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.

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 spatio-temporal distribution and level of expression during ontogenesis; these changes are especially dynamic during heart development. The formation of the heart in
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
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
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 atroventricular canal, takes place by the fusion of the cardiac cushion mesenchyme and its later replacement by myocardioctyes 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
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 myocardocytes. 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**

<table>
<thead>
<tr>
<th>Cardiac crest (stage I)</th>
<th>Structural genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogeneous</td>
<td>Structural genes</td>
</tr>
<tr>
<td>R/L axis</td>
<td>A/P axis</td>
</tr>
<tr>
<td>Nkx, GATA4-6, MEF2C, HANDs SRF, CARP, pCMF1, Midori cCLP-1, Mesp1, Tbx5</td>
<td>Ptbx2 (L)</td>
</tr>
<tr>
<td>Nkx, GATA4-6, MEF2C, HTR3 SRF, CARP, pCMF1, Mesp1, Midori dHANd</td>
<td>Ptbx2 (L)</td>
</tr>
<tr>
<td>MLC, troponin C/T, tropomyosin, tropomodulin</td>
<td>cMHC, MLC2a, actin-c, ( \beta )MHC (A), SERCA2 (P), PLB (A)</td>
</tr>
<tr>
<td>Nkx, GATA4-6, MEF2C, HTR3 SRF, CARP, pCMF1, Midori dHANd</td>
<td>Ptbx2 (L)</td>
</tr>
<tr>
<td>MLC, troponin C/T, tropomyosin, tropomodulin</td>
<td>HTR1 (P), HTR2 (A)</td>
</tr>
<tr>
<td>cMHC, MLC2a, actin-c, ( \beta )MHC (A), SERCA2 (P), PLB (A)</td>
<td></td>
</tr>
<tr>
<td>(L) indicates left; (A), anterior; (P), posterior; actin-c, cardiac actin.</td>
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</tr>
</tbody>
</table>
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...
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 Tbx5, 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 antero-posterior axis, either in expression gradients (SERCA and PLB) or discrete regions (Irx4; HRT), and that there are genes that are expressed differentially in the right and left axis (Pitx2). 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.

### Table 2. Patterns of expression in the embryonal and fetal heart

<table>
<thead>
<tr>
<th></th>
<th>Inflow tract</th>
<th>Atria</th>
<th>AV canal</th>
<th>Ventricles</th>
<th>Outflow tract</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Embryonal heart</strong> (stage IV)</td>
<td>Contractile proteins</td>
<td>α and βMHC, MLC1a/v, MLC2a/v, Troponin I</td>
<td>αMHC, MLC1a, MLC2a/v</td>
<td>α and βMHC, MLC1a/v, MLC2a/v</td>
<td>α and βMHC, MLC1a/v, MLC2a/v</td>
</tr>
<tr>
<td></td>
<td>Calcium metabolism</td>
<td>SERCA2</td>
<td>SERCA2</td>
<td>PLB</td>
<td>PLB</td>
</tr>
<tr>
<td></td>
<td>Intercalated disks ion channels</td>
<td>Cx45, Scn5a, KCNH2, Kcnq1, Kcne2/e3</td>
<td>Cx45, Scn5a, KCNH2, Kcnq1, Kcne2/e3</td>
<td>Cx45, Scn5a, KCNH2, Kcnq1, Kcne2/e3</td>
<td>Cx45, Scn5a, KCNH2, Kcnq1, Kcne2/e3</td>
</tr>
<tr>
<td></td>
<td>Transcription factors</td>
<td>Pitx2 (L), Tbx5, Tbx2, HRT1, eHAND, Pitx2 (L), Tbx5, HRT1, Inx3</td>
<td>Pitx2 (L), Tbx5, Tbx2, e/HAND</td>
<td>Tbx5 (L), Pitx2 (V), Inx1, 2, 3, 4, HTR2, d/HAND</td>
<td></td>
</tr>
<tr>
<td><strong>Fetal heart</strong> (stage V)</td>
<td>Contractile proteins</td>
<td>αMHC, MLC1a, MLC2a</td>
<td>αMHC, MLC1a, MLC2a</td>
<td>βMHC, MLC1a, MLC2a</td>
<td>βMHC, MLC1a, MLC2a</td>
</tr>
<tr>
<td></td>
<td>Calcium metabolism</td>
<td>SERCA2</td>
<td>SCN5A, KCNH2, Kcnq1, Kcne2/e3</td>
<td>SCN5A, KCNH2, Kcnq1, Kcne2/e3</td>
<td>SCN5A, KCNH2, Kcnq1, Kcne2/e3</td>
</tr>
<tr>
<td></td>
<td>Intercalated disks ion channels</td>
<td>Cx40, Scn5a, KCNH2, Kcnq1, Kcne2/e3</td>
<td>Cx40, Scn5a, KCNH2, Kcnq1, Kcne2/e3</td>
<td>Cx43, SCN5A, KCNH2, Kcnq1, Kcne2/e3</td>
<td>Cx43, SCN5A, KCNH2, Kcnq1, Kcne2/e3</td>
</tr>
<tr>
<td></td>
<td>Transcription factors</td>
<td>βMHC, Tbx5, HTR1, e/hAND</td>
<td>βMHC, Tbx5, HTR1, Inx3</td>
<td>βMHC, HTR2, d/hAND, e/hAND</td>
<td>βMHC, HTR2, d/hAND, e/hAND</td>
</tr>
</tbody>
</table>

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.
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 caudal region than in the cephalal 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 caudal region than in the cephalal 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 cephalal zone and decreases towards the caudal 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 (cephalocaudal) in the primitive cardiac tube that they presented in earlier stages; HRT1 is expressed in the caudal region whereas HRT2 is expressed in the cephalal 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 caudal or cephalal 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-
The pattern of expression of the embryonal ventricle entails the displacement of cardiac looping. The movement to the right of the fetal loop presents an interesting pattern of expression during myocardial specification, but are not necessary for the postulate that these factors have a primordial role in cardiac tube. GATA5 and GATA6 disappear gradually as the myocardial expression becomes preferentially at the ends of the cardiac tube. Similarly, the eHAND transcription factor, which in the left regions of the ends of the cardiac tube shows displacement of its expression toward the prospective zone of differentiation, 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 regionalization of pattern of expression. αMHC, MLC1α, and MLC2α expression are restricted mainly to the venous pole, including the inflow tract, atrium, and atrioventricular channel. βMHC, MLC1β, and MLC2β 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 MLC2α show residual expression in the outflow tract, as it regresses toward the atrial pole takes place, whereas βMHC and MLC2β 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 connexins. In the mammalian heart, the main connexin 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 connexin 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 connexins 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, *Irxl* and *Irxx*. 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 apparent 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 inflow 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. Rentschler, 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 sinoatral 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 posttranscriptional 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 bundle 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 connexin in the mammalian heart, connexin 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 posttranscriptional 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 connexin 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 posttranscriptional 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**

Franco D, et al. Genic expression in the embryonal myocardium

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 Renthals 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 atroventricular 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.

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