIAC CREST (E7.5 MOUSE)

The cardiac crest forms during gastrulation as a mesodermal region derived from the neural tube. This anterior portion of the mesoderm responds to a series of signals from the ectoderm and adjacent endoderm, inducing the expression of different specific genes of the future myocardiocytes. With the possible exception of the mRNA that encodes the proteins of calcium metabolism (SERCA2 and PLB), only expression of different transcription factors is observed in this stage (Table 1).

Genes that encode proteins of calcium metabolism

The expression of SERCA2 (calcium pump of the endoplasmic reticulum) and PLB (phospholamban) in such early stages is interesting, although myocardial contraction has not yet begun, there is already polarity in the gene expression. SERCA2 is more abundant in the anterior region of the cardiac crests and decreases towards the posterior regions, whereas PLB has a complementary distribution.

TABLE 1. **Patterns of expression in initial stages of heart development**

		Homogeneous	R/L axis	A/P axis	Dorsal/ventral axis
Cardiac crest (stage I)	Structural genes			SERCA2 (A), PLB (P)	
	Transcription factors	Nkx, GATA4-6, MEF2C, HANDs SRF, CARP, pCMF1, Midori cCLP-1, Mesp1, Tbx5	Pitx2 (L)	HTR1 (P), HTR2 (A), Irx4 (A)	
Cardiac tube (stage II)	Structural genes	MLC, troponin C/T, tropomyosin, tropomodulin		αMHC, MLC2a, actina-c, βMHC (A), SERCA2 (P), PLB (A)	
	Transcription factors	Nkx, GATA4-6, MEF2C, HTR3 SRF,CARP, pCMF1, *Midori* dHAND	Pitx2 (L)	Irx4 (A) HTR1 (P), HTR2 (A)	eHAND (V)
	Structural genes	MLC, troponin C/T, tropomyosin, tropomodulin		αMHC,MLCa, actin-c, βMHC (A), SERCA2 (P), PLB (A)	
Cardiac loop (stage III)	Transcription factors	Nkx, GATA4, MEF2, HTR3, SRF,CARP, pCMF1, *Midori*	Pitx2 (L)	HTR1 (P), HTR2 (A), GATA5, GATA6	Irx4, eHAND (V)

(L) indicates left; (A), anterior; (P), posterior; actin-c, cardiac actin.

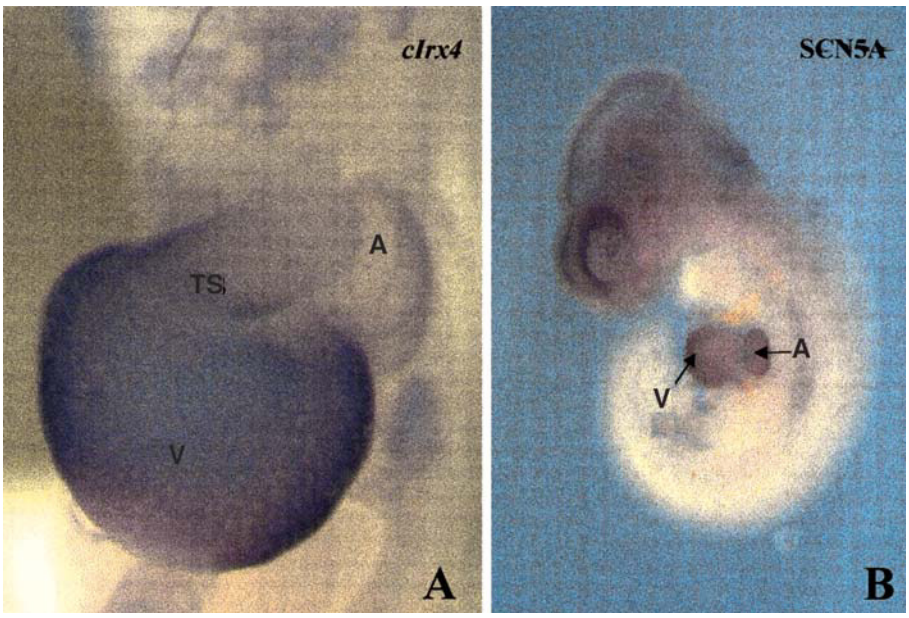

Fig. 5. Hybridization in situ in embryonal hearts of chicken (HH 20 stage; panel A) and mouse (E10.5; panel B) with probes for the Irx4 (A) and SCN5A (B) transcription factors. Irx4 is expressed in the ventricle and outflow tract, but not in atria. SCN5A is distributed homogeneously throughout the heart. A indicates atria; V, ventricles; OT, outflow tract.

Transcription factors

Several families of transcription factors present expression of some of their members in stages as early as in the promyocardium. The main families are homeobox genes, GATA, bHLH and MEF2, as is detailed below.

Among the homeobox genes, the expression of *Nkx2.5* is homogeneous throughout precardiac crests. It is interesting to note that in *Xenopus*, the expression of *Nkx2.5* seems to be restricted to the internal lateral plate of the precardiac crests (medial region). This could be interpreted as the first sign of dorsoventral differentiation, although this regionalization has not described in other models. The transcription factor *Pitx2* already evidences clear regionalization in its pattern of expression in these stages. *Pitx2* is expressed in the left precardiac crest, but not in the right crest, being configured as the first sign of molecular asymmetry during heart development. Recently a new homeobox factor has been described, *Irx4*, which has an expression restricted to the anterior region of the crests. These authors have postulated that cells that express *Irx4* constitute the primordium of the ventricular myocardium at this early age.

Several members of the GATA family of transcription factors are already expressed in the precardiac crests and have a primordial role in myocardial specification. The expression of GATA4, GATA5, and GATA6 takes place from the earliest stages of myocardial formation. GATA4, at least, is essential in the first stages of gestation since its absence leads to the formation of cardia bifida. The expression of GATA4, GATA5, and GATA6 is homogeneous throughout the precardiac crests. This distribution suggests that the GATA factors act as cofactors in myocardial specification but not in the acquisition of cellular heterogeneity.

In the first stages of heart development, one of the components of the MEF (*myocyte enhancer factor*) family of transcription factors, MEF2C, is expressed homogeneously in the precardiac crest. Studies of gene suppression in mice have of shown that the lack of this factor generates cardia bifida, as well as the suppression of different cardiac molecular markers. Like GATA4, MEF2 seems to be an essential cofactor, although the molecular mechanism of action is still not known.

The bHLH (*basic helix loop helix*) family of transcription factors includes, for example, numerous transcription factors specific to striated muscle. MyoD, Myf5, and Mrf 4 have an important role in the regulation of expression in skeletal muscle, but none of them is expressed in the myocardium in normal conditions. In recent years, two new members of the bHLH family have been discovered (dHAND and eHAND) that are expressed in embryonal heart and have an important role in heart morphogenesis. In the first stages of development dHAND and eHAND are expressed homogeneously, although they are later expressed asymmetrically in the ventricular chambers.

Recently it has been reported that different members of the family of transcription factors related to the gene *hairy* (*hairy related transcription factors*; HRT) present regionalization in the anteroposterior axis, even in stages as early as the precardiac crests. HRT1 is expressed in the most posterior region, whereas HRT2 is expressed in the most anterior region. Since the Notch Delta system regulates *hairy* expression and has a fundamental role in establishing the cellular and tissue barriers of the fruit fly (*Drosophila*

TABLE 2. **Patterns of expression in the embryonal and fetal heart**

		Inflow tract	Atria	AV canal	Ventricles	Outflow tract
Embryonal heart (stage IV)	Contractile proteins	α and βMHC, MLC1a/v, MLC2a/v. Troponin, MLC2a. Troponin I	αMHC, MLC1a, MLC2a/v	α and βMHC, MLC1a/v, MLC2v	βMHC, MLC1v, MLC2a/v	α and βMHC, MLC1a/v
	Calcium metabolism	SERCA2	SERCA2	PLB	PLB	PLB
	Intercalated disks	Cx45	Cx40, Cx45	Cx45	Cx40, Cx43, Cx45	Cx45
	ion channels	Scn5a, KCNH2, KCNQ1, Kcne2/e3	Scn5a, KCNH2, KCNQ1, Kcne2/e3	Scn5a, KCNH2, KCNQ1, KCNE3	Scn5a, KCNH2 KCNQ1, Kcne1/e3	Scn5a, KCNH2 KCNQ1, Kcne1/e3
	Transcription factors	Pitx2 (L), Tbx5, Tbx2, HRT1,	eHAND, Pitx2 (L), Tbx5, HTR1, Irx3	Pitx2 (L), Tbx5 Tbx2, e/dHAND	Tbx5 (L), Pitx2 (V), Irx1,2, 3, 4, HTR2, dHAND	Pitx2 (L), HTR2, Irx4
Fetal heart (stage V)	Contractile proteins	αMHC, MLC1a, MLC2a Troponin I	αMHC, MLC1a, MLC2a. Troponin I	βMHC, MLC1v, MLC2v	βMHC,MLC1v, MLC2v	βMHC, MLC1v, MLC2v
	Calcium metabolism		SERCA2		PLB	
	Intercalated disks		Cx40		Cx43	
	Ion channels	SCN5A, KCNH2, KCNQ1, KCNE2/E3	SCN5A, KCNH2, KCNQ1, KCNE2/E3	KCNH2, KCNQ1	KCNH2 KCNQ1, KCNE1	KCNH2 KCNQ1, KCNE1

The data of the inflow and outflow tract, and atrioventricular canal of the fetal heart talk refer to the expressions observed in the corresponding myocardial derivatives. (L) indicates left; (V), ventral.

melanogaster), Nakagawa, et al. have postulated that HRT can have similar function in the heart, for example, delimiting the atrial and ventricular regions, although there are still no experimental data to support this hypothesis.

Other transcription factors that are expressed homogeneously in the precardiac crests are *Tbx* 5, SRF (*serum response factor*), CARP (*cardiac ankyrin repeat protein*), pCMF1, *Midori*, c CLP 1, and Mesp1. The precise function of these proteins is unknown, with the exception of SRF and *Tbx5*, which seem to act as cofactors together with other transcription factors (GATA4 and *Nkx2-5*).

In summary, during the formation of the precardiac crests there is already regionalization of gene expression, which is new evidence of the complexity of cardiogenesis. It is important to emphasize that there are genes that are expressed differentially in the anteroposterior axis, either in expression gradients (SERCA and PLB) or discrete regions (*Irx*4; HRT), and that there are genes that are expressed differentially in the right and left axis (*Pitx*2). With these premises, several models of gene transcription have been conceived to explain how gradients are obtained and develop, but explanations are lacking for the right/left differences.

Transgenesis

The analysis of the regulatory elements of different cardiac-specific genes has been impressive in last decade. Knowledge of the molecular mechanisms that control the specific expression of tissue, that is, the regulatory factors and their interactions on the one hand, and the essence elements that allow the directed expression of a certain tissue compartment on the other, are the basis for future clinical gene therapy applications.

The patterns of transgene expression that have been observed can be classified into two types in this stage. Some authors indicate that transgene expression is restricted to a portion of the cardiac crest that later forms a cardiac chamber. Nevertheless, other transgenes have a similar pattern of expression in both cardiac crests. The interpretation of these observations is divergent. On the one hand, the early regionalization of transgenes indicates that the specification of the cardiac chambers (atrial/ventricular) takes place in the pro myocardium. This hypothesis is difficult to reconcile with cell labeling data that demonstrate the contribution of the cardiac crest to the right ventricle alone in the chicken and to the left ventricle in mice. On the other hand, the interpretation of a non-specified pro-myocardium contrasts with the observation of regionalized endogenous genes, although these myocardiocytes have an extremely plastic phenotype. Both hypotheses leave the door open for future experimentation to demonstrate or refute one of the theories, although in our opinion the second hypothesis seems more reasonable.

PATTERNS OF GENE EXPRESSION IN THE PRIMITIVE CARDIAC TUBE (E8 MOUSE)

The primitive cardiac tube is composed of two cell layers, the myocardium and endocardium, separated by an acellular matrix known as cardiac jelly. The myocardium that composes the cardiac tube is morphologically homogeneous. In this stage basically three types of expression patterns that are observed in the anterior stage are maintained, homogeneous expression, and regionalization in the anteroposterior and right-left axes. Then, only the genes that have a heterogeneous pattern of expression are presented, either because they change their original pattern or because their expression is initiated in this stage (Table 1).

Genes that encode for contractile proteins

Cardiac contraction is mediated by the sliding of myosin filaments over actin filaments. Contraction is modulated by the union and later release of calcium ions in the troponin-tropomyosin complex. A large variety of isoforms exists that encode different components of the sarcomere. Most of the genes that encode for contractile proteins, like heavy-chain myosin (MHC), light-chain myosin (MLC), actin, and the troponin-tropomyosin complex, have a characteristic pattern of expression in this stage of development. For example, αMHC, MLC2a, cardiac actin, and cardiac troponin I have a higher expression in the caudad region than in the cephalad region, whereas αMHC presents a reciprocal pattern. Interestingly, the expression of homologous MHC isoforms in the chicken shows more variability with respect to the regionalization of expression. In the chicken embryo, Yutzey, et al. demonstrated that AMHC1 expression is restricted to the venous pole in the cardiac tube, whereas VMHC1 is principally in the arterial pole and only in the venous pole at baseline. The other isoforms of MLC, actin, and troponin, as well as tropomyosin and tropomodulin, present a homogeneous distribution throughout the myocardium in this stage.

Genes that encode proteins of calcium metabolism

Cardiac contraction is regulated by changes in the intracellular concentration of calcium ions. The myocardial cell thus has an exhaustive mechanism for the control of calcium homeostasis that involves different membrane proteins. The SERCA pump sequesters Ca from the cytosol into the endoplasmic reticulum (ER). SERCA is controlled by phospholamban (PLB). Ca outflow to the cytosol from the ER is mediated by the ryanodin receptor (RyR), whereas the ion balance between the extracellular medium and cytosol is regulated by sodium-potassium pumps (NaK-ATPase) and the sodium-calcium exchanger (NCX). Several tissue-specific isoforms exist for several of these components. In the embryonal heart, the SERCA2a isoform is the most represented and has a gradient of expression in the anteroposterior axis, being more abundant in the caudad region than in the cephalad region. The expression of PLB, a regulator of SERCA2a, is opposed to that which is observed for SERCA2a, that is to say, it has a gradient of expression in which expression is greater in the cephalad zone and decreases towards the caudad zone. It is interesting that the expression of the RyR2 isoform, NCX, and different isoforms of cardiac NaK-ATPase is distributed homogeneously in the cardiac tube and remain there throughout the later embryonal development of the heart. Therefore, the mechanism of control of calcium homeostasis is determined by the SERCA PLB system, whereas the other translocators do not seem to influence it decisively.

Transcription factors

The *Pitx2* transcription factor in this stage maintains the regionalization of its pattern of expression. *Pitx2* only evidences expression along the left margin of the cardiac tube. On the other hand, the *Irx4* homeobox factor has a pattern of expression restricted to the future ventricular myocardium. The first evidence of regionalization in the dorsoventral axis is originated with the expression of the eHAND transcription factor in the initial cardiac tube. eHAND shows more expression in the ventral than in the dorsal region of the cardiac tube. Christoffels et al. postulate that this expression constitutes the first molecular evidence of the trabeculated ventricular myocardium. This thus implies that the ventricle is specified throughout the dorsoventral axis and not, as has been traditionally held, in the anteroposterior axis.

The HRT genes maintain the anteroposterior regionalization (cephalocaudad) in the primitive cardiac tube that they presented in earlier stages; HRT1 is expressed in the caudad region whereas HRT2 is expressed in the cephalad region.

Transgenesis

The expression of different transgenes in this stage of the cardiac tube varies as in the previous stage. Certain regulatory zones for both structural genes and transcription factors present expression of the gene marker throughout the cardiac tube. On the other hand, the expression of certain transgenes is restricted to the caudad or cephalad region. Currently, in no case has a gradient of expression of the transgene been observed throughout the myocardium of the cardiac tube, as has been observed for genes like SERCA, PLB, or αMHC. This could be because these gradients are established by other regulating sequences not contained in the re-

gion studied, or to saturation of the gene marker (e.g., β-galactosidase and GFP [green fluorescent protein] are very stable, which may be why they do not allow small changes of expression to be distinguished). The use of marker genes with signals that allow the rapid degradation of the protein (e.g., destabilized GFP) will make it possible to advance in this sense in the future.

PATTERNS OF EXPRESSION IN THE CARDIAC LOOP (E8.5 MOUSE)

The first sign of morphologic asymmetry manifested in the embryo is the looping to the right of the initial cardiac tube. The embryo thus obtains a left-right identity, and always presents the same pattern, a condition known as *situs solitus* (see, for example, the invariable looping of the heart to the right). In certain pathological cases, the control of the left-right axis is disturbed, resulting in a total inversion of this axis (*situs inversus*). In other cases a certain randomness in the distribution along the right-left axis occurs (*situs ambiguus*). At present, the are some clues as to the molecular mechanisms that control the looping of the cardiac tube, although the main protagonists are not known.

However, this process is of vital importance for future cardiac development because abnormalities in the correct looping of the cardiac tube underlie different types of congenital heart diseases. Most genes do not experience significant changes in their pattern of expression with cardiac looping, except certain transcription factors whose expression should be emphasized (Table 1).

Transcription factors

The *Pitx2* transcription factor, currently the last known link in the signaling path of the right-left axis, presents an interesting pattern of expression during cardiac looping. The movement to the right of the future embryonal ventricle entails the displacement of the pattern of expression of *Pitx2* from a left position to a ventral position in this cardiac region. Nevertheless, *Pitx2* expression remains exclusively in the left regions of the ends of the cardiac tube. Similarly, the eHAND transcription factor, which in the stage of the primitive cardiac tube is located mainly in the ventral region, shows displacement of its expression toward the prospective zone of differentiation of the embryonal ventricle with looping.

Other transcription factors, like GATA5 and GATA6, present expression preferentially at the ends of the cardiac tube. GATA5 and GATA6 disappear gradually from the most medial regions and their expression remains localized essentially in the arterial and venous poles. This pattern of expression makes it possible to postulate that these factors have a primordial role in myocardial specification, but are not necessary for the maintenance of the cardiac muscle phenotype.

PATTERNS OF EXPRESSION IN THE EMBRYONAL HEART (E10.5 MOUSE)

The embryonal heart is formed by 5 different regions, three of which (inflow tract, atrioventricular channel, and outflow tract) are continuous along the internal curvature (Figure 3). From the venous pole to the arterial pole, the following regions are distinguished: inflow tract, common atrium (with right and left regions), atrioventricular canal, ventricle (with right and left regions), and outflow tract. The inflow tract, atrioventricular canal, and outflow tract present endocardial cushions on their inner surface, whereas the atrial and ventricular chambers lack mesenchymal structures. The formation of these regions generates compartmentation of expression for many cardiac-specific genes, as will be seen below, showing evidence of the functional heterogeneity of the myocardium in these stages of development (Table 2). In this context, Christoffels, et al. recently developed a model of cardiac development that integrates current molecular data with the morphologic bases of cardiogenesis.

Genes that encode contractile proteins

With the formation of the embryonal heart, the different isoforms of MHC and MLC present a regionalizado pattern of expression. αMHC, MLC1a, and MLC2a expression are restricted mainly to the venous pole, including the inflow tract, atrium, and atrioventricular channel. βMHC, MLC1v, and MLC2v expression, in contrast, is restricted mainly to the arterial pole, that is, the atrioventricular canal, ventricle, and outflow tract. It is curious that both αMHC and MLC2a show residual expression in the outflow tract, as it regresses toward the atrial pole takes place, whereas βMHC and MLC2v have residual expression in the inflow tract. This dynamic expression of MHC and MLC is dictated by the existence of specific inhibiting factors of atrial and ventricular expression, as described by Franc, et al. In fact, it has been recently reported that the *cIrx4* transcription factor carries out this function in chicken embryos, although it does not seem to be conserved in mice.

It is important to note that the pattern of expression of the MLC3F isoform is similar to that of clearly atrial isoforms. Nevertheless, it has only been possible to clearly detect the presence of coding mRNA, but not MLC3F protein, in the embryonal heart of the mouse. At present, we do not have functional explanations for this behavior, and can only conjecture as to its evolutionary meaning.

The different isoforms of the troponin-tropomyosin complex that are expressed in the heart present a homogeneous pattern of expression in the embryonal heart, with the exception of the cardiac isoform of tropo-

nin I (cTNI), which has an anteroposterior gradient of expression. Cardiac TNI shows a greater expression in the inflow tract and a common atrium, decreases in the atrioventricular canal, and is lower in the ventricle and outflow tract, the latter having a baseline expression in its most cephalad region.

Genes that encode proteins of calcium metabolism

In the embryonal heart, the SERCA2a isoform is the most abundant and presents a regionalization of expression reminiscent of its anteroposterior gradient in the initial cardiac tube. SERCA2a is more abundant in the inflow tract and atrium than in the ventricle and outflow tract. Curiously, the expression of SERCA2a in the atrioventricular canal is lower than in atrium and ventricular myocardium. In contrast, PLB expression is opposed to that observed for SERCA2a, that is, it presents a greater expression in the atrioventricular canal, ventricle, and outflow tract, and less expression in the inflow tract and atrium. It is interesting to note that, in both cases, the expression in intermediate regions, that is, the outflow tract, atrioventricular channel, and inflow tract, is always lower than in the cardiac chambers (atrium and ventricle). This pattern of expression reinforces the model of cardiogenesis proposed by De Jong, et al., more recently supported by additional molecular evidence.

Genes that encode components of the intercalated disks

The propagation of the cardiac impulse is determined principally by the capacity to quickly transmit changes in the membrane potential. The gap junctions that mediate the propagation of the cardiac impulse are formed by membrane proteins denominated conexins. In the mammalian heart, the main conexin is 43 (Cx43), which is located principally in the ventricular myocardium and to a lesser extent in the atrial myocardium. Cx43 is detected for the first time in the embryonal stage. Cx40 has a pattern of expression similar to that of Cx43 in this stage, although its expression is more reduced. Recently, a third conexin has been described in the embryonal heart (Cx45). Cx45 has a homogeneous pattern of expression in the embryonal heart, although its expression values are very low, even controversial. Differences in the expression of the conexins are consonant with a functional embryonal model in which the working myocardium (atria and ventricles) has the capacity to transmit the cardiac impulse more quickly than the adjacent myocardium, inflow tract, atrioventricular channel, and outflow tract., This guarantees synchronized contraction without the need for a specialized conduction system.

Genes that encode ion channels

The regulation of the action potential is determined by a large variety of ion currents. Depolarization of the myocardial cell takes place through the massive passage of sodium ions to the cytoplasm, whereas repolarization of the myocardial cell is determined by a fine balance of different influx and outflux ion currents, among which potassium currents have a primordial role.

The main pore that regulates the passage of sodium through the myocardial membrane is encoded by the gene SCN5A. The permeability of this channel is modulated by an auxiliary subunit, SCN1B. The modulator role of the SNC1B subunit is well established in neuronal cells, but controversy exists regarding its function in cardiac muscle. However, a large variety of potassium ion currents exist, among which only the distribution of the components of the I Kr and I Ks currents has been studied. The I Ks current (slow entry rectifier) is mediated by the membrane pore KCNQ1 (KvLQT1) and modulated by the auxiliary subunit, KCNE1 (minK/IsK). The I Kr current (rapid entry rectifier) is mediated by the membrane pore KCNH2 (HERG) and modulated by the KCNE1 and KCNE2 subunits (MiRP1). A new isoform has been discovered recently, KCNE3 (MiRP2), whose modulating function in the myocardium is still unclear.

The distribution of different components of the sodium channels in embryonal stages has not been studied before now. The only existing data are preliminary data from our laboratory that indicate that SCN5A is distributed homogeneously throughout the embryonal heart (Franco and Bezzina, unpublished data; Figure 5). On the other hand, the expression of the potassium channels KCNQ1, KCNH2, and KCNE3 is homogeneous in the embryonal myocardium. However, it is interesting to highlight that KCNE1 presents an anteroposterior gradient, that is, greater expression in the arterial than in the venous region, while KCNE2 presents in these studies a clear regionalization of expression, being confined to the atrial myocardium.

Transcription factors

The *Pitx2* transcription factor accentuates in this stage the pattern of expression initially sketched in the cardiac loop stage. *Pitx2* is expressed in the ventral region of the ventricle, but not in the dorsal region, whereas its expression in the inflow tract, atrium, atrioventricular channel, and outflow tract is restricted to the left portion. These observations indicate that the ventricular primordia receive similar contributions from the right and left cardiac crests. The peculiarity of this finding is that cells derived from the left crest are located in the ventral region and those derived from the

right crest in the dorsal position. That is to say, there was a relocation of the right and left contributions with respect to the right/left embryonal axis that only affects the ventricles.

In the embryonal stage, three new members of the *Iroquois* family are expressed in the myocardium, *Irx1* and *Irx2*. Their patterns of expression are clearly similar; they are expressed exclusively in the crest of the interventricular septum from the onset of their formation. This distribution relates these transcription factors to the formation and/or specification of the ventricular conduction system, although there is no direct evidence of this. The third factor *Iroquois, Irx3*, has an expression restricted to the working myocardium (atrial and ventricular). The homeobox factor, *Irx4*, maintains its expression restricted mainly to the ventricular myocardium; nevertheless, it extends in a gradually decreasing way to the outflow tract and atrioventricular canal (D. Franco, unpublished data; Figure 5).

Tbx5 has a pattern of expression restricted to the left ventricle, atrioventricular canal, atrium (right and left), and inflow tract of the embryonal heart. Interestingly, the expression of *Tbx5* in the interventricular septum is located mainly in the left region, which allows it to be claimed that the interventricular septum has different right and left components. *Tbx2*, another member of the T box family, initiates its expression of clearly in the atrioventricular channel and inflow tract at this stage (D. Franco and M. Campione, unpublished data). *Tbx2* exerts an inhibiting function on expression in other tissues, and its function during cardiogenesis is apparently related to inhibition of the program of gene expression characteristic of the working myocardium (atrial and ventricular) in the zones where it is expressed (atrioventricular channel and outflow tract). This hypothesis is in agreement with molecular evidence and currently is being investigated.

Members of the bHLH family acquire in this stage a singular regionalization in their pattern of expression. eHAND expression is restricted to the left ventricle, outflow tract, and common atrium, whereas dHAND has a pattern of expression restricted mainly to the right ventricle and outflow tract. It is interesting to note that an asymmetrical expression is observed in rodents, but not in other experimental models like the chicken.

The members of the HRT family maintain the same regionalization in their expression in the embryonal heart as in earlier stages: HRT1 is expressed in the inflow tract and atrial myocardium, whereas HRT2 is expressed in the ventricular myocardium and outflow tract.

Miscellaneous

The expression of ANF (atrial natriuretic factor) is restricted in this stage to the trabeculated component of the ventricle and common atrium. It is important to emphasize that there is no ANF expression in the inflow tract in this stage, although the myocardial component of future caval veins is already differentiated. Therefore, ANF expression makes it possible to distinguish molecularly the atrial component from the venous component of the embryonal atrium, that is, the myocardium of the caval veins.

Transgenesis

In the embryonal stage, the expression of different transgenes varies. Until now, transgenes expressed throughout the heart (cTNI), in the outflow tract and right ventricle (MLC2v; SM22α), in the left ventricle and right atrium (MLC3F), in the right ventricle (GATA), in the atrioventricular channel (cTNI, cGATA), in both ventricles (MLC2v) and in both ventricles and atria, excluding the inflow and outflow tracts (MLC3F) have been documented. The heterogeneity of the transgene patterns in the embryonal stage illustrates the high degree of complexity of the regulation of the specific myocardial gene expression as well as the structural and morphologic diversity of the heart muscle.

It is interesting to highlight the association between expression in the left ventricle and right atrium that is observed in several transgene models (MLC3F and cTnI). In other models, expression is complementary (MLC2v) or englobing (αMHC and βMHC). These observations suggest that the «left ventricle-right atrium» pattern represents the primitive «single-chamber heart» of fish and that, in the course of evolution, new compartments have been added (left atrium and right ventricle) for the purpose of adapting the heart to a double circulation. This hypothesis suggests that the acquisition of new cardiac chambers would also require the addition of new «regulatory elements.» This idea is in consonance with the model proposed by Frank, et al. and Olson and Srivastava, in which the regulation of cardiac expression is modular and discrete DNA elements direct the expression of certain cardiac compartments.

Recently, two transgene models have been described (both resulting from the spontaneous insertion in a *locus* that is unknown *a priori*; *gene trap/enhancer trap*) that diverge from this general pattern. Renstchler, et al. have described a transgene whose pattern is located mainly in the prospective regions of the conduction system. The expression of the transgene En2-lacZ is located in the trabeculated myocardium (right and left bundle branches and Purkinje system), atrioventricular ring (atrioventricular nodule), interventricular septum (bundle of His), and sinoatrial junction of the right vena cava (sinoatrial node). Electrophysiological studies of the electrical activity

demonstrate that the ventricular cells that express the transgene also transmit the cardiac impulse more rapidly than cells that do not express it. At present we do not know the regulatory elements that promote the expression of this transgene.

Kelly, et al. have presented a transgene model of dynamic pattern during heart development. Initial expression is located in cells external to the cardiac tube that express FGF10. Progressively, with development, the arterial region of the cardiac tube acquires expression of the transgene, which suggests that this myocardial region is not present in the primitive cardiac tube. Cell labeling experiments have confirmed this hypothesis.

PATTERNS OF EXPRESSION IN THE FETAL HEART (E16.5 MOUSE)

The fetal heart has an architectural morphology similar to that of the adult heart. The atrial and ventricular cardiac chambers are clearly divided into right and left, and the septation of the primitive cardiac tube into two circuits is almost complete. By this means the right atrium receives blood from the caval veins, while the left atrium receives blood from the pulmonary veins. Both structures, caval veins and pulmonary veins, are covered with cardiac muscle that varies in different species, and is therefore considered an integral part of the fetal heart. Each atrium connects with its corresponding ventricle by an atrioventricular junction. Within the ventricular myocardium two clearly delimited components area can be distinguished morphologically and molecularly in this stage, that is, the compact layer and the trabeculated layer. Finally, each ventricle acquires an independent exit by septation of the outflow tract in the aortic tract and pulmonary tract. Once this configuration is attained, the myocardium shows a heterogeneous gene expression that is reminiscent of patterns of expression in the embryonal stage, which has made it possible to clarify the contribution of certain embryonal myocardial regions to the adult heart (Table 2).

The generation of different atrial and ventricular chambers with independent circuits also requires the acquisition of a specialized system of conduction of the cardiac impulse to ensure the synchronized contraction of the fetal myocardium and, above all, the establishment of a contraction pattern from the apex to the base of the heart. The cardiac conduction system is formed by the sinoatrial node, atrioventricular node (AVN), bundle of His (atrioventricular bundle), right and left bundle branches, and peripheral system of Purkinje cells. Each of these components has a different physiology and, accordingly, a differential pattern of expression. In this review, we will only briefly discuss the differential pattern of expression of the components of the conduction system because excellent reviews have been published in the literature.

Genes that encode contractile proteins

The different isoforms of MHC and MLC maintain a regionalized pattern of expression in the fetal stage, presenting a tendency to acquire this regionalization. The isoforms αMHC, MLC1a, and MLC2a progressively manifest an expression restricted to the atrial myocardium, including the caval and pulmonary veins, and the zones derived from the atrioventricular channel. It is interesting how «atrial» isoforms present a transitory differential expression between different layers of the ventricular myocardium. The trabeculated layer maintains higher levels of expression than the compact layer. In turn, the disappearance of the expression of these isoforms in the ventricular myocardium causes the right and left ventricle to show transitory differences in their expression, which indicates the existence of different transcriptional programs between the right and left ventricles. In contrast, the «ventricular» isoforms βMHC, MLC1v, and MLC2v maintain an expression restricted mainly to the arterial pole, that is, to the atrioventricular channel, ventricle, and outflow tract, although they show no substantial differences between the trabeculated and compact layers of the ventricular myocardium, or between the right and left chambers. As occurred in the previous stage (embryonal), the MLC2a isoform presents a residual expression in the outflow tract as it regresses to the atrial pole, whereas MLC2v maintains its differential expression in the inflow tract.

The cardiac isoform of troponin I (cTNI) conserves an anteroposterior gradient of expression. Cardiac TNI shows more expression in the inflow tract and common atrium, decreases in the atrioventricular channel, and is lower in the ventricle and outflow tract. The outflow tract exhibits baseline expression in its most cephalad region. The mRNA that encodes cTNI is detected throughout the embryonal heart; however, the cTNI protein is only detected in the common atrium and inflow tract in advanced fetal stages (E18.5). The functional consequences of this postranscriptional control are not known at present.

The expression of different isoforms from MHC and MLC also varies in the different structures of the cardiac conduction system. The sinoatrial nodule, as an eminently atrial region, shows expression of «atrial», but not «ventricular,» isoforms, with the possible exception of βMHC. On the other hand, the isoforms of the troponin-tropomyosin complex have a similar expression in the sinoatrial node as in the rest of atrial myocardium. The atrioventricular node, however, has an ambiguous pattern of expression, possibly due to its morphological location and embryonal origin. The

AVN shows coexpression of atrial and ventricular isoforms. It is particularly important that ventricular isoforms have a reduced expression compared with the expression of ventricular isoforms in the ventricular working myocardium. With respect to the expression of other isoforms of the sarcomere complex, it is important to emphasize that the expression of the slow skeletal muscle isoform of troponin I (ssTnI) is substantially lower throughout the ventricular conduction system (AVN, His bundle, and right and left bundles branches) than in the working ventricular myocardium. The bundle of His (atrioventricular bundle) presents a transitional expression between the atrioventricular node and right and left bundle branches. The bundle of His evidences a more tenuous expression of ventricular isoforms and no expression at all of atrial isoforms. The right and left bundle branches have a pattern of expression similar to that of the bundle of His. In contrast, no specific gene markers are known that delimit the peripheral Purkinje system in rodents, although they do exist in other species.

Genes that encode proteins of calcium metabolism

In the fetal heart, SERCA2a shows more expression in the atrial myocardium than in the ventricular myocardium. PLB expression, however, is complementary, greater in the ventricular myocardium than in the atrial myocardium. As for the pattern observed for MHC and MLC isoforms, both SERCA2a and PLB present a homogeneous expression of the different venous components, that is, in the atria and myocardium of the caval and pulmonary veins. In turn, both SERCA2a and PLB exhibit a differential expression between the trabeculated and compact layers of the ventricular myocardium. On the contrary, the expression of SERCA2a and PLB is tenuous in different components of the cardiac conduction system, atrioventricular node, and bundle of His, as befits the morphological origin of these structures in the myocardium of the atrioventricular canal. The expression of SERCA2a and PLB in the right and left bundle branches of the cardiac conduction system is similar to that of the ventricular myocardium.

The expression of the main cardiac isoforms of other components of calcium metabolism, i.e., the RyR2, NCX, and different NaK-ATPase isoforms that are expressed in the heart are distributed homogeneously in the different regions of the fetal heart, including the conduction system.

Genes that encode components of the intercalated disks

The pattern of expression of the main conexin in the mammalian heart, conexin 43 (Cx43), is restricted to the ventricular myocardium and it is only appreciable in baseline form in the atrial myocardium. It is interesting to note that Cx43 expression has not been detected in certain components of the ventricular conduction system, that is, the atrioventricular node and bundle of His, which supports the hypothesis that these structures derive from the embryonal atrioventricular canal. On the other hand, the expression of Cx43 transcripts is different in the trabeculated layer (lower) than in the compact layer (higher). Nevertheless, protein expression is higher in trabeculated layers than in compact layers, and it can be claimed that a postranscriptional control mechanism exists. These observations suggest that the transmission of the cardiac impulse is preferentially trabecular in this stage.

Cx40 expression in this stage more or less complements Cx43 expression. Cx40 is expressed mainly in atrial chambers and shows a transitory differential expression between the right and left ventricles. Cx40 expression, in turn, is substantially greater in the right and left bundle branches, although its expression in the atrioventricular node and bundle of His is similar to that of the working ventricular myocardium. The third conexin described in the mammalian heart (Cx45) presents a homogeneous pattern of expression, although some investigators claim the presence of a differential expression between the cardiac conduction system and working myocardium.

Genes that encode ion channels

SCN5A is expressed principally in the inflow tract, i.e., the myocardium of the caval veins, whereas ventricular expression is more baseline (Franco and Bezzina, unpublished data). Currently, no data exist regarding the distribution of SCN1B in the fetal heart or the distribution of these channels in the cardiac conduction system.

The expression of KCNQ1, KCNH2, and KCNE3 is homogeneous in the fetal myocardium. Nevertheless, the auxiliary subunits have a dynamic pattern of expression. KCNE1 remains limited to the ventricular myocardium, whereas KCNE2 and KCNE3 are confined to the atrial myocardium. The expression of certain subunits in the cardiac conduction system is interesting. KCNQ1 transcripts have similar levels of expression in the cardiac conduction system and working myocardium, but there is a clear increment in the amount of KCNQ1 protein in the conduction system (AV node, bundle of His, and right and left bundle branches). These differences suggest that a postranscriptional control mechanism specific to the tissue in the conduction system exists. KCNH2 expression is similar in the mRNA and protein in the myocardium.

Transcription factors

It has been documented recently that precise mutations in the *Nkx2.5* transcription factor are associated with congenital malformations of the interatrial septum and with conduction system dysfunction. Detailed study of the pattern of expression of *Nkx2.5* in the regions cited has shown that there are no differences in expression in the interatrial septum during its formation. Nevertheless, the atrioventricular nodule and the fascicle of His present a smaller expression of coding MRNA for Nkx2.5 112 in comparison with the work myocardium. Interestingly, there is a differential increment in the expression of *Nkx2.5* protein in these structures, which is due to a post-transcriptional mechanism of control specific to the conduction system

(D. Franco, unpublished data). Altogether, these observations suggest that the *Nkx2.5* transcription factor has an important role in the functional differentiation of the conduction system. The pattern of expression of *Pitx2* transcription factor varies gradually in the fetal stage of heart development. *Pitx2* is expressed mainly in the ventral region of the right ventricle, but not in the dorsal region. *Pitx2* is also expressed in the ventral and left region of the inflow tract, as well as in all the components of the left atrium, including the atrioventricular region of the mitral valve, the myocardium of the pulmonary veins and the interatrial septal complex (primary and secondary septa). In advanced and adult fetal stages, *Pitx2* expression is not observed in the myocardial tissue.

Miscellaneous

ANF expression at this stage is extremely important for understanding the molecular heterogeneity of the venous pole of the fetal heart. ANF expression is restricted to trabeculated atrial and ventricular myocardium. It is important to note that the expression in the atrial chambers is particularly interesting because it delimits the contribution of the atrioventricular canal myocardium (which does not express ANF) to the fetal atria. This also demonstrates the heterogeneity of the myocardium forming the caval and pulmonary veins with respect to the trabeculated atrial myocardium.

It is noteworthy that the gene that encodes the muscular isoform of mitochondrial creatine kinase (MCK) is first detected in the fetal stage (E14.5) of embryonal development and its expression in the myocardium is limited to the left ventricle. Later on in development, its expression is complemented in the right ventricle and atrial chambers. The existence of a differential expression in the ventricular myocardium (right/left) reinforces the hypothesis of independent transcription programs between the right and left ventricles.

Transgenesis

Aside from the patterns of expression of different transgenes observed in the embryonal myocardium, all which they are conserved in the fetal stage, new patterns of expression are established in this period. Particular emphasis should be placed on the transgenes that contain discrete regulatory elements of the MLC1F/3F locus (3F-nlacZ); the combination of different elements generates different patterns.

The combined analysis of patterns of expression in transgenes and endogenous genes has allowed the definition of four expression domains in the venous pole of the heart: embryonal atrioventricular canal, trabeculated appendages, myocardium of the caval veins, and myocardium of the pulmonary veins (the latter including the interatrial septum). The absence of ANF expression in the atrioventricular canal and analysis of the cardiac troponin I promoter has demonstrated the contribution of this embryonal region to the base of the atria. The differential expression of transgenic 3F-nlacZ and ANF has demonstrated differences between the atrial myocardium and outflow tract component (caval and pulmonary veins). The pattern of expression of the transgenes 3F-nlacZ 9E and endogenous MLC3F demonstrates that the myocardium of the caval veins is different from that of the pulmonary veins.

On the other hand, it is important to emphasize the expression of the En2-lacZ transgene described by Renthsler et al. In the fetal stage, En2-lacZ is located almost exclusively in the different components from the conduction system, i.e., the sinoatrial node, atrioventricular node, bundle of His, and right and left bundle branches. In addition, expression is evident in discrete regions of the right atrial myocardium linking the sinoatrial and atrioventricular nodes, as well as in a broad extension of the trabeculated ventricular myocardium. The regions of atrial myocardium that present transgene expression may be pathways of preferential conduction of the cardiac impulse between the atrioventricular and sinoatrial nodes, but as of yet there is no electrophysiological evidence to support this hypothesis. However, the expression in the trabeculated ventricular myocardium seems to correspond with the peripheral system of Purkinje fibers. This unique pattern of expression seems to be a faithful marker of the conduction system during heart development.

PATTERNS OF EXPRESSION IN THE ADULT HEART

In the adult heart, the patterns of expression observed in the fetal heart stay basically the same. We found 5 clear regions, four of which are included in the atrial myocardium. These 5 cell populations are the adult ventricle (including the embryonal outflow tract), the myocardium derived from the atrioventricular canal, the trabeculated atrial appendages, the myocardium of the caval veins and the myocardium of the pulmonary veins, which are defined by their particular

pattern of gene expression (e.g., ANF) and transcriptional potential (MLC3F transgenes). Interestingly, one of the significant disturbances than occur in the fetal to adult transition is experienced by the αMHC isoform in mice but not humans. αMHC passes from atrial expression in the fetal heart to the predominant isoform in the atrium and ventricle of the adult heart, thus displacing βMHC expression in ventricles.

CONCLUSIONS AND FUTURE PERSPECTIVES

As can be deduced from the previous sections, the heart muscle is a highly dynamic structure with an elevated degree of heterogeneity in its gene expression. This heterogeneity not only is characteristic of the myocardium in formation, but persists in the totally formed heart, which therefore has clinical implications.

The molecular heterogeneity of the myocardium is closely related with surgical practice. An example is the variability of results obtained by point ablation of certain atrial regions in patients with paroxysmal atrial fibrillation. Perhaps the reason for this variation in results does not depend so much on the effectiveness of the procedure (although it does in part) as it does on the heterogeneity of the underlying myocardium. The atrial myocardium is fairly homogeneous during development, in contrast with the situation in adults, which is highly heterogeneous. This means that perhaps not all regions are susceptible to ablation, in accordance with the underlying molecular characteristics. Consequently, a clear correlation should be established, for example, between zones of «atrial» myocardium with paroxysmal fibrillation and the limits of different myocardial regions (at the molecular level) in order to correctly interpret success in this type of intervention.

On the other hand, important efforts are being made to treat different myocardial dysfunctions by gene therapy. In experimental models, major advances have been made, but there has been little success in human patients. There are certain technical obstacles in the way that should be mentioned. On the one hand, our knowledge of the specific gene regulation system of the myocardium is still incipient, we only know certain elements, but not the principal mechanism («fundamental elements») of this regulation. On the other hand, we are ignoring the broad muscular heterogeneity of the adult myocardium, which limits the possibilities of successfully applying gene therapy targeting the cardiac muscle affected. In summary, the composition of the adult heart is a far cry from the classic conceptual classification of atrial and ventricular myocardium. There are many domains of gene expression, particularly in the inflow tract of the heart, which allow new (molecular) explanations for certain paradoxes of clinical practices to be postulated.

We must learn much more about the essential modules of gene regulation, and develop and optimize new strategies for patients that take molecular heterogeneity into consideration. Our efforts in basic research must aim at ascertaining the molecular mechanisms that control this cellular heterogeneity and thus provide a basis for not-too-distant future clinical applications. The key to the success of this *modus operandi* will be the disposition and willingness of specialists (basic and clinical) to contribute their knowledge to a combined effort for advancing work perspectives.

REFERENCES

1. Massage J, Chen Y-G. Controlling TGF-β signalling. Gen Dev 2000;14:627-44.
2. Schultheiss TM, Burch JBE, Lassar AB. A role of bone morphogenetic proteins in the induction of cardiac myogenesis. Gen Dev 1997;11:451-62.
3. Shi Y, Katsev S, Cai C, Evans S. BMP signalling is required for heart formation in vertebrates. Dev Biol 2000;224:226-37.
4. Manasek FJ. Embryonic development of the heart: I. Electron microscopic study of myocardial development in the early chicken embryo. J Morphol 1968;125:329-66.
5. Brown NA, Anderson RH. Symmetry and Laterality in the human heart: developmental implications. In: Harvey RP, Rosenthal N, editors. Heart Development 1999. Academic Press NY.
6. Moorman AFM, Lamers WH. Molecular anatomy of the developing heart. Trends Cardiovasc Med 1994;4:257-64.
7. Franco D, Lamers WH, Moorman AFM. Patterns of gene expression in the developing myocardium: towards a morphologically integrated transcriptional model. Cardiovasc Res 1998;38: 25-53.
8. De Jong F, Viragh SZ, Moorman AFM. Cardiac Development: a morphologically integrated molecular approach. Cardiol Young 1997;7:131-46.
9. Van den Hoff MJB, Moorman AFM, Ruijter JM, Lamers WH, Bennington RS, Markwald RR, et al. Myocardialization of the cardiac outflow tract. Dev Biol 1999;212:477-90.
10. Webb S, Brown NA, Anderson RH. The structure of the mouse heart in late fetal stages. Anat Embryol 1996;194:37-47.
11. Becker AE, Anderson RH. Cardiac pathology. Edinburgh: Churchill Livingstone, 1983.
12. García Martínez V, Schoenwolf GC. Primitive-streak origin of the cardiovascular system in avian embryos. Dev Biol 1993; 159:706-19.
13. Moorman AFM, Vermeulen JML, Koban MU, Schwartz K, Lamers WH, Boheler KR. Patterns of expression of sarcoplasmic reticulum Ca2+ATPase and phospholamban mARNs during rat heart development. Circ Res 1995;76:616-25.
14. Moorman AFM, Schumacher CA, de Boer PAJ, Hagoort J, Bezstarosti K, van den Hoff MJB, et al. Presence of functional sarcoplasmic reticulum in the developing heart and its confinement to chamber myocardium. Dev Biol 2000;223:279-90.
15. Fishman MC, Chien KR. Fashioning the vertebrate heart: earliest embryonic decisions. Development 1997;124:2099-117.
16. Lints TJ, Parsons LM, Hartley L, Lyons I, Harvey RP. Nkx2.5: a novel murine homeobox gene expressed in early heart progenitor cells and their myogenic descendants. Development 1993;119:419-31.
17. Raffin M, Leong LM, Rones MS, Sparrow D, Mohun T, Mercola M. Subdivision of the cardiac Nkx2.5 expression doamin into

myogenic and nonmyogenic compartments. Dev Biol 2000;218: 326-40.
18. Piedra ME, Icardo JM, Albajar M, Rodríguez-Rey JC, Ros MA. Pitx2 participates in the late phase of the pathway controlling left-right asymmetry. Cell 1998;94:319-24.
19. Campione M, Steinbeisser H, Schweickert A, Deissler K, van Bebber F, Lowe LA, et al. The homeobox gene Pitx2: mediator of asymmetric left-right signalling in vertebrate heart and gut looping. Development 1999;126:1225-34.
20. Campione M, Ros MA, Icardo JM, Piedra E, Christoffels VM, Schweichert A, et al. Pitx-2 expression defines a left cardiac lineage and provides evidence for the existence of ventricular isomerism in iv mice. Dev Biol 2001;231:252-69.
21. Bruneau BG, Bao Z-Z, Tanaka M, Schott JJ, Izumo S, Cepko CL, et al. Cardiac expression of the ventricle-specific homeobox gene Irx4 is modulated by Nkx2.5 and dHAND. Dev Biol 2000;217:266-77.
22. Molkentin JD, Lin Q, Duncan SA, Olson EN. Requirement of the transcription factor GATA4 for heart tube formation and ventral morphogenesis. Genes Dev 1997;11:1061-72.
23. Kuo CT, Morrisey EE, Anandappa R, Sigrist K, Lu MM, Parmacek MS, et al. GATA4 transcription factor is required for ventral morphogenesis and heart tube formation. Genes Dev 1997;11: 1048-60.
24. Koutsourakis M, Langeveld A, Patient R, Beddington R, Grosveld F. The transcription factor GATA6 is essential for early extraembryonic development. Development 1999;126:723-32.
25. 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;164:361-73.
26. 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;177:309-22.
27. 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.
28. Durocher D, Charron F, Warren R, Schwartz R, Nemer M. The cardiac transcription factors Nkx2.5 and GATA-4 are mutual cofactors. EMBO J 1997;16:5687-96.
29. Edmondson DG, Lyons GE, Martin JF, Olson EN. Mef2 gene expression marks the cardiac and skeletal muscle lineages during mouse embryogenesis. Development 1994;120:1251-63.
30. Lin Q, Schwarz J, Bucana C, Olson EN. Control of mouse cardiac morphogenesis and myogenesis by transcription factor MEF2C. Science 1997;276:1404-7.
31. Buckingham M. Skeletal muscle formation in vertebrates Curr Opin Genet Dev. 2001;11:440-8.
32. Kern MJ, Argao EA, Potter SS. Homeobox genes and heart development. Trends Cardiovasc Med 1995;5:47-54.
33. 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;170:664-78.
34. Srivastava D, Cserjesi P, Olson EN. A subclass of bHLH Proteins Required for Cardiac Morphogenesis. Science 1995;270:1995-9.
35. 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 Genetics 1997;16:154-60.
36. Riley P, Anson-Cartwright L, Cross JC. The Hand1 bHLH transcription factor is essential for placentation and cardiac morphogenesis. Nature Genetics 1998;18:271-5.
37. Nakagawa O, Nakagawa M, Richardson JA, Olson EN, Srivastava D. HRT1, HRT2 and HTR3: a new subclass of bHLH transcription factors marking specific cardiac, somitic and pharyngeal arch segments. Dev Biol 1999;216:72-84.
38. McGrew MJ, Pourquie O. Somitogenesis: segmenting a vertebrate. Curr Opin Genet Dev 1998;8:487-93.
39. Croissant JD, Kim JH, Eichele G, Goering L, Lough J, Prywes R, et al. Avian serum response factor expression restricted primarily to muscle cell lineages is required for α-actin gene transcription. Dev Biol 1996;177:250-64.
40. Saga Y, Hata N, Kobayashi S, Magnuson T, Seldin MF, Taketo MM. MesP1:a novel basic helix-loop-helix protein expressed in the nascent mesodermal cells during mouse grastrulation. Development 1996;122:2769-78.
41. Saga Y, Miyagawa-Tomita S, Takagi A, Kitajima S, Miyazaki J, Inoue T. MesP1 is expressed in the heart prescursor cells and required for teh formation of a single heart tube. Development. 1999;126:3437-47.
42. Zou Y, Evans S, Chen J, Kuo HC, Harvey RP, Chien KR. CARP, a cardiac ankyrin repeat protein, is downstream in the Nkx2.5 homoebox gene pathway. Development 1997;124:793-804.
43. Ghatpande S, Goswami S, Mathew S, Rong G, Cai L, Shafiq S, et al. Identification of a novel cardiac lineage-associated protein(cCLP-1): A candidate regulator of cardiogenesis. Dev Biol 1999;208:210-21.
44. Wei Y, Bader D, Litvin J. Identification of a novel cardiac-specifi transcript critical for cardiac myocyte differentiation. Development 1996;122:2779-89.
45. Hosoda T, Monzen K, Hiroi Y, Oka T, Takimoto E, Yazaki Y, et al. A novel myocyte-specific gene Midori promotes differentiation of P19CL6 cells intro cardiomyocytes. J Biol Chem 2001;276:35978-89.
46. Charron F, Paradis P, Bronchain O, Nemer G, Nemer M. Cooperative interaction between GATA-4 and GATA-6 regulates myocardial gene expression. Mol Cel Biol 1999;19:4355-65.
47. Hiroi Y, Kudoh S, Monzen K, Ikeda Y, Yazaki Y, Nagai R, et al. Tbx5 associates with Nkx2.5 and synergistically promotes cardiomyocyte differentiation. Nature Genet 2001;28:276-80.
48. Christoffels VM, Habets PEMH, Franco D, Campione M, de Jong F, Lamers WH, et al. Chamber formation and morphogenesis in the developing mammalian heart. Dev Biol 2000;223: 266-78.
49. Kelly R, Franco D, Moorman AFM, Buckigham M. Regionalization of transcriptional potential in the myocardium. In: Harvey RP, Rosenthal N,editors. Heart Development. New York: Academic Press, 1999.
50. Xavier-Neto J, Neville CM, Shapiro MD, Houghton L, Wang GF, Nikovits W, et al. A retinoic acid-inducible transgenic marker of the sino-atrial development in the mouse heart. Development 1999;126:2677-87.
51. He C-Z, Burch JBE. The chicken GATA-6 locus contains multiple control regions that confer distinct patterns of heart region-specific expression in transgenic mouse embryos. J Biol Chem 1997;272:28550-6.
52. Franco D, Kelly R, Lamers WH, Buckingham M, Moorman AFM. Compartment-specific myosin light chain 3F transgene expression in the embryonic mouse heart. Dev Biol 1997;188:17-33.
53. 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;165:121-31.
54. De la Cruz MV, Markwald RR. Living morphogenesis of the heart. Birkhauser. 1999.
55. Franco D, Kelly R, Moorman AFM, Lamers WH, Buckingham M, Brown NA. MLC3F transgene expression in iv mutant mice reveals the importance of left-right signalling pathway for the acquisition of atrial but not ventricular compartment identity. Dev Dyn 2001;221:206-15.
56. Satin J, Fujii S, de Haan RL. Development of the cardiac heartbeat in early chick embryos is regulated by regional cues. Dev Biol 1988;129:103-33.
57. De Haan RL, Fujii S, Satin J. Cell interactions in cardiac development. Dev Growth Differ 1990;32:233-41.
58. Schiaffino S, Reggiani C. Molecular diversity of myofibrillar proteins: gene regulation and functional significance. Physiol Rev 1996;76:371-423.

59. Yutzey KE, Bader D. Diversification of cardiomyocytes cell lineages during early heart development. Circ Res 1995;77:216-9.
60. 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;120:871-83.
61. Bao Z-Z, Bruneau BG, Seidman JG, Seidman CE, Cepko CL. Regulation of chamber-specific gene expression in the developing heart by Irx4. Science 1999;283:1161-4.
62. Biben C, Harvey RP. Homeodomain factor Nkx2-5 controls left/right asymmetric expression of bHLH gene eHand during murine heart development. Genes Dev 1997;11:1357-69.
63. Moore KL. The Developing Human: Clinically oriented Embryology. 3rd ed. Philadelphia: W.B. Saunders Co, 1982.
64. Zou Y, Chien KR. EFIA/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;15:2972-82.
65. Li Z, Marchard P, Humbert J, Babinet C, Paulin D. Desmin sequence elements regulating skeletal muscle-specific expression in transgenic mice Development 1993;117:947-59.
66. 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;132:849-59.
67. Kelly R, Alonso S, Tajbakhsh S, Cossu G, Buckingham M. Myosin light chain 3F regulatory sequences confer regionalized cardiac and skeletal muscle expression in transgenic mice. J Cell Biol 1995;192:383-96.
68. Ross RS, Navankasattusas S, Harvey RP, Chien KR. An HF-1a/HF-1b/MEF-2 combinatorial element confers cardiac ventricular specificity and establishes an anterior-posterior gradient of expression. Development 1996;122:1799-809.
69. Wright CVE. Mechanisms of left-right asymmetry: what´s right and what´s left? Dev Cell 2001;1:179-86.
70. Franco D, Kelly R, Zammit P, Buckingham M. Moorman AFM. The transcriptional building blocks of the heart. In: Doevendans P, Reneman RS, Van Bilsen M, editors. Cardiovascular specific gene expression. Dordrecht: Kluwer Academic Publish, 1999.
71. Franco D, Markman MWM, Wagenaar GTM, Ya J, Lamers WH, Moorman AFM. Myosin light chain 2a and 2v identifies the embryonic outflow tract myocardium in the developing rodent heart. Anat Rec 1999;254:135-46.
72. Kelly R, Zammit P, Mouly V, Butler-Browne G, Buckingham M. Dynamic left/right regionalization of endogenous myosin light chain 3F transcripts in the developing mouse heart. J Mol Cell Cardiol 1998;30:1067-81.
73. Kelly RG, Zammit PS, Buckingham ME. Cardiosensor mice and transcriptional subdomains of the vertebrate heart. Trends Cardiovasc Med 1999;9:3-10.
74. Ausoni S, de Nardi C, Moretti P, Gorza L, Schiaffino S. Developmental expression of rat cardiac troponin I mARN. Development 1991;112:1041-51.
75. Gros DB, Jongsma HJ. Connexions in mammalian heart function. Bioessays 1996;18:719-30.
76. 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;68:1638-51.
77. van Kempen MJA, Vermeulen JLM, Moorman AFM, Gross DB, Paul DL, Lamers WH. Developmental changes of connexin40 and connexin43 mARN-distribution patterns in the rat heart. Cardiovasc Res 1996;32:886-900.
78. Delorme B, Dahl E, Jarry-Guichard T, Marics I, Briand JP, Willecke K, et al. Developmental regulation of connexin40 gene expression in mouse heart correlates with the differentiation of the conduction system. Dev Dyn 1995;204:358-71.
79. Coppen SR, Dupont E, Rothery S, Severs NJ. Connexin45 expression is preferentially associated with the ventricular conduction system in mouse and rat heart. Circ Res 1998;82:232-43.
80. Coppen SR, Severs NJ, Gourdie RG. Connexin45 (α6) expression delineates and extended conduction system in the embryonic and mature rodent heart. Dev Genet 1999;24:82-90.
81. Alcoléa S, Théveniau-Ruissy M, Jarry-Guichard T, Marics I, Tzouanacou E, Chauvin JP, et al. Downregulation of connexin 45 gene products during mouse heart development. Circ Res 1999;84:1365-79.
82. 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: p. 22845.
83. Bezzina CR, Rook MB, Wilde AAM. Cardiac sodium channel and inherited arrythmia syndromes. Cardiovasc Res 2001;49:257-71.
84. Wang Q, Curran ME, Splawski I, Burn TC, Millohlland JM, VanRaay TJ, et al. Positional cloning of a novel potassium channel gene: KvQTL1 mutations cause cardiac arrhythmias. Nat Genet 1996;12:17-23.
85. Barharin J, Lesage F, Guillemare E, Fink M, Lazdunski M, Romey G. KvLQT1 and IsK (minK) proteins associate to form I(Ks) cardiac potassium current. Nature 1996;284:78-80.
86. McDonald TV, Yu Z, Ming Z, Palma E, Meyers MB, Wang KW, et al. A minK-HERG complex regulates the cardiac potassium current IKr Nature 1997;388:289-92.
87. Abbot GW, Sesti F, Splawski I, Buck ME, Lehman MH, Timothy KW, et al. MiRP forms IKr potassium channels with HERG and is associated with cardiac arrythmia. Cell 1999;97:175-87.
88. Schroeder BC, Waldegger S, Fehr S, Bleich M, Warth R, Greger R, et al. A constitutively open potassium channel formed by KCNQ1 and KCNE3. Nature 2000;403:196-99.
89. Tinel N, Diochot S, Borsotto M, Lazdunski M, Barhanin J. KCNE2 confers background current characteristics to cardiac KCNQ1 potassium channel. EMBO J 2000;19:6326-30.
90. Franco D, Demolombe S, Kupershmidt S, Dumaine R, Domínguez J, Roden D, et al. Divergent expression of delayed rectifier K+ channel subunits during mouse heart development Cardiovas Res 2001;52:65-75.
91. Christoffels VM, Keijser AGM, Houweling AC, Clout DEW, Moorman AFM. Patterning the embryonic heart: identification of five Iroquois homeobox genes in the developing heart. Dev Biol 2000:224:263-74.
92. Bruneau BG, Logan M, Davis N, Levi T, Tabin CJ, Seidman JG, et al. Chamber-specific cardiac expression of Tbx5 and heart defects in Holt-Oram syndrome. Dev Biol 1999;211:100-8.
93. Zammit PS, Kelly RG, Franco D, Brown N, Moorman AFM, Buckingham ME. Suppression of atrial myosin gene expression occurs independently in the left and right ventricles of the developing mouse heart. Dev Dyn 2000;217:75-85.
94. Franco D, Campione M, Kelly R, Zammit PS, Buckingham M, Lamers WH, et al. Multiple transcriptional domains, with distinct left and right components, in the atrial chambers of the developing heart. Circ Res 2000;87:984-91.
95. Di Lisi, R, Sandri C, Franco D, Ausoni S, Moorman AFM, Schaiffino S. An atrioventricular canal domain defined by cardiac troponin I transgene expression in the embryonic myocardium. Anat Embryol 2000;202:95-101.
96. 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;266:24613-20.
97. Knotts S, Sánchez A, Rindt H, Robbins J. Developmental modulation of a β-myosin heavy chain promoter-driven transgene. Dev Dyn 1996;206:182-92.
98. Olson EN, Srivastava D. Molecular Pathways Controlling Heart Development. Science 1996;272:671-6.
99. Renstchler S, Vaidya DM, Tamaddon H, Degenhardt K, Sassoon D, Morley GE, et al. Visualization and functional characterization of the developing musrine cardiac conduction system.

Development 2001;128:1785-92.
100. Kelly RG, Brown NA, Buckingham ME. The arterial pole of the mouse heart forms from FGF10-expressing cells in pharyngeal mesoderm. Dev Cell 2001;1:435-40.
101. Endo H, Mifune H, Kurohmaru M, Hayashi Y. Cardiac musculature of the cranial vena cava in the rat. Acta Anat 1994;151:107-11.
102. Kim JS, Viragh S, Moorman AFM, Anderson RH, Lamers WH. Development of the myocardium of the atrioventricular canal and the vestibular spine in the human heart. Circ Res 2001;88: 395-402.
103. Pexieder T. The conotruncus and its septation at the advent of the molecular biology era. In: Clark EB, Markwald RR, Takao A, editors. Developmental Mechanisms of Heart Disease. p 249-54, 1995.
104. Moorman AFM, Lamers WH. Development of the conduction system of the vertebrate heart. In: Harvey RP, Rosenthal N, editors. Heart Development. Academic Press, 1999.
105. Franco D, Icardo JM. Molecular characterization of the ventricular conduction system in the developing mouse heart: topographical correlation in normal and congenitally malformed hearts. Cardiovasc Res 2001;49:417-29.
106. Moorman AFM, de Jong F, Denyn MMFJ, Lamers WH. Development of the conduction system. Circ Res 1998;82:629-44.
107. 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;229:355-68.
108. Scott JJ, Benson DW, Basson CT, Pease W, Silberbach M, Moak JP, et al. Congenital heart disease caused by mutations in the transcription factor Nkx2.5 Science 1998;281:108-11.
109. Benson DW, Silberbach GM, Kavanuagh-McHugh A, Cottrill C, Zhang Y, Riggs S, et al. Mutations in the cardiac transcription factor Nkx2.5 affect diverse cardiac developmental pathways. J Clin Invest 1999:104:1567-73.
110. Biben C, Weber R, Kesteven S, Stanley E, McDonald L, Elliot DA, et al. Cardiac septal and valvular dysmmorphogenesis in mice heterozygous for mutations in the homeobox gene Nkx2.5. Circ Res 2000;87:888-95.
111. Thomas PS, Kasahara H, Edmonson AM, Izumo S, Yacoub MH, Barton PJ, et al. Elevated expression of Nkx2.5 in developing myocardial conduction cells. Anat Rec 2001;263:307-13.
112. Lyons, G. In situ analysis of the cardiac muscle gene program during embryogenesis. Trends Cardiovasc Med 1994;4(2):70-7.
113. Lompre AM, Nadal-Ginard B, Mahdavi V. Expression of the cardiac ventricular alpha and beta myosin heavy chain genes is developmentally and hormonally regulated. J Bio Chem 1984;259:6437-46.