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Hox and Tale transcription factors in heart development and disease

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ABSTRACT *Hox* genes are highly conserved transcription factors with critical functions during development, in particular for patterning the antero-posterior axis of the embryo. Their action is very often associated with cofactors including the TALE family transcription factors. From *Drosophila* to vertebrates, *Hox* genes have been shown to have a major role in heart development. In this review, we focus on the increasing evidence implicating the anterior *Hox* genes and the Tale family members during heart development both in the cardiac mesoderm and in neural crest cells. Congenital heart defects are the leading cause of death in the first year of life and a better understanding of the role of *Hox* and *Tale* factors is highly relevant to human pathologies and will provide novel mechanistic insights into the underlying defects.

KEY WORDS: *Hox, heart development, congenital heart disease, cardiovascular system*

Introduction

Hox genes are highly conserved homeodomain transcription factors, originally described as critical for segmental identity along the antero-posterior (A-P) axis in *Drosophila* (Lewis, 1978). These properties appeared to be conserved, as Hox proteins also establish positional identity along the A-P axis during vertebrate development (Deschamps and van Nes, 2005). In mammals, *Hox* genes are organized in four paralogous clusters on distinct chromosomes and are expressed in defined and often overlapping domains along the body axis in a manner corresponding to their position along the chromosome. This propriety is known as spatial collinearity (Duboule and Dolle, 1989). *Hox* genes show also temporal collinearity, which coordinates their expression along the rostro-caudal embryonic axis.

Hox functions are flexible and their activity is highly context-dependent. Several examples show that the same Hox can act as an activator or a repressor depending on the circumstances (Krasnow *et al*., 1989). The high conservation of the homeodomain results in low sequence specificity. This can be enhanced via interaction with various cofactors. An example is provided by the TALE (three amino acid loop extension) proteins (Moens and Selleri, 2006). Most Hox proteins bind DNA cooperatively with the Extradenticle (Exd)/Pbx family of proteins, whereas others interact preferentially with Homothorax/Meis family members. Hox proteins also present variable sequences outside the homeodomain, allowing them to interact with a large combination of cofactors. Several factors use the same interaction site, such that only one can be bound at a time, suggesting that several "Hox complexes" may exist, which vary in subunit composition, and most likely in function (Ladam and Sagerstrom, 2014). Moreover, a recent study found that Hox proteins operate as tissue-specific factors to modulate the ground state binding of TALE cofactors to instruct anatomic identity (Amin *et al*., 2015).

Over the past several years, studies have clearly demonstrated that *Hox* function is required for invertebrate and vertebrate heart morphogenesis. In the fruit fly *Drosophila melanogaster* the dorsal vessel (a pulsatile organ) is subdivided into two functionally and morphologically distinct regions – the heart (segments middle of A4 to A7) and the aorta (segments T1 to middle of A4) (Fig. 1). The anterior-posterior patterning of the dorsal vessel is also demonstrated by the localization of the various cardiac and associated cells types (Fig. 1). In the thoracic 'anterior aorta', each segment is constituted of four pairs of cardiomyocytes expressing the homeodomain-containing factor Tinman (Tin), whereas in the abdominal region each segment contains six pairs of cardiac cells. The two anterior cardiomyocytes express the orphan nuclear receptor Seven-up (Svp) while the four posterior ones express Tin

Abbreviations used in this paper: NCC, neural crest cell; OFT, outflow tract; PAA, pharyngeal aortic arch; SHF, second heart field; SMC, smooth muscle cell; TALE, three amino acid loop extension; VSD, ventricular septal defect.

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(with the exception of A7, which is composed of only two pairs of Tin-positive cardiomyocytes). Several studies have shown that the anterior-posterior patterning of the dorsal vessel is controlled by *Hox* genes (Lo and Frasch, 2003; Monier *et al.*, 2007). Four *Hox* genes are expressed in essentially non-overlapping domains in the dorsal vessel: *Antennapedia* (*Antp*, from segment T3 to A1), *Ultrabithorax (Ubx*, from segment A1 to A4), *abdominal-A* (*abd-A*, from segment A4 to A7) and *Abdominal-B* (*Abd-B*, in the last pair of cardiomyocytes in segment A7) (Lo and Frasch, 2003; Monier *et al.*, 2007). The four other *Drosophila Hox* genes (*labial*, *proboscipedia*, *Deformed* and *Sex combs reduced* (*Scr*)) are not expressed in the dorsal vessel. Furthermore, functional experiments have demonstrated that *Antp*, *Ubx*, *abd-A* and *Abd-B* genes play critical roles in multiple phases of heart development in *Drosophila* (Lo and Frasch, 2003; Monier *et al.*, 2007). Studies using loss- and gain-of-function approaches have shown that *abd-A*, which is expressed precisely in the heart portion, is necessary for the specification of this domain of the dorsal vessel. Indeed, ectopic expression of *abd-A* in the thoracic mesoderm before the stage 11 transforms the identity of thoracic segments into abdominal segments (Lo and Frasch, 2001; Perrin *et al.*, 2004). Deletion of the five Hox genes (*Scr*, *Antp*, *Ubx*, *abd-A* and *Abd-B*) results to a complete transformation of all abdominal segments into thoracic segments based on the number subtypes of cardiomyocytes (Tin+ and Svp+) as well as the pericardial cells (Perrin *et al.*, 2004). Interestingly, Ubx, abd-A double mutant embryos have a similar transformation, indicating the crucial function of these two genes in the control of abdominal versus thoracic cardiac cell identity. These studies and others have shown that Hox genes have pleiotropic functions in the choice of cell lineages within cardiac mesoderm, in the differentiation of cardiac cells and in the remodeling of the larval cardiac tube into the adult heart.

In vertebrates, the heart is a more complex organ, with four chambers and different cardiac cell types ensuring the oxygenation of the blood and transport of oxygen and nutrients throughout the organism during fetal and post-natal life. Cardiovascular development is a complex but ordered process that is spatially and temporally regulated (Poelmann *et al.*, 2008). Embryonic heart development begins at the early stage of gastrulation, around embryonic day (E) 6.5 in the mouse, with the specification of a pool of cardiovascular progenitor cells expressing the transcription factor Mesp1 (Saga *et al.*, 1999). After gastrulation these cells migrate from the primitive streak to the anterior part of the embryo where they differentiate in the cardiac crescent at E7.5 (Fig. 2A-B). Convergence of the bilateral structures results in the formation of the linear heart tube. The heart tube is a transient structure composed of an inner layer, the endocardium, and outer myocardial layer. Rapid growth of the heart tube is driven by progressive addition of second heart field (SHF) cells at the arterial and venous poles (Fig. 2C). The SHF is located in pharyngeal mesoderm (Buckingham *et al.*, 2005; Rochais *et al.*, 2009; Zaffran and Kelly, 2012). As SHF progenitor cells are added to the forming heart, they are exposed to a number of surrounding signals (Rochais *et al.*, 2009; Zaffran and Kelly, 2012). After rightward looping, the heart is shaped by expansion of the myocardium, which leads to the formation of four cardiac chambers, two atria and two ventricles (Fig. 2D). The forming heart is connected to the bilateral dorsal aorta by the outflow tract (OFT), aortic sac and pharyngeal arch arteries (PAAs). Cardiac chambers and OFT septation is required for separation of systemic and pulmonary blood flows. Simultaneous development of the great arteries that supply the head, neck and upper limbs is also crucial for the developing embryo. This process involves extensive vascular remodeling of the five pharyngeal arch arteries beginning at around E11.5 to form the definitive arterial architecture. Although initially symmetric, the most rostral PAAs in the 1st and 2nd arches mostly regress, whereas the caudal PAAs are remodeled into adult vascular structures. This results from the rotation of the myocardium and the preferential development of the aorta on the left side to the detriment of the right side (Yashiro *et al.*, 2007). Development of the OFT and PAAs requires a specific subpopulation of neural crest cells (NCCs) originating in the posterior hindbrain and located in the neural plate between the otic placode and the third pair of somites, termed the "cardiac neural crest" for their important contribution to heart development (Kirby *et al.*, 1983; Kirby and Waldo, 1995). NCCs migrate through pharyngeal arches 3, 4 and 6 to invade the heart through the arterial pole. During this migration, NCCs are closely apposed to SHF cells, allowing interactions between these two cell types (Rochais *et al.*, 2009). They contribute to the smooth muscles of the arteries, to the OFT endocardial cushions as well as to the aortico-pulmonary septum separating the aorta and the pulmonary trunk (Kirby *et al.*, 1983; Le Lievre and Le Douarin, 1975). Genetic lineage tracing of NCCs using the *Wnt1*^{Cre} mouse line revealed their contribution to smooth muscle cells (SMCs) of the ascending aorta, of the aortic arch, proximal carotid and coronary arteries and to conotruncal cushion mesenchyme during formation of the aortic valve (Jiang *et al.*, 2002). This lineage tracing was followed over time until adulthood and revealed that the distribution of NCCs is dynamic over time and that leads to a refinement of NCCs contribution in the mature heart (Jiang *et al.*, 2002). The first cells invading the OFT participate to the formation of the aortico-pulmonary septum and those migrating

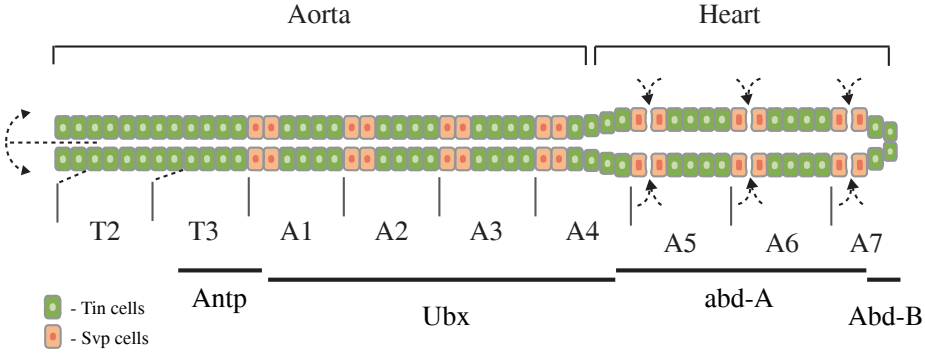

Fig. 1. Schematic representation of the *Drosophila* embryonic dorsal vessel. *The dorsal vessel comprises a posterior heart region and an anterior aorta, each of which contains* Tinman *(*Tin*) and* Seven-up *(*Svp*) expressing cells. In the heart region, three pairs of ostia (inflow tract) forming by Svp-positive cells allow the entrance of hemolymph. Expression patterns of the Hox proteins Antennapedia (Antp), Ultrabithorax (Ubx), abdominal-A (abd-A) and Abdominal-B (Abd-B) in the dorsal vessel are also indicated.*

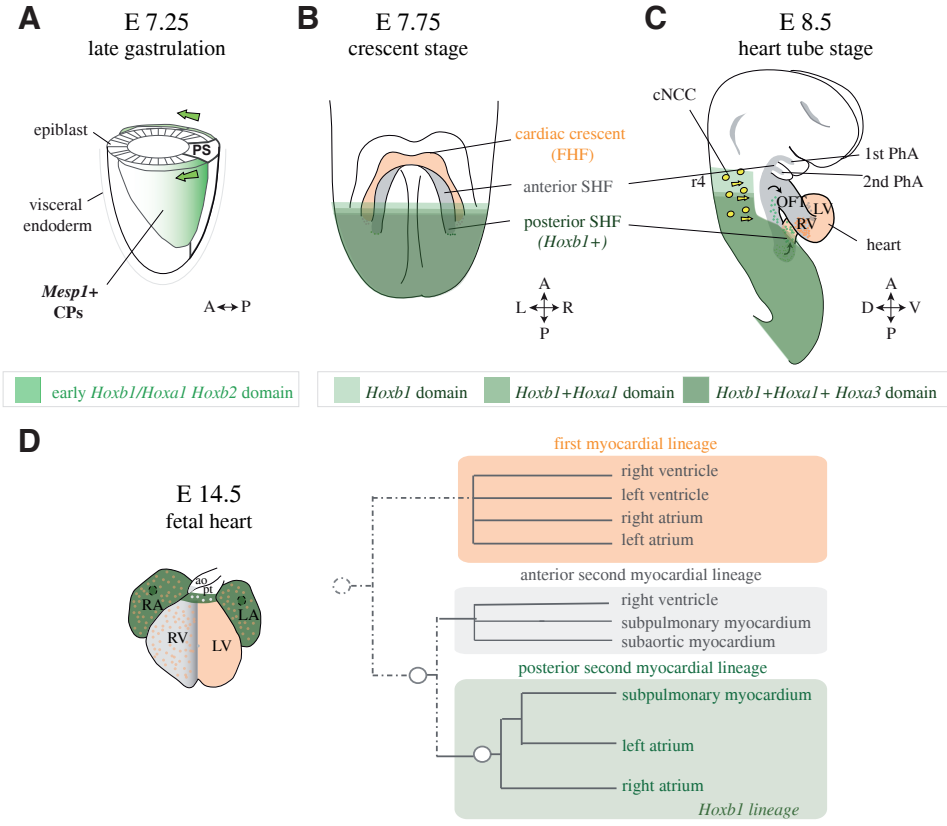

Fig. 2. Hox expression and lineage during mouse heart development. (A-D) *Schemes of the early steps of cardiac morphogenesis. The expression domains of* Hox *genes are indicated in green.* **(A)** *Lateral view of an E7.25 embryo during late gastrulation. Cardiovascular progenitors (CPs) expressing* Mesp1 *ingress the primitive streak (PS) and migrate toward the anterior part of the embryo (green arrows). The onset of the most anterior Hox genes expression (*Hoxb1/Hoxa1 *and* Hoxa2 *in green) is found at E7.25 in a domain close to the primitive streak (Lescroart* et al., *2018).* **(B)** *Frontal view is shown for embryonic day 7.75 (E7.75). The cardiac crescent corresponding to the first heart field (FHF in orange) is formed at the anterior side of the embryo. The limit of* Hox *genes expression domain (in green) is found at the posterior limit of second heart field (SHF).* **(C)** *Lateral view for E8.5. An heart tube is formed and cells of the SHF are added to the cardiac tube at both its arterial and venous pole. Cardiac neural crest cells (cNCC - in yellow) expressing the anterior Hox genes migrate to the heart.* **(D)** *Early* Hoxb1/a1/a3 *expressing cells characterize distinct sub-domains along the antero-posterior axis in the SHF. Later, in the fetal heart, these cardiac progenitor cells contribute to both atria and to myocardium at the base of pulmonary trunk (Bertrand* et al., *2011). Dots represent cell contributions from the FHF (orange) or from the anterior SHF (grey). The lineage tree for myocardial cells is depicted on the right.* Hoxb1 *lineage is indicated in green. A, anterior; Ao, aorta; CC, cardiac crescent; D, dorsal; L, left; LA, left atria; LV, left ventricle; OFT, outflow tract; P, posterior; PhA, pharyngeal arch, Pt, pulmonary trunk; R, right; RA, right atria; RV, right ventricle; r4, rhombomere 4; V, ventral.*

later contribute to the remodeling of the PAAs, in particular to the smooth muscle wall of the arteries themselves (Boot *et al.*, 2003). Recent studies have further characterized the contribution of pre-otic and post-otic NCCs to the forming heart and showed that while pre-otic NCCs contribute to coronary arteries and adjacent cardiac tissues, post-otic NCCs are essential to aortic and pulmonary valve leaflet formation (Arima *et al.*, 2012, Odelin *et al.*, 2018). These findings suggest a great heterogeneity in NCC contributions during the formation of the heart.

Heart morphogenesis is tightly regulated such that a slight perturbation during the early and late steps of heart morphogenesis may result in a spectrum of cardiovascular defects. In human, variably abnormal cardiovascular development is reflected in the high incidence of congenital heart diseases (CHD), affecting nearly 1-2% of all live births (Hoffman and Kaplan, 2002). It often difficult to determine the cause of the CHD with certitude as often multigenic causes; nonetheless, studying factors that control cardiovascular development can help better understand the etiology of these defects. Numerous molecular pathways have been implicated in heart formation (Buckingham *et al.*, 2005; Rochais *et al.*, 2009; Srivastava, 2006; Vincent and Buckingham, 2010; Zaffran and Kelly, 2012).

In this review, we will discuss different studies documenting the expression and contribution of *Hox* and/or *Tale* positive cells to the development of the vertebrate heart. Increasing evidence now indicates that Hox and Tale factors play critical roles during cardiac morphogenesis as cardiac malformations or other congenital heart diseases are often found in animal models or patients with mutations in Hox or Tale genes.

Hox genes in heart development and disease

Hox *expression and lineage*

During gastrulation, cardiovascular progenitors are among the first mesodermal cells to emerge from the primitive streak between E6.5 and E7.5 in the mouse embryo. Lineage tracing experiments have shown that cardiovascular progenitors are marked by the expression of the transcription factor Mesp1 and that these cells will contribute to most of the heart, excluding NCC derivatives (Lescroart *et al.*, 2014; Saga *et al.*, 1999). At the same time, activation of genes along the *Hox* clusters occurs sequentially in the primitive streak throughout axis elongation (Deschamps and van Nes, 2005). Several signaling pathways, including the Bone Morphogenetic protein (BMP), Fibroblast growth factor (FGF) and Wnt pathways are required for specification of cardiogenic mesoderm (Zaffran and Frasch, 2002). Lengerke *et al.,* have shown that coordination of these pathways induces patterning of the mesoderm and specification by activating Cdx/Hox genes, emphasizing the role of Hox factors during this process (Lengerke *et al.*, 2008). More recently, the transcriptional profiling of single *Mesp1*+ cardiovascular progenitors has shown the heterogeneity of the progenitor cell population with the identification of 5 different subpopulations

characterized by different transcriptional signatures and localized at distinct locations relative to the primitive streak (Lescroart *et al.*, 2018) (Fig. 2A). Interestingly one *Mesp1+* subpopulation is characterized by the expression of the most anterior *Hox* genes *Hoxa1*, *Hoxa2*, *Hoxb1* and *Hoxb2*. This *Hox* positive subpopulation of cardiovascular progenitor cells is located close to the primitive streak at E7.25 suggesting that it may correspond to the last cardiovascular progenitor population to emerge from the primitive streak (Lescroart *et al.*, 2018). Only a fraction of the *Mesp1+* pool of cardiovascular progenitors expresses *Hox* genes and it is unclear if other homeodomain transcription factors act to pattern the *Hox*-negative population. Very interestingly, clonal analysis of single *Mesp1+* cardiovascular has revealed that cardiovascular progenitors are already restricted in their lineage so that an early *Hox*-negative cardiovascular progenitor population is unipotent contributing to either the muscular or endocardial lineages of the left ventricle, while a late population is bipotent contributing to cardiomyocytes and smooth muscle cells or to cardiomyocytes and endocardial cells (Devine *et al.*, 2014; Lescroart *et al.*, 2014). *Hoxb1* has been shown to be upregulated in the late bipotent population suggesting that *Hoxb1+* cardiovascular progenitors might be bipotent in contrast to the *Hox* negative left ventricular progenitors (Lescroart *et al.*, 2014).

After the migration and differentiation of cardiovascular progenitor cells to form the cardiac crescent and primitive cardiac tube, elongation of the tube occurs by addition of SHF progenitor cells at the arterial and venous poles of the forming heart. Recent studies have clarified the origin of these cardiac progenitors and their regulation (Buckingham *et al.*, 2005; Rochais *et al.*, 2009; Vincent and Buckingham, 2010; Zaffran and Kelly, 2012). SHF cells are characterized by an elevated proliferation and delayed differentiation, and by expression of the transcription factors *Islet 1* (Cai *et al.*, 2003), *Nkx2-5* (Prall *et al.*, 2007) and *Tbx1* (Baldini, 2005). Since its discovery, evidence has accumulated that the SHF is pre-patterned along the antero-posterior axis (Galli *et al.*, 2008; Zaffran *et al.*, 2004). Anterior and posterior SHF populations have been identified that contribute to the formation of OFT and right ventricular myocardium at the arterial pole and a large part of the atrial myocardium at the venous pole, respectively (Vincent and Buckingham, 2010) (Fig. 2B-D). However, the factors required for the A-P patterning of the SHF were, until recently, unknown. Analysis of the expression pattern of the most anterior *Hox* genes (including the most anterior *Hoxa1-3* and *Hoxb1-2* genes) in the early embryo at around E8.5 to E9.5, demonstrated that in SHF mesoderm, the most anterior expression domains of *Hoxa1*, *Hoxb1* and *Hoxa3* overlap with the posterior limit of *Islet1* and *Tbx5* expression (Fig. 2B-C) (Bertrand *et al.*, 2011). This suggests that an interaction between T-box transcription factors such as Tbx5 and the anterior *Hox* genes may also mediate the patterning of the SHF in a similar model to that described in the limbs where Tbx4/5 interact with posterior Hox genes to direct limb specific gene expression (Jain *et al.*, 2018).

Genetic lineage tracing confirmed that *Hox*-expressing SHF cells contribute to the heart (Fig. 2D). Surprisingly, this study revealed that *Hoxb1^{Cre}*-labeled cells contribute to both poles of the heart tube: the inferior wall of the OFT, which give rise to the sub-pulmonary myocardium at the arterial pole, the atrio-ventricular canal and atrial myocardium at the venous pole (Bertrand *et al.*, 2011). Similarly, lineage tracing of *Hoxa1-enhIII-Cre-* and *Hoxa3^{Cre}*-labeled cells showed contributions of the *Hoxa1-* and *Hoxa3*-expressing cells to both the atria and the distal part of the OFT (Bertrand *et al.*, 2011; Makki and Capecchi, 2010). Consistent with these observations, retrospective clonal analysis confirmed that sub-pulmonary and atrial myocardial cells are clonally related (Fig. 2D) (Lescroart *et al.*, 2012). Furthermore, this clonal analysis also demonstrated a clonal relationship between the sub-pulmonary and atrial myocardium with a subset of skeletal muscles of the neck that do not derive from the somites (Lescroart *et al.*, 2015). This suggest that *Hoxb1+* SHF progenitors might include bipotent progenitors for the Trapezius and Sternocleidomastoid neck muscles and for myocardium of the OFT and atria. Recent single cell analysis of *Isl1+* cells (between E7.5 and E9.5) also showed the expression of more posterior *Hox* genes including *Hoxa7*, *Hoxa9*, *Hoxb6*, *Hoxc8* and *Hoxd8* (unpublished data). Furthermore, studies in mouse embryonic stem cells showed that Hoxa10 cooperates with Nkx2-5 to regulate the timing of cardiac differentiation *in vitro* (Behrens *et al.*, 2013). Lineage tracing experiments would be required to follow the descendants of these more posterior Hox positive cells and address whether they really have a significant contribution to heart development.

Cardiac NCCs also express a specific set of *Hox* genes, depending on their rostro-caudal origin. Genetic lineage tracing analysis in mice shows that *Hoxa1* is expressed in the NCCs that contribute to OFT cushions (Bertrand *et al.*, 2011). *Hoxb1* is expressed in rhombomere 4 and from rhombomere 6 to the posterior side of the embryo (Studer *et al.*, 1998). NCCs migrating to the third pharyngeal arch express normally *Hoxa3* as seen in the *Hoxa3-lacZ* transgenic mice (Diman *et al.*, 2011).

Regulation of Hox expression

It is well established that major signaling pathways, such as FGF, retinoic acid (RA) and Wnt, play important roles in the establishment of ordered domains of *Hox* expression. In particular, exogenous RA can induce anterior expansions of *Hox* expression domains. Consistently, several Hox genes have well characterized RA-response elements (RAREs) in their enhancers or proximal promoters (Alexander *et al.*, 2009; Duester, 2008; Niederreither and Dolle, 2008). Several novel enhancers containing RAREs have been identified and characterized in the *HoxA* and *HoxB* gene cluster as well as *Hoxa3* genes (Diman *et al.*, 2011; Nolte *et al.*, 2013). Interestingly, studies in several animal models have shown that discrete levels of RA signaling are required for the A-P patterning of the heart tube (Zaffran and Niederreither, 2015). In the mouse, loss of function of retinaldehyde dehydrogenase 2 (*Raldh2*), which encodes an enzyme for the synthesis of endogenous RA in the early embryo, results in cardiac defects including a hypoplastic inflow tract region (IFT) (Niederreither *et al.*, 1999). Further investigations of *Raldh2* mutant mouse embryos have shown that RA signaling is required to define the posterior boundary of the SHF (Ryckebusch *et al.*, 2008; Sirbu *et al.*, 2008). Expression of several SHF markers, including *Islet1*, *Tbx1, Fgf8* and *Fgf10*, are caudally expanded in *Raldh2$^{-/-}$*. In addition, disruption of RA signaling causes a failure in the deployment of SHF-derived cells that contribute to the inferior wall of OFT myocardium (Bertrand *et al.*, 2011). In zebrafish, RA signaling has also been shown to regulate the size of the cardiac field through indirect regulation of *hoxb5b* expression in the adjacent forelimb field (Waxman *et al.*, 2008). Furthermore, increased Hoxb5 activity or excess of RA

result in similar cardiac phenotypes (Waxman and Yelon, 2009). When mouse embryos are treated with a teratogenic dose of RA, an anterior shift of the rostral border of *Hoxa1*, *Hoxb1* and *Hoxa3* expression domains is observed (Bertrand *et al.*, 2011). Hence, these data suggest that *Hox* genes are susceptible to the dose of RA and that effect of RA signaling on heart development may be mediated via *Hox* function.

However, there is also evidence for a RA-independent regulation of *Hox* genes. Indeed, in a RA deficient context, the *Hoxa1* lineage disappears while the *Hoxb1* lineage is maintained (Bertrand *et al.*, 2011). Mesp1 ChipSeq experiments on mouse ES cells show binding of Mesp1 to *Hoxb1* sequences suggesting that Mesp1 could directly regulate *Hoxb1* expression in a subset of cardiovascular progenitors, before the onset of RA expression (Lescroart *et al.*, 2014; Lescroart *et al.*, 2018). In addition, long intergenic non-coding RNAs, (lincRNA) have been shown to be important for the regulation of *Hox* genes (Soshnikova, 2014). Recently the role of a *Hoxb* locus associated lincRNA, termed *HoxBlinc* has been reported in mesoderm specification and regulation of *HoxB* gene transcription (Deng *et al.*, 2016). This study showed that *HoxBlinc* RNA regulates specification of mesoderm-derived Flk1+ toward cardiac and hematopoietic lineages. Deng *et al.,* demonstrated that *HoxBlinc* RNA activates transcription through recruiting the Setd1a/MLL1 complexes and facilitates the organization of a specific 3D chromatin structure that activates the anterior *Hoxb* genes, resulting in cardiogenic-hematogenic mesoderm differentiation. Loss of *HoxBlinc* RNA leads to a decrease in *Hoxb1-b6* gene expression as well as a down-regulation of four important transcription factors *Islet1*, *Nkx2-5*, *Mef2c*, and *Gata4* involved in cardiac differentiation (Deng *et al.*, 2016). Thus, this study demonstrated a novel cooperation between lincRNA and *Hox* genes during the specification and differentiation of a specific lineage.

Role of Hox genes during heart development and disease

Hox genes control many aspects of cardiovascular development and *Hoxa1* genome wide binding analysis in mouse ES cells, for example, revealed that Hoxa1 is able to target genes associated with biological processes including heart development and NCC migration (De Kumar *et al.*, 2017).

During the early steps of cardiogenesis, *Mesp1+* cardiovascular progenitors have to migrate from the primitive streak to the anterior part of the embryo to form the cardiac crescent (Fig. 2A). Studies in the chick have shown that cardiovascular progenitor migration is triggered by Wnt3a signaling (Yue *et al.*, 2008). Interestingly, Lengerke *et al.,* (2008) demonstrated that addition of Wnt3a to embryonic stem (ES) cells is sufficient to induce posterior *Hox* gene expression (Lengerke *et al.*, 2008). This was the first study to links Wnt3a to Hox genes. We could thus wonder whether a relationship between Wnt3a and the most anterior Hox triggers the migration of a subset of Mesp1+ cardiovascular progenitors.

In addition, while it has been shown *in vitro* that Mesp1 promotes cardiovascular progenitor migration by direct activation of *Prickle1* and *Rasgrp3* (Chiapparo *et al.*, 2016), studies have demonstrated the activation of *prickle1b* by Hoxb1a during facial neuron migration in zebrafish (Rohrschneider *et al.*, 2007). This further suggests that *Hoxb1* might play a role in the migration of cardiovascular progenitors.

Perturbation of the SHF during elongation of the forming heart tube results in a spectrum of conotruncal congenital defects, ranging from OFT misalignment, including double outlet right ventricle (DORV), overriding aorta, and tetralogy of Fallot (ToF), to ventricular septal defects (VSD) (Ward *et al.*, 2005). Several studies showed that human patients carrying a homozygous truncating mutation in *HOXA1* have OFT malformations (Holve *et al.*, 2003; Tischfield *et al.*, 2005). Interestingly, similar defects have recently been reported for *Hoxa1* deficient mice (Table 1), demonstrating that *Hoxa1* is

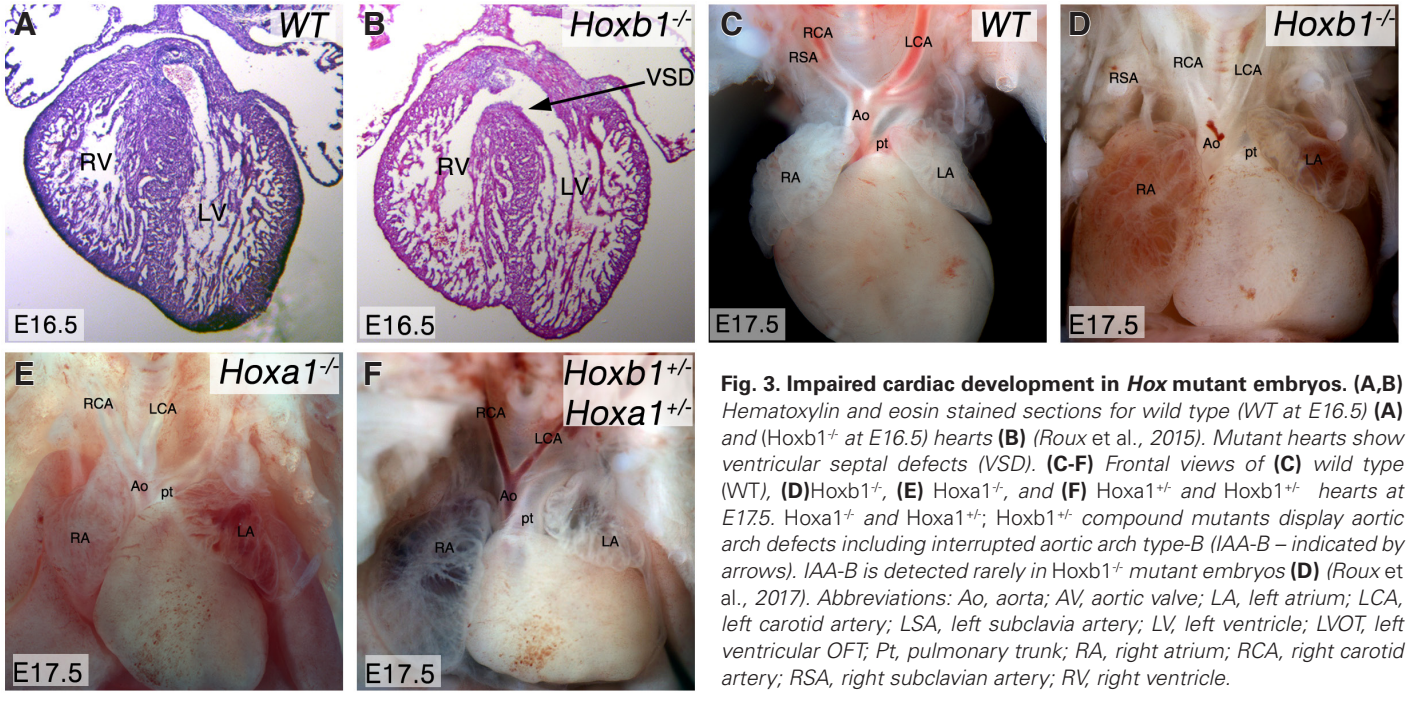

Fig. 3. Impaired cardiac development in *Hox* mutant embryos. (A,B) *Hematoxylin and eosin stained sections for wild type (WT at E16.5)* **(A)** *and* (Hoxb1$^{-/-}$ *at E16.5) hearts* **(B)** *(Roux* et al., *2015). Mutant hearts show ventricular septal defects (VSD).* **(C-F)** *Frontal views of* **(C)** *wild type* (WT*),* **(D)**Hoxb1$^{-/-}$*,* **(E)** Hoxa1$^{-/-}$*, and* **(F)** Hoxa1$^{+/-}$ *and* Hoxb1$^{+/-}$ *hearts at E17.5.* Hoxa1$^{-/-}$ *and* Hoxa1$^{+/-}$; Hoxb1$^{+/-}$ *compound mutants display aortic arch defects including interrupted aortic arch type-B (IAA-B – indicated by arrows). IAA-B is detected rarely in* Hoxb1$^{-/-}$ *mutant embryos* **(D)** *(Roux* et al., *2017). Abbreviations: Ao, aorta; AV, aortic valve; LA, left atrium; LCA, left carotid artery; LSA, left subclavia artery; LV, left ventricle; LVOT, left ventricular OFT; Pt, pulmonary trunk; RA, right atrium; RCA, right carotid artery; RSA, right subclavian artery; RV, right ventricle.*

required for patterning of the OFT of the heart (Makki and Capecchi, 2012). More recently, we identified a previously unknown role for the *Hoxb1* gene in the forming heart (Roux *et al.*, 2015). Mouse embryos carrying homozygous *Hoxb1* null allele have VSD and OFT defects (Table 1 and Fig. 3). These malformations are the consequence of a failure in the deployment of SHF cells during the formation of the OFT (Roux *et al.*, 2015). Interestingly, the balance between proliferation and differentiation in the SHF is disrupted in embryos lacking *Hoxb1* with premature differentiation of SHF cells in the mutant embryos. *Hox* genes might also play a role in the differentiation of cardiac progenitor cells. Indeed, reduction of the length of the OFT and increased incidence of heart defects were observed in compound *Hoxa1*$^{-/-}$; *Hoxb1*$^{+/-}$ embryos compared to *Hoxa1*$^{-/-}$ mutants, demonstrating an overlap in function between these two genes during the formation of the OFT (Roux *et al.*, 2015).

Several studies have demonstrated the role of Hox genes in patterning NCCs, particularly in the cranial region of the embryo. Using chick embryos Gouti *et al.,* showed that the most anterior *Hox* genes participate in NCC induction (Gouti *et al.*, 2011). This study established that *Hox* play a central role in NCC specification by rapidly inducing the transcription factors *Snail2* and *Msx1/2*. The same study demonstrated that anterior *Hox* genes impose a neural to NCC fate switch by a reduction in proliferation and changes in cell adhesion that lead to epithelial-to-mesenchymal transition (Gouti *et al.*, 2011). The most anterior *Hox* genes play also a role in cardiac NCCs. Cardiac NCCs contribute to the development of the circulatory system including the formation of the great arteries. Great artery defects have been reported in *Hoxa3*$^{-/-}$ mice including malformation of the carotid arteries, which probably results from bilateral degeneration of the 3rd aortic arch artery (Chisaka and Capecchi, 1991; Chisaka and Kameda, 2005; Kameda *et al.*, 2003). Bosley-Salih-Alorainy syndrome (BSAS) patients have homozygous mutations in *HOXA1* and exhibit cardiac malformations including interrupted aortic arch type B (IAA-B), aberrant subclavian artery, VSD, and ToF (Bosley *et al.*, 2008; Tischfield *et al.*, 2005). Two recent studies have reported cardiac defects including VSD in *Hoxa1*$^{-/-}$ mice consistent with a role in NCCs (Makki and Capecchi, 2012; Roux *et al.*, 2015). Indeed, *Hoxa1* acts upstream of the transcription factors *Zic1* and *Foxd3*, two major players in neural crest development (Makki and Capecchi, 2012). More recently, the analysis of single or compound *Hoxa1*; *Hoxb1* mutants has shown defects in pharyngeal arch artery development. IAA-B defects were identified in a large number of *Hoxa1*$^{-/-}$ or *Hoxb1*$^{+/-}$; *Hoxa1*$^{+/-}$ embryos but more rarely in *Hoxb1*$^{-/-}$ mutant embryos (Fig. 3, Table 1). This confirmed a major role for *Hoxa1* in PAA development and revealed that while *Hoxb1* alone has a minor role, it acts in a synergistic interaction with *Hoxa1* during the remodeling of the great arteries. The PAA defects in *Hoxa1* or *Hoxa1; Hoxb1* compound mutants are likely due to a defect in NCC migration (Roux *et al.*, 2017). Interestingly the overexpression and ectopic expression of *Hoxb1* in all NCCs also leads to cardiovascular defects with the frequent observation of misaligned great arteries, abnormally branched or connected PAAs and biscupid aortic valve defects (Zaffran *et al.*, 2018).

Tale factors in heart development and disease

TALE factors (PBC, MEIS), which are associated as cofactors of Hox transcription factors, are also expressed in cardiovascular

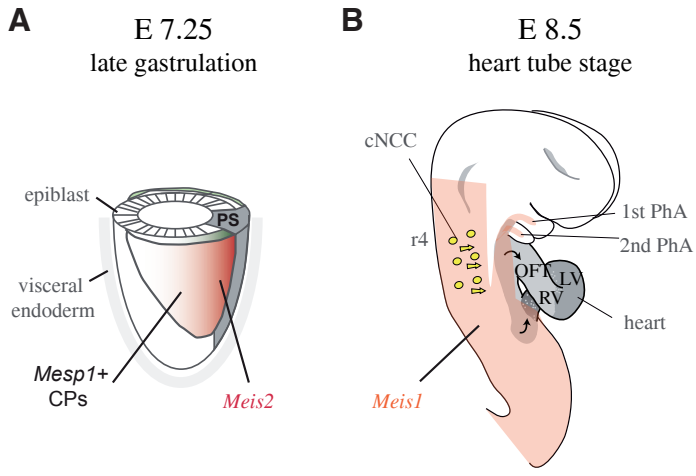

Fig. 4 Meis expression during mouse heart development. *The expression domains of* Meis *genes are indicated in red.* **(A)** *Lateral view of an E7.25 embryo during late gastrulation.* Meis2 *expression has been found at E7.25 in the population of* Mesp1+ *cardiovascular progenitors (CPs) expressing* Hoxb1 (Lescroart et al., 2018). **(B)** *Lateral view for E8.5. Meis1 expression has been shown in cardiac neural crest cells (cNCC - in yellow) as well as in the anterior second heart field (Dupays* et al., *2015). cNCC, cardiac crescent; LV, left ventricle; OFT, outflow tract; PhA, pharyngeal arch, PS, primitive streak; RV, right ventricle; r4, rhombomere 4.*

progenitors and cardiac NCCs. The function of TALE factors in cardiovascular development has not yet been fully addressed however there is increasing evidence that they might act together with anterior *Hox* genes during heart development.

In vertebrates, the PBC includes four PBX (pre B cell leukemia homeobox), whereas the PREP and MEIS subfamilies include two PREP (prolyl endopeptidase) and three MEIS (myeloid ecotropic viral integration site 1 homolog) proteins. PBX proteins bind also to other factors including HDACs (Histone deacetylase) and HATs (Histone acetyltransferase), which suggests a role in the regulation of Hox complex activity (Saleh *et al.*, 2000). Studies have reported that the formation of trimeric complexes involves HOX, PBX, and MEIS proteins (Ferretti *et al.*, 2000). Such interactions may modulate the transcriptional activity of the Hox complex (Ladam and Sagerstrom, 2014). Choe *et al.,* demonstrated that Hoxb1-regulated promoters are highly acetylated on histone H4 and occupied by Hoxb1, Pbx and Meis in zebrafish tissues (Choe *et al.*, 2009). Inhibition of Meis blocks gene expression and reduces acetylated Histone H4 levels. In addition, Meis proteins promote CBP (CREB binding protein) recruitment by probably displacing HDACs to permit CBP binding. In conclusion, Meis acts by controlling access of HDAC and CBP to Hox-regulated promoters.

A recent study in zebrafish showed that Pbx/Meis association occurs on *hoxb1a* promoter but is not sufficient for transcriptional activation (Choe *et al.*, 2014). Recruitment of hoxb1b releases "poised" RNA-PolII, allowing transcription to start. Thus, the authors suggest that TALE factors access promoters during early embryogenesis to poise them for activation but that Hox proteins are required to trigger efficient transcription. In mouse ES cells, De Kumar *et al.,* have used genomic approaches to characterize the binding properties of Hoxa1 and have shown very frequent co-occupancy of Pbx and Meis on Hoxa1 bound regions (De Kumar *et al.*, 2017). Interestingly, they also uncover associations with other

TALE family members such as Prep1, Prep2 and Tgif suggesting that the TALE family proteins represent a wider repertoire of Hox cofactor. The genome wide binding analyses have also pointed to an important series of auto – and cis-regulatory interactions among *Hoxa1* and *Tale* genes in ES cells (De Kumar *et al.*, 2017).

TALE expression and regulation during heart morphogenesis

As for Hox genes, TALE factors are detected very early during heart development such that *Meis1* and *Meis2* have been reported to be transiently and robustly activated at the cardiovascular progenitor stage during mouse ESC differentiation assays (Wamstad *et al.*, 2012). In the gastrulating mouse embryo, *Meis2* expression is associated with *Hoxb1* expression in a subpopulation of *Mesp1*+ cardiovascular progenitors (Fig. 4). As for *Hoxb1*, Mesp1 has been shown to bind *Meis2* enhancer region suggesting that it could also regulate its expression (Lescroart *et al.*, 2018). Mesp1 induced expression in mouse ES cells increases the level of *Pbx1* expression indicating that Pbx1 might also be important at the early stages of cardiovascular specification.

The expression of TALE factors is not restricted to the earliest steps of cardiogenesis such that *Meis1* expression has been reported between E8.5 and E9.5 in both the anterior region of the SHF and in the distal part of the OFT (Dupays *et al.*, 2015) (Fig. 4). *Meis2* is expressed in the NCCs (Machon *et al.*, 2015). Pbx proteins have also been detected widely in tissues relevant to the heart such as NCCs, the pharyngeal arches and the myocardium and endocardium between E8.5 and E9.5. In contrast to *Hoxa1*, *Hoxb1* or *Hoxa3*, the expression of Pbx proteins are thus maintained in differentiated cardiac cells such that in the fetal heart they are detected in the endocardial and smooth muscle cells of the great arteries but are downregulated in myocardium (except for Pbx2) (Stankunas *et al.*, 2008). Interestingly, the fish *meis2b*, described as *Meis1* homologue, is expressed, as early as the 12 somites stage, in the cardiogenic posterior lateral mesoderm. From 4 days post-fertilization its expression is switched off from the ventricle and become restricted to the left part of the atrium until adulthood (Guerra *et al.*, 2018). It would be interesting to investigate whether this bilateral expression of *Meis* is conserved in mammals.

Similarly to *Hox*, *Tale* genes are likely to be regulated by RA signaling. A recent study in human ES cells has interestingly shown that *Meis2* expression is immediately induced upon RA treatment (Quaranta *et al.*, 2018). This common regulation and the overlapping expression domains between the most anterior *Hox* genes and *Tale* factors in cardiac mesoderm or neural crest cells suggest a cooperation between the Hox factors and their Tale cofactors during heart development.

Role of TALE factors during heart development and disease

Pbx and *Meis* genes have been involved in cardiovascular development (Chang *et al.*, 2008; Kao *et al.*, 2015; Machon *et al.*, 2015; Mahmoud *et al.*, 2013; Maves *et al.*, 2009; Moens and Selleri, 2006). *Pbx1*$^{-/-}$ mutants have anomalies of the great arteries and OFT septation defects (Chang *et al.*, 2008). Interaction between *Pbx1, Pbx2* and *Pbx3* has been demonstrated as compound mutants have more severe cardiac defects than single mutant (Stankunas *et al.*, 2008). *Pbx1* displayed the most severe phenotype, with PTA (persistent truncus arteriosus) (Table 1). Chang *et al.,* showed that Pbx function is required in NCCs to promote *Pax3* expression, which is necessary for OFT development (Chang *et al.*, 2008). In zebrafish, *pbx4* is required to form a proper OFT, probably through the formation of the SHF (Kao *et al.*, 2015). Absence of *Meis1* results in cardiac defects such as VSD similar to *Pbx* mutations (Stankunas *et al.*, 2008). This result led the authors to propose that Pbx1 interacts with Hox and/or Meis1 proteins to control a subset of target genes important for OFT development. In addition, Meis1 has also been described as a key factor for proliferation of postnatal cardiomyocytes (Mahmoud *et al.*, 2013). It remains to be elucidated what genes are direct targets of Pbx/Meis factors. Interestingly together with Hox or independently from Hox genes, TALE factors seem to also act with other key cardiac transcription factors. Wamstad *et al.,* have identified a functional relationship between Gata and Meis transcription factors (Wamstad *et al.*,

TABLE 1

CARDIAC PHENOTYPES REPORTED IN *HOX* OR *TALE* MUTANT EMBRYOS

Genes	Mutants	Cardiac phenotypes	References
Hoxa1	*Hoxa-1.6*$^{-/-}$	No cardiac phenotype, VSD, IAA-B, CAA, Ab-RSA, RAA	(Lufkin *et al.*, 1991; Roux *et al.*, 2017)
	Hoxa1$^{GFPneo/GFPneo}$	No cardiac phenotype	(Godwin *et al.*, 1998)
	Hoxa1$^{-/-}$	IAA-B, ASC, BAV, RAA, VSD, ToF	(Makki and Capecchi, 2012)
Hoxa3	*Hox-1.5*$^{-/-}$	No cardiac phenotype	(Chisaka and Capecchi, 1991)
	Hoxa3$^{-/-}$	Degeneration of the 3rd arch artery malformation of the carotid artery system	(Chisaka and Kameda, 2005; Kameda *et al.*, 2003)
Hoxb1	*Hoxb1*$^{GFP/GFP}$	OFT defects, VSD, rare IAA-B	(Roux *et al.*, 2015; Roux *et al.*, 2017)
	Wnt1-Cre; CAG-Hoxb1-IRES-GFP	BAV	(Zaffran *et al.*, 2018)
Hoxa1/Hoxb1	*Hoxa1-1.6*$^{-/-}$*; Hoxb1*$^{GFP/+}$	OFT defects, VSD, IAA-B, CAA, Ab-RSA, RAA	(Roux *et al.*, 2015; Roux *et al.*, 2017)
HoxA/HoxB	*Hoxa*$^{-/-}$*; Hoxb*$^{-/-}$	Heart looping defects	(Soshnikova *et al.*, 2013)
Pbx1	*Pbx1*$^{-/-}$	PTA and VSD	(Stankunas *et al.*, 2008)
Pbx2	*Pbx2*$^{-/-}$	No cardiac phenotype	(Stankunas *et al.*, 2008)
Pbx3	*Pbx3*$^{-/-}$	No cardiac phenotype	(Stankunas *et al.*, 2008)
Pbx1/ Pbx2/ Pbx3	*Pbx1*$^{+/-}$*; Pbx2*$^{+/-}$*; Pbx3*$^{+/-}$	BAV	(Stankunas *et al.*, 2008)
	Pbx1$^{+/-}$*; Pbx2*$^{-/-}$	Overriding aorta, VSD, BAV, bicuspid pulmonary valve	(Stankunas *et al.*, 2008)
	Pbx1$^{+/-}$*; Pbx2*$^{-/-}$*; Pbx3*$^{+/-}$	ToF	(Stankunas *et al.*, 2008)
Meis1	*Meis1*$^{-/-}$	Overriding aorta, VSD	(Stankunas *et al.*, 2008)
	Meis1$^{ECFP/ECFP}$	VSD	(Gonzalez-Lazaro *et al.*, 2014)
	a-MHC-CRE; Meis1$^{f/f}$	Increased postnatal cardiomyocyte proliferation	(Mahmoud *et al.*, 2013)
Meis2	*Meis2*$^{-/-}$	PTA and VSD	(Machon *et al.*, 2015)
	AP2a-Cre; Meis2$^{f/f}$	DORV, abnormal semilunar valves	(Machon *et al.*, 2015)

Ab-RSA, abberrant origin of the right subclavian artery including retroesophageal right subclavian artery; BAV, bicuspid aortic valve; CAA, cervical aortic arch; DORV, double outlet right ventricle; IAA-B, interrupted aortic arch; PTA, persistent truncus arteriosus; RAA, right side aortic arch; ToF, tetralogy of Fallot; VSD, ventricular septal defect.

2012) and Dupays *et al.,* also reported an overlapping binding site for Nkx2-5 and Meis1 (Dupays *et al.*, 2015). Moreover, genome wide association studies have linked both *NKX2*-5 and *MEIS1* to conduction defects in human patients (Pfeufer *et al.*, 2010).

Machon *et al.,* (2015) showed that the loss of *Meis2* function results in OFT defect (Machon *et al.*, 2015). Although cardiac defects have also been reported in a neural crest-targeted deletion no OFT defects were observed, suggesting a role for non-crest *Meis2*-expressing cells in OFT formation (Table 1). Recently, *MEIS2* mutations have been reported in patients with cardiac defects such as atrial septal defects (ASD) and VSD (Crowley *et al.*, 2010; Fujita *et al.*, 2016; Louw *et al.*, 2015). Paige *et al.* studied the temporal chromatin alterations in human ES cells as these cells differentiate into cardiomyocytes, thus, providing a model to determine key regulator genes of heart development (Paige *et al.*, 2012). This work characterized those "key" genes as tightly regulated since the consequence of their inappropriate expression could lead to extremely severe defects caused by cell-fate change. *MEIS2* was among the genes identified. Using the zebrafish model, these authors demonstrated that *Meis* is critical for cardiac development. Indeed, embryos that received splice-blocking morpholino directed against *meis2b* had defective cardiac morphogenesis (Paige *et al.*, 2012). A recent study further showed that *meis2b*$^{-/-}$ zebrafish embryos have abnormal atrial growth due to an increased atrial proliferation as well as conduction defects that increase over time. In addition, Guerra *et al.,* showed that both the fish and mouse MEIS1/2 regulate the expression of *pitx2* (Guerra *et al.*, 2018). Furthermore, MEIS2 might also be very important for posterior SHF development as Quaranta *et al.,* showed that MEIS2 activation significantly suppresses Isl1 and activates Nr2f1 and thus promotes atrial cardiomyocytes specification in human ES cells (Quaranta *et al.*, 2018). Despite these studies, the function of TALE factors in cardiovascular development has clearly been under investigated and their role is not yet fully understood.

Conclusions

In this review, we have reported studies documenting expression of the genes encoding for the Hox and Tale factors during heart development from gastrulation to later embryonic stages in both cardiac mesoderm and neural crest cells. There is now increasing evidence that Hox and Tale factors can act together and have major roles during heart development as illustrated by the phenotypes of mutant mice and the association of mutations in human patients. Lineage tracing as well as genetic approaches aimed at deleting one or more alleles of the *Hox*, *Pbx* and *Meis* genes will provide valuable information regarding the interaction of these genes during heart development. In addition, tissue specific deletion of these factors will help better understanding the origin of the defects in NCCs or cardiac mesoderm. Finally, it will be crucial to identify the common direct targets of Hox and Tale transcription factors, which could provide insights into potential mechanisms underlying CHDs.

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Disclosure statement

The authors have nothing to declare

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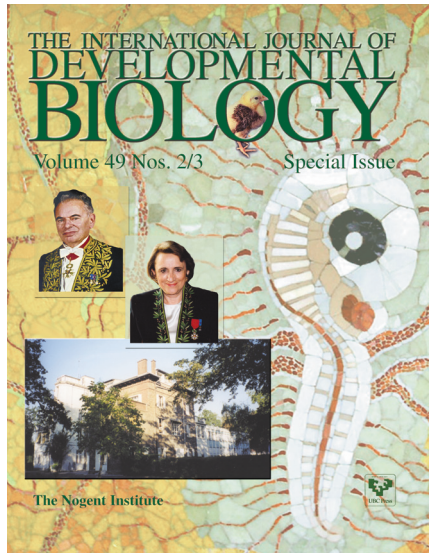

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