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Mesp1: A Key Regulator of Cardiovascular Lineage Commitment

Cardiopoietic Factors: Extracellular Signals for Cardiac Lineage Commitment

Developmental and Regenerative Biology of Multipotent Cardiovascular Progenitor Cells

Regulation of Smooth Muscle Cell Commitment

Michael Schneider, Guest Editor

Mesp1
A Key Regulator of Cardiovascular Lineage Commitment

Antoine Bondue, Cédric Blanpain

Abstract: In mammals, the heart arises from the differentiation of 2 sources of multipotent cardiovascular progenitors (MCPs). Different studies indicated that an evolutionary conserved transcriptional regulatory network controls cardiovascular development from flies to humans. Whereas in Drosophila, Tinman acts as a master regulator of cardiac development, the identification of such a master regulator in mammals remained elusive for a long time. In this review, we discuss the recent findings suggesting that Mesp1 acts as a key regulator of cardiovascular progenitors in vertebrates. Lineage tracing in mice demonstrated that Mesp1 represents the earliest marker of cardiovascular progenitors, tracing almost all the cells of the heart including derivatives of the primary and second heart fields. The inactivation of Mesp1/2 indicated that Mesp genes are essential for early cardiac mesoderm formation and MCP migration. Several recent studies have demonstrated that Mesp1 massively promotes cardiovascular differentiation during embryonic development and pluripotent stem cell differentiation and indicated that Mesp1 resides at the top of the cellular and transcriptional hierarchy that orchestrates MCP specification. In primitive chordates, Mesp also controls early cardiac progenitor specification and migration, suggesting that Mesp arises during chordate evolution to regulate the earliest step of cardiovascular development. Defining how Mesp1 regulates the earliest step of MCP specification and controls their migration is essential to understand the root of cardiovascular development and how the deregulation of these processes can lead to congenital heart diseases. In addition, these findings will be very useful to boost the production of cardiovascular cells for cellular therapy, drug and toxicity screening. (Circ Res. 2010;107:1414-1427.)

Key Words: Mesp1 ■ stem cells ■ cardiovascular progenitors ■ transcription ■ migration

The heart is generated soon after gastrulation from the differentiation of multipotent cardiovascular progenitors (MCPs) into the different cell lineages that constitute the mature heart, including cardiomyocytes, pacemaker cells, vascular cells, and smooth muscle cells.1 Two sources of MCPs contribute to the formation of the heart in mammals.2 The primary heart field MCPs give rise to the left ventricle and cells of both atria, whereas the second heart field MCPs give rise to the right ventricle, atrial cells, and cells of the vascular outflow tract.2,3 Although the genes expressed within the primary and second heart field progenitors and regulate their function are relatively well known,3 the molecular and cellular mechanisms that govern the specification of the early MCPs that give rise to these 2 sources of progenitors...
and guide their migration from the primitive streak (PS), a transient structure in which the cells of the early embryo ingress into to form the mesoderm and the endoderm, to the anterolateral pole of the embryo are not well characterized.

Phylogenetic studies support the idea that the heart has evolved by the modification of an ancestral gene regulatory network consisting of transcription factors of the Nkx, Hand, Gata, and Mef families, which through gene duplication and cooption of novel transcriptional regulators, allow the increasing complexity of heart development during evolution from the simple heart tube in *Drosophila* to the 4-chambered heart of mammals.\(^4,5\) Whereas Tinman, a homeodomain transcription factor and the homolog of Nkx2-5, acts as a master regulator of the gene regulatory network that promotes cardiac cell fate specification in flies,\(^5\text{-}^8\) the loss of Nkx2-5, although leading to severe cardiac malformations, does not prevent cardiac cell fate specification, suggesting that other transcription factors could have substituted for the early functions of Tinman.\(^9,10\)

In this review, we discuss the possibility that Mesp1, a basic helix-loop-helix transcription factor, has taken the role of key transcriptional regulator of cardiovascular progenitor specification during vertebrate evolution. We discuss the different studies that recently showed that Mesp1 promotes cardiovascular differentiation and resides at the top of cellular and transcriptional hierarchy during embryonic development and embryonic stem cell differentiation. A better understanding of the gene regulatory network and cellular hierarchy that govern cardiovascular progenitor specification and early cardiovascular lineage commitment will improve the means of producing cardiovascular cells for cellular therapy in humans, drug and toxicity screening, as well as studying the molecular mechanisms of congenital heart diseases.

**Mesp1 Represents the Earliest Marker of Cardiovascular Progenitors in Mice**

Mesp1 has been first identified following a screen for transcripts enriched in the posterior region of the mouse embryo at embryonic day (E)7 to E7.5 and was called "mesoderm posterior 1," or Mesp1, referring to its expression in the posterior part of the embryonic mesoderm.\(^11\) Mesp1 is expressed in the nascent mesoderm at the onset of gastrulation (from E6.5) in the early mesodermal cells that ingress into and exit the PS (Figure 1A).\(^11,12\) As Mesp1-expressing cells leave the PS, the expression of *Mesp1* is rapidly downregulated. A weaker expression of *Mesp1* is seen as a pair of bands in the presomitic mesoderm, which correspond to the precursors of cranial mesoderm.\(^11,12\) Beyond E8.5, *Mesp1* is expressed with *Mesp2* in the presomitic mesoderm. Mice expressing β-galactosidase (β-gal) under the regulatory region of *Mesp1*, in which the β-gal was retained longer than Mesp1 mRNA expression, demonstrate that Mesp1-expressing cells that migrate out of the PS are incorporated into the heart field and the head mesenchyme.\(^12\)

The definitive demonstration that Mesp1-expressing cells give rise to cardiac cells came from genetic lineage tracing experiments in mice. Mice in which the recombinase CRE was knocked in under the control of the regulatory sequence of *Mesp1* (Mesp1-CRE) were crossed with lacZ reporter mice.\(^12\) In their double transgenic offsprings (Mesp1-CRE/R26R-β-gal), all cells that expressed *Mesp1* at one point of the development are irreversibly marked and all their progeny will express β-gal whether or not the cells continue to express endogenous *Mesp1* (Figure 1B). At E9.5, β-gal staining was mainly observed in the heart, dorsal aorta, intersomitic and cranial vessels, and the amnion contiguous to the closing foregut (Figure 1C).\(^12\) β-Gal–positive cells were present in all cardiac lineages including the myocardium, the endocardium, the conduction cells and the epicardium (Figure 1D).\(^12,13\) Although it was initially suggested that Mesp1 derived cells do not give rise to endocardium cells,\(^12\) studies showed that Mesp1-expressing cells also contribute to the development of the endocardium.\(^13,14\) *Mesp1* is expressed in the precursor of MCPs of both heart fields because both ventricles and atria, as well as the cells of the outflow tract, which represent an exclusive second heart field derivative, were stained with β-gal.\(^2,12,13\) Only 20% of cells of the conductive system in the interventricular septum and some cells of the cardiac outflow tract cushions are not marked by β-gal, suggesting that these cells derived from non–Mesp1-expressing cells and may represent neural crest derivatives.\(^14\) Alternatively, Mesp1 might have been expressed only too transiently or at low levels, in the ancestors of unstained cells, which may also explain the discrepancies reported in different Mesp1-CRE lineage tracing experiments, such as the labeling of endocardium cells. Altogether, these data indicate that almost all cardiac cells are derived from *Mesp1*-expressing cells including MCPs of both heart fields. Beside its predominant contribution to the development of the cardiovascular system and according to its transient expression in the first somites at E8.5, *Mesp1*-expressing cells also contribute to the development of some muscles and bones of the face.\(^15-17\) In addition, Mesp1-derived cells also give rise to mesothelial cells and perivascular mesenchymal cells of the embryonic liver and possibly to some fetal hematopoietic stem cells.\(^18\) However, in adult mice, <3% of hematopoietic cells derived from Mesp1-expressing cells, suggesting a minor contribution of Mesp1-derived cells to the hematopoietic lineages.\(^19\)
Mesp1 and Mesp2 Are Redundantly Required During Early Mesoderm Development

Inactivation of Mesp1 in mice results in severe cardiac malformations called “cardia bifida,” leading to embryonic lethality around E10.5. In Mesp1-null embryos, cardiac bifida has been attributed to a defect of cardiac mesoderm migration and is likely attributable to a failure of the ventral fusion of the cardiac mesoderm. Supporting this notion, in Mesp1<sup>lacZ/+/H11002</sup> mice (corresponding to Mesp1 mice but allowing to track Mesp1-expressing cells), Mesp1-expressing cells initially accumulate in the PS and reach the anterior region of the embryo with some delay. Beside their morphological defect, Mesp1-null mice still display preserved signs of myocardium and endocardium differentiation, suggesting that other genes can compensate for the loss of Mesp1 during cardiac differentiation.

In the absence of Mesp1, Mesp2 its closest homolog located on the same chromosome and separated by only 23 kb, is massively upregulated in cardiac mesoderm and may compensate for the loss of Mesp1. As compared with Mesp1, Mesp2 expression is detected at a much lower level than Mesp1 in the same primitive mesoderm regions as Mesp1 before E8.5. The main expression of Mesp2 occurs in the presonomic mesoderm after E8.5, where Mesp2 mediates critical aspects of somitogenesis by controlling the segmentation clock and Notch signaling. Mesp2-null mice do not present cardiac malformation but rather severe defects in somitogenesis and segmentation, which could be rescued by inserting 4 copies of Mesp1 in the Mesp2-null locus in the Mesp2 locus demonstrating a functional overlap between these 2 genes during development.

To determine whether Mesp2 compensates for the loss of Mesp1 during cardiac development, Mesp1/2-null mice were generated. Inactivation of both Mesp1 and Mesp2 induces a profound defect of gastrulation, leading to embryonic lethality around E9.5. Strikingly, the PS is formed in Mesp1/2-deficient embryos but no mesodermal cells seem to exit the PS. The abnormal accumulation of cells within the PS indicated a possible defect in specification and/or migration of early mesodermal cells. Thus, Mesp1 and Mesp2 play a redundant role during the early stages of gastrulation and control the exit of mesoderm precursors out of the PS.
whereas Mesp1 and Mesp2 play a nonredundant role during the later stages of mesodermal development, in which Mesp1 controls MCPs migration and Mesp2 controls somitogenesis. The dramatic and early developmental defects in Mesp1/2-null mice preclude to study the role of Mesp genes during cardiovascular specification in mice and the molecular mechanisms that connect Mesp1 and Mesp2 to the expression of cardiovascular transcriptional gene network.

**Mesp1 Gain of Function Strongly Promotes MCP Specification and Cardiovascular Differentiation During Embryonic Development and Embryonic Stem Cell Differentiation**

To define more precisely how Mesp1 controls cardiovascular development in vertebrates, Mesp1 gain-of-function studies have been undertaken in different cellular and animal models. The injection of a plasmid expressing human Mesp1 in two-cell stage embryos of *Xenopus laevis* induced the formation of ectopic beating zones expressing myosin light chain in different regions of the developing embryos (Figure 3A), which were either electrically coupled to heart or presented autonomous rhythm. These data demonstrated that the expression of Mesp1 was sufficient to promote the formation of functional cardiomyocytes ectopically.

By taking advantage of the natural propensity of embryonic stem cells (ESCs) to differentiate into cardiovascular lineages and the ease of their genetic manipulation, different groups used ESCs to study the role of Mesp1 during cardiovascular differentiation. 

During ESC differentiation, MCPs are naturally specified and give rise upon differentiation to the different cardiovascular cell lineages, as it occurred during embryonic development. Early differentiating ESCs undergo a transition through a PS-like stage that mimic early gastrulation, and depending on the concentration of Wnt and Activin, the PS-like cells will adopt either a mesoderm or an endoderm fate. During ESC differentiation, transcription factors implicated in mesoderm and cardiovascular cell fate specification are expressed in a similar temporal pattern as during embryonic development. Brachyury, a marker of the early PS is expressed and peaks within the first 2 days of ESC differentiation. Mesp1 is expressed soon after the onset of Brachyury expression, peaks at D3-4 and is rapidly downregulated thereafter, mimicking its early and transient expression during mouse gastrulation. Transcription factors of the core gene regulatory network of cardiovascular differentiation machinery such as Nkx2-5, Gata4, Hand2, and Mef2c are expressed soon after Mesp1 and precede the expression of cardiac structural genes.

To explore the possibility that Mesp1 can promote the differentiation of cardiac cells, the different groups used slightly different approaches to express Mesp1 during ESC differentiation. In one study, the authors used a constitutive expression of human Mesp1 under the control of a CMV promoter, whereas in the other studies the authors used doxycycline (Dox)-inducible Mesp1 ESCs allowing the temporal regulation of Mesp1 expression. In all cases, Mesp1 expression accelerates cardiac differentiation, as identified by the precocious appearance of beating areas and cardiac troponin T (cTnT) expression, but also massively enhances cardiac differentiation (around 5 fold) as determined by cTnT and α-MHC expression (Figure 3B). Importantly, only transient expression of Mesp1 promotes and accelerates cardiac differentiation because continuous expression of Mesp1 throughout the course of ESC differentiation using the Dox-inducible system inhibits cardiac differentiation rather than promoting it. In that view, the observed rapid extinction of Mesp1 expression following constitutive expression of human Mesp1 under the control of a CMV promoter was potentially beneficial to reveal the cardiac promoting effect of Mesp1.

Functional studies demonstrated that increased Mesp1 expression promotes the differentiation of ESCs into all types of cardiomyocytes presented in the mature heart, including atrial and ventricular cardiomyocytes, as well as pacemaker-like cells. This observation was confirmed by electrophysiological studies showing that the electric patterns typical of all 3 types of cardiomyocytes could be recorded in a cellular preparation obtained following Mesp1 expression in ESCs. In addition these cells were able to respond to pharmacological agents such as isoprenaline, supporting a full maturation of some cardiomyocytes, even though in the presence of serum, a large proportion of the cells were still characterized by an immature phenotype. Addition of Dkk1 during ESC differentiation increases the proportion of cardiomyocytes with electrophysiological characteristics of more mature cardiomyocytes, supporting the importance of the inhibition of Wnt signaling activity for full cardiomyocyte maturation. In addition to promoting cardiac differentiation, Mesp1 promotes endothelial and smooth muscle cell fates during ESC differentiation (Figure 3C through 3E). Mesp1 expression promotes the differentiation of primary and second heart field derivatives and altogether the 3 main lineages arising from the differentiation of MCPs are strongly enriched following Mesp1 expression and represent about two-thirds of the differentiated cells, suggesting that Mesp1 promotes the specification of MCPs of both heart fields during ES cell differentiation. Supporting this notion, forced expression of Mesp1 during ESC differentiation accelerates the appearance of cells coexpressing markers (Isl1, Flk1 and Nkx2-5) of second heart field MCPs. However, because Mesp1 derived cells also give rise to endothelial cells of the aorta and the cranial vessels as well as perivascular mesenchymal cells of the embryonic liver, which also express smooth muscle actin, future studies using clonal analysis would be required to clarify at the single cell level whether Mesp1 promotes the differentiation of MCPs into the different cardiovascular lineages or whether the differentiation induced by Mesp1 is skewed to the cardiomyocyte lineage. It is important to note that Mesp1 does not promote cardiovascular differentiation in all cells types because forced expression of Mesp1 in fibroblast is not sufficient to induce cardiac cells, suggesting that Mesp1 may promote the differentiation of cardiovascular lineages only in PS like cells and other factors are required together with Mesp1 to promote cardiovascular differentiation.
In accordance to the later expression of Mesp1 in the presomitic mesoderm and the contribution of Mesp1 deriving cells to facial muscles, Mesp1 expression in ESCs increased Myogenin expression, a marker of skeletal muscle differentiation.\textsuperscript{25} It has been recently suggested that during zebrafish development, Mesp1 is a target gene of Trf3, and positively regulates hematopoiesis.\textsuperscript{40} However, in mouse ESC colony forming assays in methylcellulose, Mesp1 expression represses the differentiation of hematopoietic colonies and the expression of hematopoietic markers.\textsuperscript{19,25} In addition, lineage tracing analysis of Mesp1 derived blood cells in mice demonstrated that the majority of hematopoietic cells are not Mesp1 derived, and only few percents of hematopoietic cells corresponding to less than 2\% of bone marrow progenitor cells and 3\% of splenic B and T cells were derived from Mesp1-expressing cells.\textsuperscript{19} In a serum containing medium, Mesp1 expression in ESCs also lead to an increase in hepatocyte marker expression such as Tcf1, albumin, and \( \alpha \)-fetoprotein, which could correspond to a cellular non-autonomous function of cardiac cells in promoting liver development, as it has been previously suggested in embryonic development.\textsuperscript{41} Altogether these data indicate that

![Figure 3. Mesp1 promotes cardiovascular differentiation. A, In situ hybridizations for myosin light chain (MLC) expression in stage 45 Xenopus tadpoles injected with a control (top) or Mesp1-expressing plasmid (bottom). Asterisks show the expression of myosin light chain mRNA in cardiac tissue. Mesp1 expression induces ectopic cardiac tissue, as depicted in the inset of the bottom panel. Scale bars are 500 \( \mu \)m. Reproduced from David et al\textsuperscript{24} with permission from Nature Publishing Group. B through D, Immunostainings for cardiac (cTroponinT) (B), endothelial (Pecam) (C), and smooth muscle cell (smooth muscle actin (SMA)) markers at the indicated days of ESC differentiation in control (left) and Mesp1 stimulated cells (right). Scale bars are 500 \( \mu \)m (B), 200 \( \mu \)m (C), and 100 \( \mu \)m (D). B and D, Reproduced from Bondue et al\textsuperscript{25} with permission from Elsevier. C, Reproduced from Lindsay et al\textsuperscript{19} with permission from Elsevier. E, Fluorescence-activated cell sorting (FACS) quantification of CD31 (Pecam) expression in control and Mesp1-expressing cells at D6 of ESC differentiation. Reproduced from David et al\textsuperscript{24} with permission from Nature Publishing Group.]

![Figure 4. Mesp1 promotes cardiovascular differentiation by a cellular autonomous mechanism. A through C, Mesp1/2-null cells do not contribute to the formation of the heart in chimeric mice. A, Mesp1/2-null embryos at 8-cell stage that constitutively express \( \beta \)-gal were aggregated with control embryos, and the reimplanted chimeric embryos were analyzed for \( \beta \)-gal expression at E9.5. B, \( \beta \)-Gal staining of whole-mounted embryo at E9.5 shows the absence of contribution of Mesp1/2-null cells to the cardiac region despite an important contribution of Mesp1/2-null cells to the rest of the embryo. C, Cross-section of B showing cardiac cells derived from control but not from Mesp1/2 double-knockout cells, illustrating that Mesp1/2 functions are required in a cellular autonomous manner to allow cardiac differentiation. OFT indicates outflow tract; PLV, primitive left ventricle; PRV, primitive right ventricle. A through C, Reproduced from Saga et al\textsuperscript{13} with permission from Elsevier. D and E, Cell-autonomous promotion of cardiac differentiation by Mesp1 in ESCs. Dox-inducible Mesp1-IRES-GFP cells are mixed together with control DsRed-expressing cells, and the cardiac-promoting effect of Mesp1 is assessed in the control (red) and Mesp1-expressing cells (green). E, FACS quantification of cTnT expression in all cells (black bars), Mesp1-expressing cells (green bars), or control cells (red bars) at 8 days of ESC differentiation demonstrates that the cardiac promoting effect of Mesp1 is restricted to Mesp1-expressing cells, supporting the cellular-autonomous role of Mesp1 during cardiovascular differentiation. Reproduced from Bondue et al\textsuperscript{25} with permission from Elsevier.]
Mesp1 preferentially promotes the differentiation of ESCs into all cardiovascular lineages by inducing MCP specification and/or promoting their differentiation. In that view, Mesp1 could direct the transition between the first wave of hemangioblast specification and the second wave of cardiovascular progenitor specification that occurred in early Flk1 positive mesoderm.19,24,25,34

Mesp1 Promotes Cardiac Cell Fate by an Intrinsic and Cellular Autonomous Mechanism

To determine the cellular versus noncellular autonomous functions of Mesp during embryonic development, chimeric mice were generated using wild type and Mesp1/2-null 8-cell stage embryos marked with β-gal (Figure 4A).21 Despite obtaining chimeric embryos that were almost entirely derived from Mesp1/2-null cells, no heart cell derived from Mesp1/2-deficient cells was detected (Figure 4B and 4C), suggesting a cellular autonomous requirement of Mesp1/2 expression for cardiovascular progenitor specification and migration.21 On the other hand, other developmental defects of Mesp1/2-null mice such as those seen in the paraxial mesoderm appear to be non cellular autonomous.21

During ESC differentiation, conditioned medium of Mesp1-stimulated cells does not promote and/or accelerate cardiac differentiation, suggesting that Mesp1 does not promote cardiac differentiation through the secretion of soluble factors.25 Similarly to the chimeric experiments in mice, chimeric embryoid bodies were generated by mixing ESCs expressing Mesp1-IRES-GFP and ESCs expressing DsRed (Figure 4D), and the cardiac promoting effect of Mesp1 expression was assessed independently in both cell types.25 These experiments demonstrated that the cardiac promoting
Mesp1 Acts As a Key Molecular Switch Promoting the Expression of the Cardiovascular Gene Network

Cell fate specification can occur either by promoting the survival or expansion of a preexisting progenitor (selective mechanism), or by directly inducing a new cell fate in a precursor population (inductive mechanism). To determine whether Mesp1 acts through a selective mechanism to induce MCP specification in differentiating ESCs, prolif-
eration and apoptosis were assessed.\textsuperscript{25} No increase in bromodeoxyuridine incorporation and only a small de-
crease in activated caspase-3 expression were observed following Mesp1 expression, strongly suggesting that
promotion of MCP specification by Mesp1 does not occur through a selective mechanism but rather through an
inductive mechanism.\textsuperscript{25}

The inductive and cellular autonomous mechanism of cell fate specification usually occurs through the expres-
sion of transcription factors that, upon transcriptional activation and repression, allow the expression of the
different genes that promote the acquisition of a particular cell fate. To determine Mesp1 target genes, transcriptional
profiling following Mesp1 gain of function was performed in ESCs.\textsuperscript{19,25} These studies reveal that 12 hours after the
expression of Mesp1 in the nucleus, only 423 single annotated genes, representing 1.3% of the murine transcrip-
tional regions, were differentially regulated by Mesp1, suggesting that Mesp1 promotes cardiovascular differentiation by
modulating the expression of a restricted set of genes.\textsuperscript{25} Very interestingly, Mesp1 rapidly promotes the expression of the
majority of cardiovascular transcription factors involved in primary and second heart field development, such as
hand2, Myocardin, Nkx2-5, Gata4, Mef2c, Tbx20,

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<th>Transcription factors</th>
<th>Upregulated Genes</th>
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<td>Ripply2 (43.7), Cited1 (36.8), Trim9 (22.7), Foxd2 (17.9), Hey2 (13.8), Otx1 (13.6), dHAND (6.7), Ebf2 (6.3), Lhfp (5.5), Sna1 (4.9), Lef1 (4.3), Nfatc1 (4.0), Pdlim4 (4.0), Myocd (3.7), Pdlim2 (3.5), Asx3 (3.3), Foxc1 (3.2), sNaiv (3.1), Twist1 (3.1), Filii (2.9), Fosl2 (2.8), Kih6 (2.8), Zeb1 (2.7), Ankrd6 (2.6), Insnt1 (2.6), Gata4 (2.4), Hes6 (2.6), Spic (2.5), Hmgap2 (2.5), Gbx2 (2.3), Pdlim5 (2.3), Dmrt1 (2.3), Ankrd1 (2.3), Fosf (2.3), Hmip (2.2), Dactil (2.1), Zfp571 (2.1), Pbx1 (2.1), Zfp238 (2.1), Spec1 (2.1), Hidprp (2.1), Dachshund1 (2.1), Etv2 (2.0), Tbx3 (2.0), Hoxd13 (2.0), Sox1 (1.9), Lmo1 (1.9), Tbx20 (1.9), Cebpl (1.9), Tbx3 (1.9), Lbx1 (1.9), Gata6 (1.8), Psc2 (1.8), Lmnb3 (1.8), Neurod1 (1.7), Foxf1 (1.7), Dicer (1.7), Tceai (1.7), Fhilt (1.7), Sap30l (1.6), Pplip (1.6), Zeb2 (1.6), Cbx2 (1.6), Sox4 (1.6), Smad1 (1.6), Pitx2 (1.6)</td>
<td>T (8.5), Foxa2 (6.3), Sox17 (6.2), Ldb2 (4.6), Kih6 (4.3), Gsc (3.3), Sp8 (3.1), Id2 (2.7), Eras (2.7), Mx1 (2.4), Zic5 (2.4), Irf6 (2.1), Foxd3 (2.1), Hlhh2 (2.1), Nrf5a2 (1.9), Hoxp (1.9), Tox3 (1.8), Nkx3.6 (1.8), Pdrt1 (1.8), Tfcp2l1 (1.8), Dmrt1 (1.8), Esrb (1.7), Myo1 (1.7), Mctf2 (1.7), Nsolv (1.7), Pycard (1.7)</td>
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Dox inducible ESCs allowing forced Mesp1 expression were stimulated or not at D2 of ESC differentiation for 24 hours. Difference in mRNAs expression induced by Mesp1 expression was compared in 2 biologically separated experiments. Fold changes are indicated between parentheses, and transcription factors expressed or implicated during cardiovascular differentiation are highlighted in bold. Reproduced from Bondue et al\textsuperscript{25} with permission from Elsevier.

The modulation of the expression of these genes was extremely rapid, starting as soon as 12 hours following Dox addition, corresponding to the beginning of Mesp1 expression in the nucleus (Figure 5A and 5D).\textsuperscript{25} Moreover, chromatin immunoprecipitation experiments during ESC differentiation showed that Mesp1 directly binds gene regulatory regions containing conserved basic helix-loop-helix binding sites within the genomic regions of Hand2, Myocardin, Nkx2-5, and Gata4 (Figure 5B, 5C, 5E, and 5F) that encompass the previously identified cardiac enhancers for Nkx2-5 and Hand2, strongly suggesting that Mesp1 directly activates the expression of these cardiovascular transcription factors by binding directly to their promoter region.\textsuperscript{25,42,43} Consistent with the direct promoting effect of Mesp1 on the expression of these transcription factors, Nkx2-5 and Gata4 are no longer expressed in ESCs following loss of Mesp1 expression using short hairpin RNA.\textsuperscript{24} Among the direct Mesp1 target genes, Myocardin, a transcriptional coactivator that regulates SRF and Mef2c activity and activates cardiac and smooth muscle gene expression,\textsuperscript{44} plays a critical role downstream of Mesp1 in promoting cardiac and smooth muscle cell differentiation.\textsuperscript{19,25} The forced expression of a dominant negative isoform of Myocardin that lacks its transactivation domain blocks the promoting effect of Mesp1 on cardiac and smooth muscle cell differentiation (Figure 5G), strongly suggesting that Myocardin, by regulating SRF activity, is one of key downstream effector of Mesp1 in mediating cardiac and smooth muscle cell fate specification.\textsuperscript{19} In addition to the upregulation of the transcription factors belonging to the cardiovascular transcriptional network, Mesp1 also promotes the expression of other cardiac structural genes such as Myh6 (α-MHC), Myl1 (MLC1f), Myl2 (MLC2v), Myl7 (β-MHC), and Tmnt2 (cTnT), suggesting that Mesp1 also promotes the differentiation of MCPs into cardiomyocytes.\textsuperscript{19} Additional studies will be required to further precise how exactly Mesp1 regulates the expression of cardiogenic transcription factors. What are the other transcription factors that cooperate with Mesp1 to promote the expression of the cardiogenic

Dox inducible ESCs allowing forced Mesp1 expression were stimulated or not at D2 of ESC differentiation for 24 hours. Difference in mRNAs expression induced by Mesp1 expression was compared in 2 biologically separated experiments. Fold changes are indicated between parentheses, and transcription factors expressed or implicated during cardiovascular differentiation are highlighted in bold. Reproduced from Bondue et al\textsuperscript{25} with permission from Elsevier.

The inductive and cellular autonomous mechanism of cell fate specification usually occurs through the expression of transcription factors that, upon transcriptional activation and repression, allow the expression of the different genes that promote the acquisition of a particular cell fate. To determine Mesp1 target genes, transcriptional profiling following Mesp1 gain of function was performed in ESCs.\textsuperscript{19,25} These studies reveal that 12 hours after the expression of Mesp1 in the nucleus, only 423 single annotated genes, representing 1.3% of the murine transcriptionome, were differentially regulated by Mesp1, suggesting that Mesp1 promotes cardiovascular differentiation by modulating the expression of a restricted set of genes.\textsuperscript{25} Very interestingly, Mesp1 rapidly promotes the expression of the major of cardiovascular transcription factors involved in primary and second heart field development, such as Hand2, Myocardin, Nkx2-5, Gata4, Mef2c, Tbx20,
transcriptional program? Does Mesp1 promote indirectly the expression of certain cardiac transcription factors through the expression of other well-characterized cardiac transcription factors such as Tbx genes?

**Mesp1 Controls Epithelial–Mesenchymal Transition and Early MCP Migration**

The loss of Mesp1 function during mouse development results in a cardiac malformation attributed to a defect in migration of the cardiac progenitor.2 During ESC differentiation, Mesp1 regulates the expression of many key transcription factors implicated in EMT, such as Snail1, Twist1, FoxC1, FoxC2, Zeb1, and Zeb2 (Table), that mediate the downregulation of E-cadherin expression (Figure 5H), and the concomitant increase in mesenchymal markers such as N-cadherin, MMP2, vimentin, and fibronectin1 observed in ESCs following Mesp1 gain of function.19 Increased expression of Snail1 in differentiating ESCs, like Mesp1, represses E-cadherin expression but does not induce the expression of other markers of early mesoderm such as PDGFRa or Flk1,19 suggesting that Snail1 mediates some but not all Mesp1 functions and indicates that the induction of EMT is not sufficient to recapitulate the Mesp1 promoting effect on MCPs specification.

In addition to regulate EMT, Mesp1 also regulates the expression of many other genes implicated in cell migration such as the chemokine receptors CXCR4 and CX3CL1, Wnt5a, a ligand of non canonical Wnt signaling pathway, or the small GTPase RhoB.25

**Mesp1 Represses the Expression of Endoderm Markers**

The transcriptional profiling following Mesp1 gain of function also demonstrated that Mesp1 also represses the expression of several genes that regulate the early steps of PS formation such as Brachyury or FGFR8, and early endoderm cell fate specification such as Sox17, Gsc, Nodal, and FoxA2 (Table).25 Chromatin immunoprecipitation experiments following Mesp1 expression in ESCs demonstrated that Mesp1 directly binds to conserved E-Box sequences present in the genomic regions of these genes, suggesting that Mesp1 directly represses some of these early mesoderm-endoderm genes.25 FGFR8, FoxA2, Cer1, Gsc, and Nodal are expressed more broadly, longer and at higher level in Mesp1/2-null embryos, consistent with the repression of these genes by Mesp1 in vivo as well.21 The repression of the early mesendodermal genes by Mesp1, may ensure the specificity and the irreversibility in the cardiovascular specification induced by Mesp1 through inhibition of pluripotent state and the repression of the other cell fates that occurred within the PS at this stage of development.

**Mesp1 Regulates Its Own Expression, As Well As Mesp2 Expression**

Mesp1 is expressed very transiently during embryonic development and ESC differentiation.11,12,19,25,35,36 Using a RT-PCR strategy that allows the monitoring of endogenous Mesp1 transcripts following Dox-inducible Mesp1 gain of function, it has been shown that Mesp1 induces a biphasic effect on its own expression.25 Very rapidly following Dox addition, Mesp1 promotes its own expression, which is followed by a secondary and long lasting repression of Mesp1 mRNA expression, suggesting that Mesp1 acts as a molecular switch during cardiovascular progenitor specification (Figure 5I).25 Chromatin immunoprecipitation experiments showed that Mesp1 binds to its own promoter, suggesting that the sequential transcriptional activation and repression of Mesp1 by itself can be direct.25 The repression of Mesp1 on its own expression is also likely to occur in vivo, as demonstrated by the enhanced expression of Mesp1-LacZ in the PS of Mesp1<sup>1–<sup>11002</sup>-/-</sup> mice.21 Mesp1 also represses the expression of its closest homolog Mesp2 both in vitro and in vivo, as illustrated by the massive and prolonged upregulation of Mesp2 in the PS of Mesp1-null mice25 and the decrease of Mesp2 expression following Mesp1 expression in ESCs.25 Although the mechanisms by which Mesp1 regulates its own expression and Mesp2 expression are still unclear, one possible mediator of Mesp1/2 repression may involve the direct upregulation of Ripply2 by Mesp1.25 Ripply2 is a known Mesp2 target gene that negatively regulates Mesp2 expression.45 At least in ESCs, Ripply2 seems also to be a Mesp1 target gene, which is highly induced following Mesp1 expression.25 Future studies will be required to clarify the role of Ripply2, as a downstream mediator Mesp1, induced Mesp1/2 repression.

**Mesp1 Establishes the Heart Field and Promotes Cardiac Progenitor Migration in Primitive Chordates**

Ascidians, including Halocynthia rorestzi and Ciona intestinalis, are sessile marine invertebrates that belong to the chordate phylum, thus representing one living trace of the ancestor of vertebrates. Because of their small size, their simple organization, and their completely sequenced genome, ascidians have recently emerged as a very interesting model organism to study how gene regulatory networks control the organization of the body plans during early development.4–46 The heart field in Ciona can be traced back during early development, when the embryo only contains 110 cells, to a pair of cells called, the B7.5 cells.47 Upon asymmetrical cell division, the pair of B7.5 cells gives rise to truncal ventral cells (TVCs) and a pair of anterior tail muscle (ATM) cells.48 The TVCs differentiate on both sides of the trunk, migrate and fuse along the ventral midline, similarly to what is found during cardiac crescent development in vertebrates.47 On metamorphosis, the TVCs will further differentiate to form the mature heart (Figure 6A).47

In both C intestinalis and C savignyi, there is only one ortholog of Mesp1 (C-Mesp) present in their genome, which begins to be expressed during gastrulation exclusively in the pair of B7.5 cells (Figure 6B).47 C-Mesp is expressed transiently in the progeny of the B7.5 cells and C-Mesp expression disappeared during neurulation.47 Other genes such as the ortholog of Nlx2-5 (C-Nlx) and Hand2 (C-Hand-like) that belong to the conserved cardiovascular transcriptional network begin to be expressed in TVCs at the neurula stage.
sue development. B7.5 lineage cells into cardiac cells, generating embryos that have supernumerary cardiac progenitors leading to exuberant cardiac tissue in the region of the embryo (red arrow) and do not express Hand-like gene (middle), demonstrating the critical role of Mesp during cardiac progenitor specification in Ciona. D, Targeted expression of constitutively active Ets-VP16 in Mesp1-expressing cells transforms all B7.5 lineage cells into cardiac cells, generating embryos that have supernumerary cardiac progenitors leading to exuberant cardiac tissue development. B through D, Reproduced from Davidson\(^{46}\) with permission from Elsevier.

(Adapted from Satou et al\(^{47}\) with permission from Cell Press.)

**Mesp1 establishes the heart field and promotes cardiovascular progenitor migration in primitive chordates.** A, Representation of the lineage-tracing analysis of cardiac progenitors during ascidian development. Following an asymmetrical cell division, a pair of cells (B7.5 cells) in the 110-cell embryo gives rise to heart progenitor cells (called truncal ventral cells [TVCs]) and anterior muscle cells in tailbud embryos. TVCs differentiate, migrate, and fuse medially in the larva to give rise to the mature cardiac structures at the juvenile stage. Adapted from Satou et al\(^{47}\) with permission from The Company of Biologists. B, Detection of Mesp expression in B7.5 cells by in situ hybridization of Mesp mRNA (upper left), by β-gal staining in transgenic Mesp-LacZ embryos (upper middle), or by GFP detection in Mesp-GFP transgenic embryos (lower left). Hand-like gene is expressed in the cardiac progeny of B7.5 cells (lower middle panel and schemes). ATM indicates anterior tail muscle. C, Mesp knockdown embryos using morpholino (Mesp-MO) combined with Dil lineage tracing of B7.5 cells (depicted in red) showed that B7.5 progeny do not migrate in the anterior cardiac region of the embryo (red arrow) and do not express Hand-like gene (middle), demonstrating the critical role of Mesp during cardiac progenitor specification in Ciona. 

**Figure 6.** Mesp1 establishes the heart field and promotes cardiovascular progenitor migration in primitive chordates. A, Representation of the lineage-tracing analysis of cardiac progenitors during ascidian development. Following an asymmetrical cell division, a pair of cells (B7.5 cells) in the 110-cell embryo gives rise to heart progenitor cells (called truncal ventral cells [TVCs]) and anterior muscle cells in tailbud embryos. TVCs differentiate, migrate, and fuse medially in the larva to give rise to the mature cardiac structures at the juvenile stage. Adapted from Satou et al\(^{47}\) with permission from The Company of Biologists. B, Detection of Mesp expression in B7.5 cells by in situ hybridization of Mesp mRNA (upper left), by β-gal staining in transgenic Mesp-LacZ embryos (upper middle), or by GFP detection in Mesp-GFP transgenic embryos (lower left). Hand-like gene is expressed in the cardiac progeny of B7.5 cells (lower middle panel and schemes). ATM indicates anterior tail muscle. C, Mesp knockdown embryos using morpholino (Mesp-MO) combined with Dil lineage tracing of B7.5 cells (depicted in red) showed that B7.5 progeny do not migrate in the anterior cardiac region of the embryo (red arrow) and do not express Hand-like gene (middle), demonstrating the critical role of Mesp during cardiac progenitor specification in Ciona. D, Targeted expression of constitutively active Ets-VP16 in Mesp1-expressing cells transforms all B7.5 lineage cells into cardiac cells, generating embryos that have supernumerary cardiac progenitors leading to exuberant cardiac tissue development. B through D, Reproduced from Davidson\(^{46}\) with permission from Elsevier.

**Mesp1 establishes the heart field and promotes cardiovascular**

By generating a series of transgenic reporter animals containing different promoter regions of C-Mesp, a minimal enhancer region of 100 bp was identified in the promoter of C-Mesp that drives its expression exclusively in the B7.5 cells.

**Mesp1 establishes the heart field and promotes cardiovascular**

By generating a series of transgenic reporter animals containing different promoter regions of C-Mesp, a minimal enhancer region of 100 bp was identified in the promoter of C-Mesp that drives its expression exclusively in the B7.5 cells. This enhancer region contains several putative binding sites for Tbx6. Tbx6c is expressed just before C-Mesp expression, and at 110-cell stage Tbx6c and C-Mesp are coexpressed in B7.5 cells. Mutations of these putative Tbx6 binding sites in C-Mesp promoter decreased or abolished transgene expression, strongly suggesting that Tbx6c directly induces C-Mesp expression in the developing embryo. However, the expression of Tbx6 is not restricted to the B7.5 cells and extends throughout the tail muscles, suggesting that Tbx6 expression alone is not sufficient to ensure the specific expression C-Mesp in the prospective heart field and that other factors might collaborate with Tbx6 to stimulate C-Mesp at the right place and the right time. Knockdown of β-catenin results in a significant decrease of C-Mesp expression and about half of the β-catenin morpholino injected embryos lacks C-Mesp expression. In addition, ectopic expression of β-catenin results in ectopic expression of C-Mesp, suggesting that β-catenin can promote the expression of C-Mesp. Lhx3 is a known β-catenin target gene expressed in B7.5 cells, and B7.5 cells are the only cells coexpressing Tbx6 and Lhx3 at high level, suggesting that Lhx3 could be the elusive coactivator downstream of β-catenin signaling required in conjunction with Tbx6 to promote C-Mesp expression in cardiac progenitors. Consistent with this possibility, ectopic expression of Tbx6b and Lhx3b in blastomeres strongly induces ectopic expression of C-Mesp in these cells and morpholino knockdown of Lhx3 reduces Mesp expression in B7.5 cells. Although there are no data available suggesting that Tbx6 acts upstream of Mesp1 in mammals, Tbx6 directly regulates Mesp2 expression in presomitic mesoderm, suggesting that at least a part of the Tbx-Mesp axis has been conserved during evolution.

Surprisingly, the expression of a constitutively active form of C-Mesp (C-Mesp-VP16) in Ciona induces ectopic heart differentiation but also inhibits cardiac progenitor migration. The constitutive active form of Mesp in the caudal lineage is sufficient to transform the ATM cells into beating...
heart tissue, but blocks cardiac cell migration. These experiments demonstrate that in Ciona, cardiac cell fate specification and cardiac progenitor migration can be uncoupled and Mesp expression is sufficient to induce the competence for heart field formation. It is not clear yet why the constitutive activity of C-Mesp inhibits cardiac progenitor migration.

In the developing Ciona embryos, fibroblast growth factor (FGF) signaling plays an important role in the induction of mesoderm formation. Ets1/2 transcription factor is a Mesp target gene required to mediate signaling downstream of FGF in cardiac progenitors. Ets1/2 is expressed in the daughters of B7.5 cells and become restricted to the cells that are specified to become cardioblasts, whereas expression of Ets1/2 is lost in the prospective ATM cells after asymmetrical cell division. Treatment of embryos with MEK inhibitor or expression of a dominant negative form of FGF receptor in B7.5 cells abolishes the migration and the expression of FoxF in cardiac progenitors. Expression of a dominant negative form of Ets1/2 inhibits cardiac progenitor migration and heart formation, whereas expression of a dominant active form of Ets1/2 stimulates cardiac but also muscle progenitor migration toward the head, the expression of FoxF1 and also cardiac genes in these cells, suggesting that Ets1/2 play an important role downstream of Mesp and FGF signaling to mediate cardiac progenitor specification and migration (Figure 6D). The excess of heart progenitors that are specified following expression of a dominant active form of Ets1/2 by transforming ATM cells into heart progenitors induces the formation of excessive cardiac tissues, and in a fraction of the embryos, this excess of cardiomyocytes leads to the formation of a functional dual heart compartment, suggesting that the emergence of multiple chambered hearts in vertebrates may involve the additional recruitment of cardiac progenitors. It is interesting to note that in anniotes the dual compartment hearts involve the presence of an additional heart field, the second heart field, which also arise from the differentiation of Mesp1-expressing cells.

FoxF expression depends on Ets1/2 function, and the cells that failed to migrate following constitutive expression of Mesp do not express FoxF but express Hand and differentiate into heart cells, demonstrating that FoxF expression correlates with cardiac progenitor migration but not cardiac progenitor specification. Inhibiting FoxF function by morpholino or by expressing a dominant negative of FoxF blocks cardiac progenitor migration in the physiological situation, and following expression of a Ets1/2 dominant active mutant, demonstrating that FoxF is required downstream of FGF/MAPK/Ets axis to promote cardiac progenitor migration. A more detailed analysis of the genes involved in the migration of cardiac progenitors was performed by transcriptionally profiling mutants that present migration defects without compromising cardiac specification (constitutive active form of Mesp and dominant negative isoform of FoxF), suggesting that the activation of these cardiac genes is not so dependent on the signaling environment or that this is similar at these different locations. This study allowed to identification of a subset of genes, such as RhoDF, a small Rho GTPase, that are controlled by Mesp, FGF, and FoxF and involved in cardiac progenitor migration. RhoDF cooperates with Cdc42 to generate actin-based protrusions at the leading edge of migrating cardiac progenitors.

Altogether, these data suggest that Mesp1 functions as a key transcriptional regulator of cardiac development in both primitive chordates and mice. In both species, Mesp genes seem to be critical to regulate directly and/or indirectly the expression of the core cardiac transcriptional machinery. In both species, Mesp also controls critical aspects of cardiac progenitor migration, which seems to be regulated independently of cardiac cell fate specification, at least in Ciona. It would be interesting to determine whether the cardiac malformation observed in Mesp1-null mice is the consequence of a defect in MCP migration that involves a defect in the same Mesp regulatory mechanisms than the ones controlling Ciona cardiac progenitor migration.

**Wnt Signaling Positively Controls Cardiac Mesoderm Formation and Mesp1 Expression**

Cardiac development involves a complex sequence of events including mesoderm induction, cardiovascular progenitor specification, progenitor expansion, cardiovascular differentiation, and finally cell maturation. Several recent works have demonstrated that Wnt signaling acts in an antagonist manner at different stages of cardiac differentiation during embryonic development and ESC differentiation. Wnt signaling promotes the early steps of cardiogenesis, whereas cardiac differentiation and maturation are inhibited by Wnt signaling. The specification and the patterning of the PS are tightly regulated by multiple signaling pathways, including Wnt, Nodal, and BMPs. During early development, Wnt signaling plays a central role in the early stage of gastrulation. Canonical Wnt signaling is activated at E6.5 in the posterior region of the PS, in which the prospective mesoderm ingress into the PS and migrates antero-laterally, as demonstrated by the Axin2-LacZ Wnt reporter mice. At E7.5, the Wnt reporter activity was presented throughout the posterior PS and in the antero-lateral mesoderm. The expression of Wnt activity could be superimposed to the expression of Mesp1 during the early stage of gastrulation and cardiovascular progenitor specification. The critical role of Wnt/β-catenin signaling during early mesoderm formation was also illustrated by the phenotype of mice deficient for different components of the Wnt/β-catenin signaling pathway. Wnt3a is expressed throughout the PS, and its expression is essential for PS and mesoderm formation, as demonstrated by the absence of PS and mesoderm in Wnt3a-null mice. Lrp5/ Lrp6 double-null mice (both critical coreceptors that transduce Wnt signaling) present an absence of PS and posterior axis patterning. Similarly, the loss of β-catenin, the essential cofactor associated with Lef/TCF transcription factors to relay Wnt signaling into the nucleus, and which transactivates Wnt target genes, also results in a complete absence of mesoderm formation. The expression of many key genes of early mesoderm formation such as Brachyury or Eomes were strongly reduced in these mouse mutants and promoter reporter analysis suggested that Brachyury could be a direct target gene of Wnt/β-catenin signaling in the nascent meso-
Gain of function of canonical Wnt signaling in the epiblast results in the acceleration of epithelial mesenchymal transition (EMT), suggesting that Wnt signaling may regulate directly or indirectly cardiac mesoderm migration. Although, Mesp1 expression has not been directly investigated in these different studies, the results of these experiments suggest that canonical Wnt signaling is critical for the specification and migration of the prospective cardiac mesoderm during early embryonic development.

The first evidence that Wnt/β-catenin signaling positively regulates cardiogenesis came from a study using pluripotent stem cell differentiation into cardiomyocytes. Blocking Wnt ligands through the addition of a soluble Wnt antagonist strongly inhibited the expression of cardiac transcription factors and cardiac structural genes. Moreover, activation of Wnt pathway by Wnt3a or lithium chloride accelerates and enhances the expression of cardiac genes, demonstrating the early requirement of Wnt/β-catenin in the induction of cardiac gene expression during pluripotent stem cell differentiation.

Similarly, during ESC differentiation addition of Dkk1, a soluble Wnt inhibitor, completely blocks the expression of markers of early mesoderm and cardiac progenitors such as Brachyury, Mesp1, PDGFRα, Flk1, or Snail1. Although during the initial stages of ESC differentiation, Wnt signaling promotes cardiac progenitor specification, later Wnt signaling inhibits cardiac terminal differentiation and the upregulation of cardiac structural genes such as Myh6 (α-MHC), Myh7 (β-MHC), Myl2 (MLC2v), Myl7 (MLC2a), Tnnt2 (cTnT), and Nppa induced by Mesp1 expression was further enhanced in the presence of Dkk1. The biphasic effect of Wnt/β-catenin signaling on cardiogenesis was also demonstrated in zebrafish, in which Wnt/β-catenin promotes heart specification at the pregastrula stages, whereas it inhibits heart formation at gastrula stages, confirming the biphasic effect of Wnt signaling during embryonic development.

Mesp1 expression in ESCs is dependent on Wnt activity as demonstrated by the profound downregulation of Mesp1 expression on Dkk1 addition and the strong upregulation of Mesp1 expression following Wnt3a addition. Similarly in Ciona, the expression of C-Mesp in cardiac progenitors is also regulated by β-catenin signaling. In addition to be upstream of Mesp1, Wnt signaling seems also to acts downstream of Mesp1. Whereas the promotion of cardiovascular gene expression by Mesp1, such as Myocardin, Hand1, Hand2, Gata4, Foxe1, Foxe2, Tbx1, Tbx5, Tbx20, and Smyd1(Bop), is independent of Wnt signaling, addition of Dkk1 partially blocks the cardiac promoting effect of Mesp1, suggesting that early Wnt signaling promotes cardiogenesis by other means than only inducing Mesp1 expression. Interestingly, Mesp1 expression stimulates the expression of Lef1, a transcription factor relaying Wnt signaling and pangolin, the Drosophila ortholog of Lef1, is required for dorsal vessel development in flies. It has been suggested that Mesp1 regulates Dkk1 expression to promote cardiac differentiation. Although endogenous Dkk1 expression is not rapidly modulated on Mesp1 expression, it is possible that the modulation of Dkk1 represents a late effect of Mesp1 functions, increasing the terminal differentiation of cardiomyocytes. Clearly, more studies will be required to precise how Wnt signaling controls the expression Mesp1 and how Mesp1 in turn regulates Wnt signaling activity during the early stage of cardiovascular progenitor specification and early lineage commitment.

**Clinical Importance of Mesp1**

Cardiovascular diseases remain the leading cause of death in Western countries. Several cardiovascular diseases are associated with an acute or chronic loss of functional cardiomyocytes attributable to ischemia, hypertension, viral infections, and other pathological conditions and resulting in cardiac failure. Despite recent advances in treatment of cardiac failure, cardiac transplantation remains up to now the only available treatment for end-stage cardiac failure. In that context, the paucity of available organs has underlined the need for other sources of transplantable cardiac cells. Experimental and clinical studies have highlighted the complexity of cardiac cell therapy and underlined the need of identifying the optimal source of cells that should be used for cardiac cell therapy in humans.

Cardiomyocytes or cardiovascular progenitor cells arising from the differentiation of ESCs, or induced pluripotent cells (iPS cells), have been suggested as a valuable source of cardiomyocytes for cell therapy. Importantly, the recent demonstration that expression of only 4 factors can induce the reprogramming of differentiated human somatic cells into pluripotent stem cells opens the possibility that autologous cardiomyocytes can be obtained by the differentiation of patient-specific pluripotent stem cells to cardiovascular lineages, but also opens new avenues in the diagnosis and the understanding of the molecular mechanisms underlying cardiovascular diseases.

In that respect, the major increase in the generation of cardiovascular cells following Mesp1 gain of function in ESCs represents a method of choice for obtaining differentiated cardiovascular cells for therapeutic purpose. Cardiovascular cells obtained from Mesp1-expressing ESCs could also be used for drug and toxicity screening, as well as a means for understanding the molecular mechanisms of congenital and inherited cardiovascular diseases.

**Conclusions**

Emerging during evolution with chordate specification, Mesp1 is the earliest marker of cardiovascular development in vertebrates. Mesp1 promotes cardiovascular differentiation by stimulating cardiovascular progenitor specification and delineating heart field in a cell autonomous manner. Mesp1 acts as a key regulator of the cardiovascular transcriptional network by inducing directly and/or indirectly the expression of the majority of key cardiovascular transcription factors including Hand2, Myocardin, Nkx2-5, Gata4, Mef2c, Foxe1 and Foxe2. Mesp1 also promotes EMT in early mesodermal cells and regulates the migration of MCPs.

Although important progress has been made in our understanding of the role of Mesp1 during cardiovascular lineage commitment, how precisely Mesp1 controls early MCP specification, migration, and cardiovascular differentiation during embryonic development remains to be answered. Do early Mesp1-expressing cells represent a homogeneous cell...
population common for both heart fields, or do they represent a heterogeneous population already committed to one or the other heart field? Do early Mesp1-expressing cells express cell surface markers allowing their prospective isolation using monoclonal antibodies? At what stages of embryonic development is Mesp1 protein expressed? What are the direct Mesp1 target genes at the different stages of cardiovascular specification and differentiation during embryonic development? What are the transcription factors that, in collaboration with Mesp1, promote early MCP specification and cardiovascular lineage commitment? A better understanding of the mechanisms that control Mesp1 expression and how Mesp1 target genes in turn promote MCP specification and migration will be required to better delineate the molecular events that control the earliest step of cardiovascular specification in vertebrates and will be helpful to increase the production of cardiovascular cells for cellular therapy and drug screening.

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