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Ingression, migration and early differentiation of cardiac progenitors

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1. ABSTRACT

During vertebrate embryogenesis the heart is the first functioning organ and cardiac progenitor cells (CPCs), which form the future heart, are among the first cell types to be established during gastrulation. A large number of studies indicate that cardiac development is tightly regulated by a series of molecular signaling pathways and morphological events. The cellular and molecular events that control early cardiac development are conserved among vertebrates. The favorable experimental characteristic of the chicken embryo and the ease in which cell labeling and imaging can be performed has allowed direct observation of the process of gastrulation and cell migration trajectories. This has enabled the study of the signaling proteins and molecular pathways required to specify early embryonic cells to the myocardial lineage. In this review we discuss the major morphogenetic and regulatory events that control gastrulation and migration of CPCs in the chicken embryo. We also describe the signaling mechanisms critical for early CPC specification in pre-gastrula, gastrula and early neurula stage embryos.

2. INTRODUCTION

Gastrulation is one of the major events that occur during development as it is the process in which a single layer of epithelial cells is transformed into the three germ layers of the embryo; endoderm, ectoderm and mesoderm as well as being the developmental stage during which the embryonic body plan is established by convergence and extension movements (CE). In amniotes, gastrulation starts with the formation of the primitive streak (PS), which forms as a thickening of the epiblast at the posterior margin of the embryo and extends as a morphologically distinct structure in the midline of the embryo. Epithelial cells within the epiblast are recruited to the PS where they undergo epithelial to mesenchymal transition (EMT), ingress through the PS and migrate ventrally and laterally to form the endoderm and mesoderm (1-3). As soon as these cells migrate away from the PS, other cells move from the epiblast into the streak to replace them.

Fate mapping studies in mouse and chicken embryos have demonstrated that the time and location of

cell ingression through the PS determines the spatial distribution and the fate of mesodermal cells (4-7). For example, in chicken embryos at the mid-gastrulation stage, cells at the anterior section of the PS, anterior streak cells, give rise to somites, whereas middle streak cells predominantly form intermediate and lateral plate mesoderm, and posterior streak cells form extra-embryonic mesoderm and cells that give rise to the haematopoietic system (6, 8). This suggest that the movement and fate of these cells depends on their position within the streak and is not a cell autonomous property but is determined by extrinsic signal(s) produced by surrounding cells.

Mesoderm CPCs are among the first cell lineages to be established and in chicken embryos ingress through the PS during early gastrulation (9, 10). In pre-streak embryos, future cardiac cells have been identified in the posterior lateral region of the epiblast layer (5, 11) and in the anterior-mid section of the PS at stage Hamburger Hamilton (HH) 3 (9, 12). After gastrulation, CPCs occupy bilaterally symmetrical heart-forming regions in the lateral plate mesoderm (13). By HH10 the bilateral progenitor cell populations fuse at the ventral midline, forming a simple primary heart tube consisting of a myocardial outer layer and an endocardial inner layer (14).

Multiple extracellular cues have been implicated in the migration and initial specification of cardiac precursor cells during early heart formation. Studies in embryos have uncovered crucial roles for bone morphogenic proteins (BMPs), fibroblast growth factors (FGFs), platelet-derived growth factor (PDGF) and Wnt signaling in one or both of these processes (15-23). The specification of cardiac precursor cells appears to occur concomitantly with cell migration and morphogenetic movements during early embryogenesis (14). However, the way in which these two distinct processes are coordinated is, at present, not completely understood.

The cellular and molecular events that control early cardiac development are conserved among vertebrates (24, 25). Thus, a model organism that allows real time observation of cell movements in the whole embryo would be beneficial to provide further insights into CPC migration, specification and heart development in vertebrates. The chicken embryo is easily accessible *in ovo* and can also be harvested and cultured on semi-solid media, *ex ovo* (26). Both *in ovo* and *ex ovo* settings make chicken embryos suitable for manipulations (e.g. grafting and transplants, electroporation, bead or cell implants, cell labeling, dissection or ablation) and time-lapse imaging, which can be used to study embryo gastrulation and cell migration in real time. These favourable experimental characteristics along with the development of techniques such as grafting green fluorescent protein (GFP)-expressing PS cells into cultured unlabeled host embryos and long-term live video microscopy have enabled the characterization of the movement patterns of lateral and paraxial plate mesoderm cells that emerge from the PS (8, 27- 29 to reference only a few examples).

In this review we discuss the signaling pathways, which regulate gastrulation movements, mesoderm cell migration and early cardiac cell fate specification during development. We specifically focus on the movement and commitment of cardiac progenitor cells (CPCs) within the PS and cardiogenic mesoderm in chicken embryos.

3. PRIMITIVE STREAK FORMATION AND INGRESSION OF CARDIAC PROGENITOR CELLS

At the blastula stage the chicken embryo is a flat, concentric disc composed of two layers, the epiblast (upper layer) and the hypoblast (lower layer). The outer region/periphery of the embryo is called the area opaca (ao) and the central part of the embryo is called the area pellucida (ap). Posterior epiblast cells at the boundary between the ao and ap move into the midline 'marginal zone' and form Koller's Sickle. Signals from the marginal zone and area opaca induce nodal expression in the epiblast overlying Koller's Sickle and the nodal-expressing cells then differentiate to form a triangular-shaped initial PS at the posterior midline of the epiblast layer (30). From this structure, cells that will give rise to mesoderm and endoderm ingress into the sub-epiblast space, resulting in the formation of the PS. When the PS reaches half-maximum extension, epiblast cells in the PS undergo EMT and start to migrate between the epiblast and the hypoblast to form axial and lateral mesoderm, and definitive endoderm (1). It has been shown that FGF and Wnt signaling is required for the formation of the PS (21, 22, 31-34) and interestingly, Vasiev *et al* (35) have suggested, based on computational modeling, that differential chemotaxis and cell-cell adhesion could be sufficient for the formation and anterior extension of the PS. Once the PS is fully extended, the Hensen's node is formed at its tip before the PS begins to regress. Hensen's node plays a key role as the organiser of cell movement during later gastrulation, especially during streak regression.

CPCs that contribute to the avian heart are found in blastula stage embryos within the posterior half of the epiblast (5, 36). It is these posterior cells, but not the anterior epiblast that have been shown to have cardiogenic potential in culture (11, 37). It has also been demonstrated that the pre-gastrula hypoblast possesses cardiogenic-inducing capabilities and is required to induce cardiac myogenesis in the early epiblast (11). Transforming growth factor beta (TGF-beta) and activin are expressed in the chicken pre-gastrula hypoblast and activin is sufficient to substitute for the hypoblast to induce myocardial cell formation in quail posterior pre-gastrula epiblast (11, 38). In fact, the function of the hypoblast to induce myocardial cell formation in the pre-gastrula embryo has been shown to be mediated by activin/TGF-beta signaling (37). Additionally, BMP-antagonists such as Chordin, Noggin or Follistatin at the pregastrula stage are also necessary for heart mesoderm induction/cardiac precursor cell specification (37, 39).

In chicken embryos, CPCs ingress early during gastrulation (at the early streak stage) and are found within the anterior-middle region of the PS at HH3, yet caudal to the location where Hensen's node will later form (9). Cells from the posterior region of the PS give rise to lateral plate

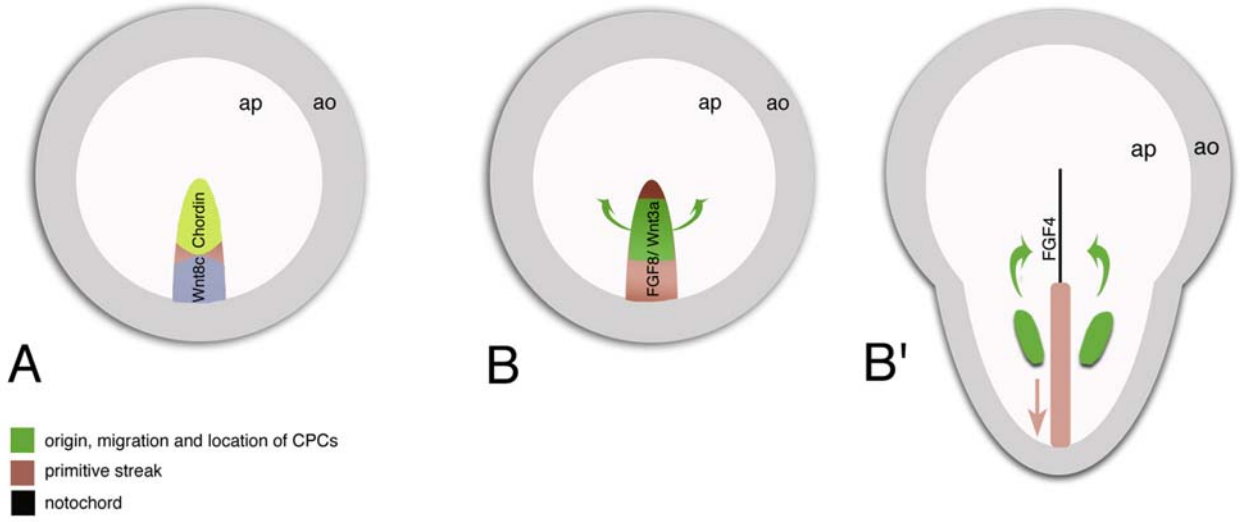

Figure 1. Origin and migration of cardiac progenitor cells (CPC)s. (A) In embryos at stage HH3-HH4 *Chordin* RNA is expressed in the anterior part of the streak and *Wnt8c* RNA is expressed in the posterior part of the streak. (B) In gastrula stage embryos at HH3 CPCs are located in the anterior-middle region of the primitive streak (PS) and migrate away from the PS in an anterior-lateral direction, indicated by green arrows. FGF8 and Wnt3a mediated chemorepulsion regulates the movement of these cells away from the streak. (B') Once the PS begins to regress (pink arrow) CPCs migrate toward the midline of the embryo (green arrows). FGF4 mediated chemoattraction regulates the movement of these cells towards the midline. ao= area opaca, ap= area pellucida

mesoderm, extraembryonic mesoderm and endoderm (40). It has been shown that cells in the anterior part of the PS express different genes compared to the cells in the posterior part of the PS. Some of these encode candidate signals important for early cardiac specification and it has been demonstrated that 'non-cardiogenic' progenitor cells that are transplanted to the PS are recruited into the heart (10, 41). For example, the BMP inhibitor Chordin and the Wnt ligand, Wnt8c, are initially expressed in the sickle-shaped mesoderm, of Koller's Sickle. However, during PS formation and elongation the expression of Chordin becomes restricted to cells in the anterior PS, while Wnt8c becomes restricted to the posterior PS (Figure 1A, 35). Wnt8c can signal through a beta-catenin dependent mechanism and may negatively regulate cardiac differentiation (17). On the other hand, Chordin is an inhibitor of BMP signaling and BMP2 and BMP4 have been shown to inhibit cardiac myogenesis prior to gastrulation (37).

4. EMT AND THE SIGNALING MECHANISMS THAT REGULATE EARLY GASTRULATION IN THE CHICKEN EMBRYO

Explantation and cell labeling studies in early and mid-gastrulation stage chicken embryos have demonstrated that cardiac precursors cell are present in the PS at HH3 and migrate out from the streak by HH4 (6, 9, 36, 42, 43). At the mid-gastrulation stage/early neurula, cardiac progenitors occupy bilaterally symmetrical heart-forming regions in the lateral plate mesoderm. It is important to understand the mechanisms, which regulate cell ingression through the streak and the signals that guide the migration of these progenitors to their final destination.

4.1. Role of FGF in cell movements associated with gastrulation

Chemotactic signals coordinate movements in a variety of developmental systems and are very important during vertebrate development. Most commonly, these signals are members of the FGF family of growth factors. Strong evidence that FGF signaling is essential for gastrulation comes from studies in the mouse where embryos homozygous null for FGF receptor 1 (Fgfr1) do not gastrulate correctly due to the inability of cells to go through EMT (44-46). It has been shown that FGFR1 regulates the expression of *Snail* and *E-cadherin* (47). *E-cadherin* is expressed in the epiblast and is downregulated by the repressor, Snail, as cells undergo EMT at the PS. Downregulation of *E-cadherin* expression has been directly implicated in the differentiation and migration of mesoderm at gastrulation (48). In *Fgfr1* null embryos, *E-cadherin* is ectopically expressed at the PS, whilst *Snail* expression is lost. In chicken embryos, FGFR1 is necessary for the PS to form (31, 33, 49) thus it appears that FGF signaling during PS formation and EMT is one of the essential signaling mechanisms required during early gastrulation. In addition, cells that ingress start to express *N-cadherin*, which is required for the migration of the mesoderm cells away from the PS. PDGF signaling through the ligand, PDGFA, and the receptor, PDGFRα, has been shown to be responsible for the control of *N-cadherin* expression in these cells through a PI3 dependent mechanism (50). Thus, PDGF signaling is also required for the movement of cells away from the PS during gastrulation.

4.2. Wnt signaling in convergence and extension movements

Convergence and extension (CE) movements during gastrulation also play a major role in shaping the embryo body. In vertebrates, the beta-catenin-independent

Wnt/planar cell polarity pathway (PCP) is a key regulator of CE movements (51-53). PCP is one of several Wnt-driven, beta-catenin-independent signaling pathways identified. Wnt ligand interacts with the transmembrane receptor, Frizzled, to transduce signals through the protein Dishevelled (Dvl). Downstream of Dvl, effectors of the PCP pathway signal through the small GTP-binding protein RhoA, to coordinate actin polymerization, organization and polarized cell movements. In zebrafish, embryos carrying mutations in the Wnt/PCP pathway have defective elongation and CE cell movements (53-55). In particular, mutations in the homolog of *Drosophila prickle* (*pk*) gene and also the zebrafish mutants, silberblick (slb that lacks Wnt11 activity) and pipetail (Wnt5), demonstrate that the Wnt/PCP pathway has an important role in this process.

In the chicken embryo, *Wnt5a*, *Wnt5b* and the homolog of the zebrafish *Wnt11* gene, *Wnt11b* are expressed within and surrounding the PS (56, 57). Altered levels of Wnt11b and Wnt5a activity, i.e. gain of function experiments (overexpression of the gene, thus an increase in signaling) or dominant negative inhibition of signaling, result in an increased number of cells remaining in the epiblast and inhibit the migration of cells away from the PS (57). These authors also demonstrated that the inhibition of beta-catenin-independent signaling at HH3-4, using a dominant negative Dvl construct thought to specifically block signaling through the PCP pathway, results in cells remaining in the epiblast and PS due to a reduced ability to ingress and migrate through the PS (57). This suggests that Wnt11b, Wnt5a and beta-catenin-independent Wnt signaling have important roles during early gastrulation and that a precisely regulated level of Wnt11b and Wnt5a is required for normal cell migration away from the PS during avian gastrulation.

5. THE REGULATION OF CARDIAC PRECURSOR CELL MIGRATION TRAJECTORIES BY EXTRACELLULAR SIGNALS

Classic mapping studies using radioactive tracers, fluorescent labels and chick-quail chimeras have provided extensive information about the fate of cells at different stages during gastrulation. It has been demonstrated that the anterior-posterior position of cells within the streak correlates well with their eventual mediolateral position within the embryo (4, 5, 6, 10). However, until recently little was known about the migration trajectories or the molecular signals that guide cells from the PS to their final destination. Studies in HH3-HH4 chicken embryos show that the movement pattern of cells in the node, anterior-middle streak and caudal streak are very different and are controlled by extrinsic cues from surrounding cells (27). Cells at the anterior-middle PS, give rise to lateral plate mesoderm (where prospective cardiac cells reside) and they initially move out laterally, away from the streak in a semi-circular fashion. Once Hensen's node has regressed past the cells, they move towards the midline of the embryo (27).

5.1. Chemotactic signals

FGF signaling plays a fundamental role during mouse and chicken gastrulation as observed in Fgfr1 null mutant mice and the requirement of Fgfr1 for the formation of the PS in chicken embryos. The FGF ligands, FGF4 and FGF8 have also been shown to play a direct role in guiding cell movements. In mouse embryos, *Fgf4* and *Fgf8* are expressed in cells within the PS, however *Fgf4* mutant embryos die before the streak is formed and thus it has not been possible to determine whether it has a role in gastrulation (58). However, homozygous *Fgf8* mutant embryos do survive past E7.0, mid-streak stage. In *Fgf8* mutant embryos epiblast cells move to the PS and undergo EMT, but the majority of these cells fail to migrate away from the PS (59). These embryos also no longer express *Fgf4* in the PS at stage E7.25 to E8.5. Thus at E7.25 (mid-streak stage) *Fgf8* mutant embryos are also null for *Fgf4* and this leads to an accumulation of cells on the posterior side of the embryo, a result of the failure of most cells, which have entered the PS and have undergone EMT, to migrate away from the PS (59). Consequently, no embryonic mesoderm or endoderm-derived tissues develop, but extraembryonic tissues do form. Time-lapse microscopy of gastrulating mouse embryos has shown that migration away from the distal region of the streak is an active process (60), thus FGF8 and FGF4 mediated signaling participate in regulating the migration of cells which ingress at this position along the PS. On the other hand, future extraembryonic cells ingress at the proximal portion of the streak and their movement may be a more passive process or not dependent on FGF signaling but on other signaling mechanisms (61).

In chicken embryos, FGF receptors are expressed initially in the epiblast and subsequently in the migrating mesoderm (62). Following the emergence of mesoderm cells from the PS it appears that FGF signaling, in particular FGF4 and FGF8 mediated signaling, influences mesoderm migration. Streak cells from HH3-4 embryos are attracted by sources of FGF4 and repelled by sources of FGF8 (27, 28). Based on the expression pattern and observed chemotaxis effects of FGF4 and FGF8 it was proposed that the movement patterns of anterior-middle streak cells are due to an FGF8-mediated chemorepulsion of cells away from the streak followed by chemoattraction toward an FGF4 signal produced by the developing notochord (Figure 1B, B', 27).

FGF mediated chemotaxis is involved in guiding cell movements of prospective lateral plate mesoderm cells during gastrulation (27). This includes cardiac progenitor cells (CPCs), which are located in the anterior-middle region of the PS in HH3 embryos and migrate into the developing anterior lateral plate mesoderm after ingressing through the streak. In addition to FGFs, experiments investigating the effect of Wnt signaling on cell migration after cell ingression through the streak indicate that prospective cardiac cells also alter their movement pattern in response to Wnt3a (28). Wnt3a is highly expressed in the PS (17, 28) and it has been demonstrated that it guides the movement pattern of HH3 cardiac progenitor cells through a RhoA dependent mechanism (28). *Ex-ovo* experiments showed that cardiac precursors in HH3 embryos respond to Wnt3a and are repelled by it (28). Therefore, it appears that FGF8 and Wnt3 signaling are both involved in the directed

migration of CPCs away from the streak (Figure 1B). However, these two mechanisms are independent, as inhibition of FGF receptor signaling does not impair the Wnt3a mediated repulsion of cardiac progenitor cells (28), suggesting that they act in parallel.

6. SPECIFICATION OF CARDIAC MESODERM IN GASTRULA AND EARLY NEURULA STAGE EMBRYOS

Specification of prospective cardiac cells begins while the cells are within the streak (11) and continues throughout late gastrulation as they migrate to the anterior lateral mesoderm and form bilateral heart fields, on either side of the anterior midline (63, 64). The lateral plate mesoderm splits into two layers, the splanchnic (inner) and the somatic (outer) mesoderm and cardiogenic cells are found in the splanchnic mesoderm at HH5-6. The splanchnic mesodermal layers fold ventrally and medially towards the intestinal portal and fuse at the ventral midline at HH9. The heart tube is formed by HH10 and begins to loop from HH11 onwards. This primitive heart tube hangs in the pericardial coelom via the dorsal mesocardium as well as via the arterial and venous poles of the myocardium. Heart development continues through the addition of future cardiomyocytes, which originate from the epithelium of the dorsomedial region of the pericardial cavity and pharyngeal arch mesoderm. The cardiac precursor cells located within these regions, which differentiate and enter the developing heart later, after the primary heart tube has formed, are known as 'secondary' and 'anterior heart field' cells. Various studies suggest that induction of secondary and anterior heart field cells is controlled by similar signals to those involved in the induction of the primary heart tube. This is not surprising as new evidence suggests that all heart fields are contiguous in the lateral plate mesoderm and supports the view that all CPCs originate from a single heart field, which is then spatiotemporally defined at later stages (65-68). In this review only signaling mechanisms involved in the specification of the primary heart field present in late gastrula/early neurula stage embryos will be discussed.

The bilateral fields (pre-cardiac mesoderm) are highly dynamic as cells change position in a short period of time. In addition, the specification of cardiac progenitor cells occurs concomitantly with cell migration and morphogenetic movements during early embryogenesis introducing more complexity to the molecular mechanisms that coordinate cardiac progenitor cell migration and differentiation (14, 67). Signals from the surrounding cells, including members of the BMP, FGF and Wnt families, are thought to promote the specification of myocardial fate.

Cardiac mesoderm precursors are in contact with presumptive anterior endoderm throughout their migration from the streak into the lateral plate (9). Once cardiac progenitor cells have migrated to the anterior lateral plate mesoderm, there is evidence for the anterior lateral endoderm to play a role in cardiac lineage determination and differentiation (69). Interaction between the anterior lateral mesoderm and its adjacent endoderm is required to generate beating mesoderm tissue. Endoderm derived inductive signal/s during gastrulation are required for future functional cardiomyocytes (14, 69).

Pre-cardiac anterior lateral mesoderm is defined by the expression of the transcription factors *Nkx2.5* and *GATA4*, which are detected in a crescent shape pattern at HH6. Anterior lateral endoderm can convert non-precardiac mesoderm to a cardiac phenotype but cannot induce a cardiac phenotype in epiblast cells (11, 70). At mid gastrula stage the anterior endoderm adjacent to the anterior lateral plate mesoderm (where cardiac progenitor cells are located), express a variety of signaling molecules such as Wnt/beta-catenin signaling inhibitors, FGFs and BMP2 (15, 71, 72).

6.1. Wnt signaling in early cardiogenesis

Wnt are a family of secreted proteins, which initiate several signal transduction pathways. One of these pathways is known as the beta-catenin-dependent Wnt signaling pathway and acts by stabilizing beta-catenin. As described previously, after binding of the Wnt ligand to the transmembrane receptor, Frizzled, the signal is transduced through dishevelled (Dvl); downstream of Dvl, beta-catenin phosphorylation by GSK-3beta does not occur and its subsequent degradation is inhibited. As a result beta-catenin accumulates and is translocated to the nucleus where it interacts with members of the LEF/TCF family of transcription factors. In addition to the Wnt/beta-catenin signaling pathway, Wnt ligand interaction with the Frizzled receptors and signaling through Dvl can also lead to the activation of at least two beta-catenin-independent Wnt pathways, the planar cell polarity (PCP) and the Wnt/Ca^{2+} pathway (67, 73-77). Known mediators of the Wnt/Ca^{2+} pathway are the protein kinases PKC and CamKII whereas the Wnt/PCP pathway involves the small GTPase RhoA and the protein kinase JNK. In amphibian embryos, three members of the Wnt gene family have been shown to activate these pathways, Wnt4, Wnt5A, and Wnt11 (77, 78). The Wnt signaling pathway in chicken embryos controls tissue polarity and cell movement in part through the activation of RhoA, c-Jun N-terminal kinase (JNK) (28, 57, 74). In addition, the Wnt/Ca^{2+} pathway is also important for cell adhesion and cell movements during vertebrate gastrulation (76). However, this has yet to be studied in chicken embryos.

There is evidence, which suggest that at the gastrula stage beta-catenin-dependent Wnt signaling negatively regulates cardiac differentiation and that the inhibition of the Wnt pathway is required for cardiac specification (14, 17-19, 79). On the other hand, beta-catenin-independent Wnt signaling is thought to enhance myocardial differentiation (80). In fact, small interfering RNA (siRNA) to disrupt RhoA expression, injected into anterior lateral plate mesoderm caused severe defects in cardiac tube fusion in the chicken embryo. Thus, RhoA expression (Wnt/PCP signaling) is necessary for the development of a normal heart (81). This is consistent with the model suggested by Yue *et al* (28), whereby the role of Wnt3a in cardiac progenitor cell migration is mediated via RhoA activity.

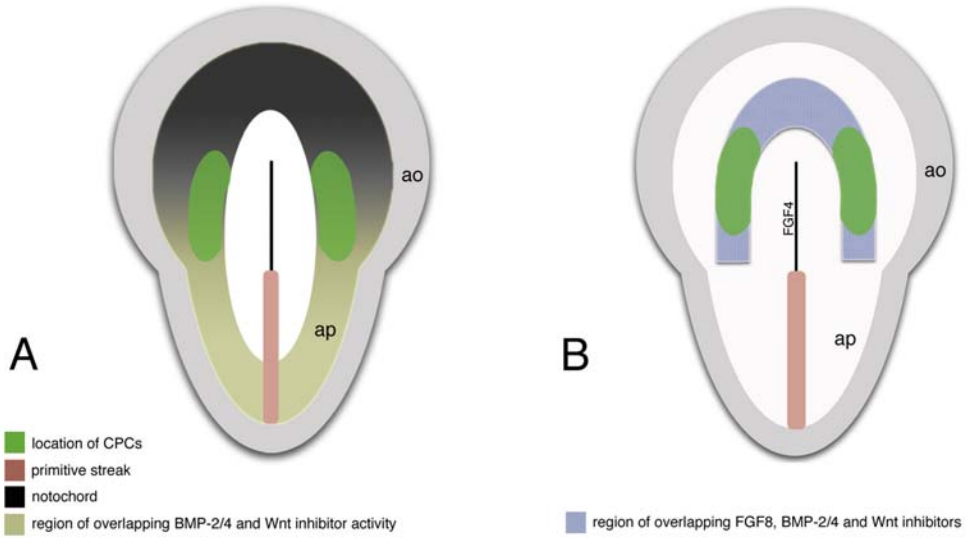

Figure 2. Factors that regulate early cardiac specification. (A) At stage HH5-6 CPCs are located in the bilateral heart field (green ovals) in the anterolateral part of the embryo. This is a region of high BMP and high beta-catenin-dependent Wnt inhibitor activity (darker shading). (B) CPCs located in the bilateral heart fields respond to inductive signals, FGF8, BMP-2 and beta-catenin-dependent Wnt inhibitors from the endoderm, BMP4 from the ectoderm and FGF4 from the notochord. ao= area opaca, ap= area pellucida

Molecules that antagonize the beta-catenin-dependent Wnt pathway also have cardiac inducing properties (17-19). Some of the antagonists of beta-catenin-dependent Wnt signaling, which act as mediators of cardiogenesis in chicken at the gastrula stage, are *dickkopf (Dkk1*) and *crescent*. In chicken embryos, crescent is expressed in the anterior endoderm during gastrulation and it can induce expression of cardiac genes in posterior, non-cardiogenic tissues *in vitro* (17, 41). During gastrulation, cells in the PS and posterior mesoderm express both Wnt3a and Wnt8c, thus prospective cardiac cells express Wnt genes at an early stage of development. At this stage of development Wnt3a has been suggested to signal through a beta-catenin-independent mechanism to guide cardiac precursor cell migration away from the PS (28). At the pre-gastrula stage of development Wnt3a and Wnt8c also signal through the beta-catenin-dependent Wnt pathway and play a prominent role in PS induction in birds and mammals (32, 34, 82, 83). However, ectopic expression of either Wnt3a or Wnt8c in precardiac mesoderm at gastrulation blocks cardiogenesis (17). In fact, beta-catenin-dependent Wnt signaling in the posterior lateral mesoderm induces hematopoiesis. Conversely, expression of the beta-catenin-dependent Wnt signaling inhibitors, *crescent*, *Dkk1* or *Wnt11* in posterior lateral plate mesoderm induces heart muscle formation (17, 18, 41). It thus appears that beta-catenin-dependent Wnt signaling, during gastrulation, is one of the principal factors preventing the induction of cardiogenesis in the posterior part of the embryo.

6.2. The role of BMP and FGF signaling during early cardiogenesis

BMP and FGF signaling molecules are expressed during the gastrula stage in the anterior endoderm situated adjacent to the anterior lateral plate mesoderm and thus adjacent to where CPCs are located. In addition, BMP4 is expressed widely in the ectoderm, including a region which overlies the location of CPCs in the lateral plate mesoderm. These signaling molecules have been found to promote cardiogenesis.

At mid-gastrulation and early neurula the anterior endoderm, which is adjacent to the anterior lateral plate mesoderm (that contains precardiac mesoderm), expresses BMP2 and the anterior lateral plate mesoderm is overlain by BMP-4 expressing ectoderm. (15). Exposure to the secreted protein Noggin, an antagonist of BMP signaling, completely inhibits differentiation of the precardiac mesoderm (15, 18). However, exogenous BMP2 can induce ectopic expression of the heart specific transcription factors, *Nkx2.5* and *GATA4* in the anterior medial region of HH6 embryos. Explanted anterior medial mesoderm from HH6 embryos can induce cardiac differentiation in response to BMP2 or BMP4 (15, 84). On the other hand BMP2 alone cannot induce a cardiac cell fate in posterior lateral mesoderm. This is consistent with loss and gain of function which show that BMP2 and 4 are necessary but not sufficient to induce the expression of heart specific transcription factors *Nkx2.5* and *GATA4*, and cardiac contractile proteins sarcomeric α-actin and sarcomeric myosin (15, 41, 85, 86).

Studies investigating BMP and beta-catenin-dependent Wnt signaling during the gastrula stage have initially suggested a model for activation of cardiac gene expression and subsequent cardiac progenitor differentiation. This model described cardiac gene expression to be initiated in the anterolateral part of the embryo because it is a region of high BMP and low Wnt/beta-catenin signalling (Figure 2A). However there is

a region in the early embryo in which both BMP and Wnt inhibitors are expressed yet cardiac genes are not expressed (87). Also, neither BMPs nor Wnt inhibitors can reproduce the cardiac inducing capability of the anterior endoderm, thus another factor must be involved. FGF signaling at this developmental stage has now been demonstrated to also promote cardiogenesis. In fact a combination of BMP2 plus FGF4 has been shown to have the same capacity as the anterior lateral endoderm to induce cardiogenic cell fates and is sufficient to convert posterior mesoderm to a myocardial cell fate (88).

As discussed earlier, *Fgf8* expression is detected in the PS in HH4 embryos. At HH5, *Fgf8* is expressed in a crescent shape pattern in the anterior endoderm, this overlaps with the expression domain of BMP2 in the endoderm and *Nkx2.5* in the mesoderm (87). Ectopic expression of *Fgf8* outside the heart field, in more lateral areas, results in the expansion of the expression domains of the cardiac markers *Nkx2.5* and *Mef2c*. However no expansion was observed if ectopic expression of *Fgf8* occurred in the medial anterior mesoderm or in posterior regions (87). Thus FGF8 is only able to include cardiac gene expression in regions where BMP signaling is present and BMP signaling induces cardiac gene expression only in regions exposed to FGF8 signaling (87). Furthermore, ectopic administration of BMP2/4 and FGF8 in non-cardiogenic mesoderm leads to the expression of *Nkx2.5*. Therefore, the current view is that, in avian embryos, beta-catenin-dependent Wnt signaling inhibitors, BMP and FGF signaling molecules act together to specify cells to cardiac fates during the late gastrula/ early neurula stage (Figure 2B).

7. CONCLUSIONS

Gastrulation is one of the key phases of development as it is the process when the three germ layers are formed and the body plan is established. These three layers give rise to all of the tissues and organs of an animal through the process of organogenesis. In amniote blastula embryos, such as the chicken, the beginning of gastrulation involves the movement of epiblast cells into the midline to form the PS. This is followed by EMT and ingression of the mesendoderm. The heart is the first organ to function during development and cardiac progenitor cells are among the first cell types to be established from mesoderm cells emerging form the PS. Data obtained from a large number of published studies indicates that cardiac development is tightly regulated by a series of molecular signaling pathways and morphological events. The molecular pathways controlling early cardiac progenitor cell migration and differentiation are not completely understood, however WNT, FGF and BMP signaling pathways are emerging as critical molecular players required for multiple aspects of early cardiac development.

7.1. Ingression and cardiac progenitor cell migration trajectories

In chicken embryos, cardiac precursor cells ingress at the early streak stage and are found within the anterior-middle region of the PS at HH3 (5, 9, 11, 12). Cells at the anterior-middle PS, give rise to lateral plate mesoderm (6, 8, 13). Long-term time-lapse video microscopy of GFP labeled PS cells at this position has demonstrated that GFP positive cells initially move out laterally, away from the streak in a semi-circular (anterior-lateral) fashion, are found in the lateral plate mesoderm at HH5 and in the bilateral heart forming-regions at HH7. They then move towards the midline of the embryo before contributing to a single heart tube by HH10 (27, 28). beta-catenin-dependent and independent Wnt signaling and FGF signaling in chicken is required for the formation of the PS (21, 22, 32, 34) and strong evidence suggests that PDGF and FGF signaling along with Wnt11b and Wnt5a are required for the movement of cells away from the PS during gastrulation (44-48, 50, 57). When investigating the movement trajectories of just lateral plate mesoderm and cardiac progenitor cells, it has been demonstrated that cell movement patterns are controlled by negative and positive chemotaxis mediated by FGF8, Wnt3a and FGF4. Streak cells are repelled by sources of FGF8 and attracted by sources of FGF4 (27). In addition, prospective cardiac cells alter their movement pattern in response to Wnt3a, are repelled by it and it has been demonstrated that Wnt3a guides the movement pattern of cardiac progenitor cells through a RhoA dependent mechanism (28). Thus, it is proposed that the movement patterns of CPCs are controlled by Wnt3a/FGF8-mediated chemorepulsion of cells away from the streak followed by chemoattraction towards an FGF4 signal at the midline of the developing embryo.

7.2. Two temporally distinct signaling events regulate early cardiac progenitor cell specification

The origin of cardiac precursor cells can be traced back before gastrulation as these cells are found in the posterior half of the blastula chicken embryo (5, 36). Furthermore, the hypoblast, which lies beneath the epiblast in the blastula embryo, has been shown to possess cardiogenic inducing capabilities. The first signaling event towards cardiac cell specification is comprised of signals derived from the hypoblast acting on epiblast cells and is mediated by activin and TGF-beta. It has been shown that at the pre-gastrula stage, activin/ TGF-beta signaling in addition to BMP-antagonists are necessary for heart mesoderm induction/myocardial cell formation (11, 37-39). At gastrulation and early neurula, the second signaling event, which controls cardiac cell differentiation is regulated by BMP2/4 and FGF8 signaling (15, 18, 37, 87, 89). Furthermore, the absence or presence of beta-catenin-dependent Wnt signaling has been shown to control the specification of cardiac cells, whereby its absence in the anterior endoderm is needed for cardiac cell specification to occur (17). Thus at the gastrulation stage, BMP2/4, FGF8 signaling and inhibitors of beta-catenin-dependent Wnt signaling are necessary for the specification of cardiac cells in the anterior lateral mesoderm.

Ectopic expression of *crescent* or *Dkk-1* in posterior lateral plate mesoderm induces cardiogenesis (18, 41). On the other hand ectopic expression of either Wnt8c or Wnt3a in the anterior lateral plate mesoderm (precardiac mesoderm) blocks cardiogenesis (17). Together this

indicates that beta-catenin-dependent Wnt signaling negatively regulates cardiac differentiation at the gastrulation stage. Consistent with this, it has been demonstrated in zebafish embryos that beta-catenin-dependent Wnt signaling inhibits cardiac differentiation during gastrulation. However, beta-catenin-dependent Wnt signaling has been shown to promote cardiac differentiation before gastrulation (89). Furthermore, *in vitro* studies using mouse embryoid bodies, aggregates formed from mouse embryonic stem cells, show that beta-catenin-dependent Wnt signaling enhances the differentiation of cells into cardiomyocytes during the early phase of embryoid body formation. In contrast, during the late phase of embryoid formation the differentiation of cells into cardiomyoctes is inhibited by this pathway (90). From these studies it has been suggested that beta-catenin-dependent Wnt signaling promotes or inhibits cardiogenesis depending on the developmental stage. The distinct developmental stage-dependent role of Wnt/beta-catenin signaling observed during cardiogenesis is not specific for this developmental process. In fact, Wnt signaling has also been observed to have stage-dependant biphasic and distinct roles during retinal development in the medaka fish (91).

After the precardiac mesoderm is formed and cardiac progenitor cell differentiation begins, expression of contractile proteins and generation of the sarcomeric apparatus (terminal differentiation) occurs. Loss-of-function experiments have shown that BMP2/4 are necessary to include the expression of sarcomeric *alpha-actin*, *tintin* and sarcomeric *myosin* (92). However the expression of other important cardiac markers such as smooth muscle *alpha-actin* and cardiac *troponin T* are not dependent on the anterior endoderm and thus not dependent on BMP2 or FGFs (92, 93). Thus BMP and FGF emitted from the endoderm regulate heart specification and terminal differentiation, but there are additional regulatory mechanisms that play a role in terminal differentiation of CPCs. What these other regulatory mechanisms are and how they cooperate with WNT, FGF and BMP signaling remains to be studied further.

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9. REFERENCES

1. A. Lawson and G.C. Schoenwolf: New insights into critical events of avian gastrulation. *Anat Rec* 262, 238-252 (2001)

2. A. Lawson and G.C. Schoenwolf: Epiblast and primitive-streak origins of the endoderm in the gastrulating chick embryo. *Developmen*t 130, 3491-3501 (2003)

3. C.D. Stern: Gastrulation in the Chick. Gastrulation: From Cells to Embryos. *Cold Spring Habor Press, Cold Spring Harbor*, pp. 219-232 (2004)

4. V. Garcia-Martinez and G.C. Schoenwolf: Positional control of mesoderm movement and fate during avian gastrulation and neurulation. *Dev Dyn* 193, 249-256 (1992)

5. Y. Hatada and C.D. Stern: A fate map of the epiblast of the early chick embryo. *Development* 120, 2879-2889 (1994)

6. D. Psychoyos and C.D Stern: Fates and migratory routes of primitive streak cells in the chick embryo. *Development* 122, 1523-1534 (1996)

7. S.J. Kinder, T.E. Tsang, G.A. Quinian, A.K. Hadjantonakis, A. Nagy and P.P Tam: The ordely allocation of mesodermal cells to the extraembryonic structures and the anterioposterior axis during gastrulation of the mouse embryo. *Development* 126, 4691-4701 (1999)

8. T. Limura, X. Yang, C.J. Weijer and O. Pourquie: Dual mode of paraxial mesoderm formation during chick gastrulation. *Proc Natl Acad Sci USA* 104, 2744-2749 (2007)

9. V. Garcia-Martinez and G.C. Schoenwolf: Primitive-streak origin of the cardiovascular system in avian embryos. *Dev Biol* 159, 706-719 (1993)

10. G.C. Schoenwolf and V. Garcia-Martinez: Primitive-streak origin and state of commitment of cells in the cardiovascular system in avian and mammalian embryos. *Cell Mol Res* 41 233-240 (1995)

11. T.A. Yatskievych, A.N. Ladd and P.B Antin: Induction of cardiac myogenesis in avian pregastrula epiblast: the role of hypoblast and activin. *Development* 124, 2561-2570 (1997)

12. G.C. Rosenquist: Location and movements of cardiogenic cells in the chick embryo: the heart-forming portion of the primitive streak. *Dev Biol* 22, 461-475 (1970)

13. R.L. DeHaan: Morphogenesis of the vertebrate heart. In: Organogenesis. New York: Holt, Rinehart, and Winston. p 377-419. (1965)

14. T Brand: Heart development: molecular insights into cardiac specification and early morphogenesis. *Dev Biol* 258, 1-19 (2003)

15. T.M. Schultheiss, J.B. Burch and A.B. Lassar: A role for bone morphogenetic proteins in the induction of cardiac myogenesis. *Genes Dev* 11, 451-462 (1997)

16. C.A. Eisenberg, and L.M. Eisenberg: WNT11 promotes cardiac tissue formation of early mesoderm. *Dev Dyn* 216, 45-58 (1999)

17. M.J. Marvin, G. Di Rocco, A. Gardiner, S.M. Bush and A.B. Lassar: Inhibition of Wnt activity induces heart formation from posterior mesoderm. *Genes Dev* 15, 316-327 (2001)

18. V.A. Schneider and M. Mercola: Wnt antagonism initiates cardiogenesis in Xenopus laevis. *Genes Dev* 15, 304-315 (2001)

19. E. Tzahor and A.B. Lassar: Wnt signals from the neural tube block ectopic cardiogenesis. *Genes Dev* 15, 255-260 (2001)

20. H. Lickert, S. Kutsch, B. Kanzler, Y. Tamai, M.M. Taketo and R. Kemler: Formation of multiple hearts in mice following deletion of beta-catenin in the embryonic endoderm. *Dev Cell* 3, 171-181 (2002)

21. M. Chuai, W. Zeng, M. Yang, V. Boychenko, J. Glazier and C Weijer: Cell movement during chick primitive streak formation. *Dev Biol* 296, 137-149 (2006)

22. O. Voiculescu, F. Bertocchini, L. Wolpert, R. Keller and C.D. Stern: The amniote primitive streak is defined by epithelial cell intercalation before gastrulation. *Nature* 449, 1049-1052 (2007)

23. X. Yang, H. Chrisman and C.J. Weijer: PDGF signalling controls the migration of mesoderm cells during chick gastrulation by regulating N-cadherin expression. *Development* 135, 3521-3530 (2008)

24. M.C. Fishman and K.R. Chien: Fashioning the vertebrate heart: earliest embryonic decisions. *Development* 124, 2099-2117 (1997)

25. D. Srivastava and E.N. Olson: A genetic blueprint for cardiac development. *Nature* 407, 221-226 (2000)

26. S.S. Chapman, J. Collignon, G.C. Schoenwolf and A. Lumsden: Improved Method for Chick Whole-Embryo Culture Using a Filter Paper Carrier. *Dev Dyn* 220, 284-289 (2001)

27. X. Yang, D. Dormann, A.E. Münsterberg and C.J. Weijer: Cell Movement Patterns during Gastrulation in the Chick are Controlled by Positive and Negative Chemotasis Mediated by FGF4 and FGF8. *Dev Cell* 3, 425-437 (2002)

28. Q. Yue, L. Wagstaff, Y. Yang, C. Weijer and A. Münsterberg: Wnt3a-mediated chemorepulstion controls movement patterns of cardiac progenitors and requires RhoA function. *Development* 135, 1029-1037 (2008)

29. D. Sweetman, L. Wagstaff, O. Cooper, C. Weijer and A. Münsterberg: The migration of paraxial and lateral plate mesoderm cells emerging from the late primitive streak is controlled by different Wnt signals. MBC. *Dev Biol* 8:63 doi:10.1186/147-213X-8-63 (2008)

30. F. Bertocchini, I. Skromme, L. Wolpert and C.D. Stern: Determination of embryonic polarity in a regulative system: evidence for endogenous inhibitors acting sequentially during primitive streak formation in the chick embryo. *Development* 131, 3381-3390 (2004)

31. E. Mitrani, Y. Gruenbaum, H. Shohat and T. Ziv: Fibroblast growth factor during mesoderm induction in the early chick embryo. *Development* 109, 387-393 (1990)

32. P. Liu, M. Wakamiya, M.J. Shea, U. Albrecht, R.R. Behringer and A. Bradley: Requirement for Wnt3 in vertebrate axis formation. *Nat Genet* 22, 361-365 (1999)

33. A. Streit, A.J. Berliner, C. Papanayotou, A. Sirulnik and C.D. Stern: Initiation of neural induction by FGF signalling before gastrulation. *Nature* 406, 74-78 (2000)

34. I. Skromne and C.D. Stern: Interactions between Wnt and Vg1 signalling pathways initiate primitive streak formation in the chick embryo. *Development* 128, 2915-2927 (2001)

35. B.V Vasiev, A Balter, M Chaplain, J.A Glazier and C.J Weijer. Modeling Gastrulation in the Chick Embryo: Formation of the Primitive Streak. *PLoS One* 5 doi:10.1371/journal.pone.0010571 (2010)

36. M.E. Rawles: The heart-forming regions of the early chick blastoderm. *Physiol Zool* 16, 22-42. (1943)

37. A.N. Ladd, T.A. Yatskievych and P.B. Antin: Regulation of avian cardiac myogenesis by Activin/TGFb and bone morphogenetic proteins. *Dev Biol* 204, 407-419 (1998)

38. E.J. Sanders, N. Hu and M.A. Wride: Expression of TGF beta 1/beta 3 during early chick embryo development. *Anat Rec* 238, 397-406 (1994)

39. H. Matsui, K. Ikeda, K. Nakatani, M. Sakabe, T. Yamagishi, T. Nakanishi, and Y. Nakajima: Induction of initial cardiomyocytes α-actin- smooth muscle α-actin- in cultured avian pregastrula epiblast: a role of Nodal and BMP antagonist. *Dev Dyn* 233, 1419-1429 (2005)

40. G.C. Schoenwolf, V. Garcia-Martinez and M.S. Dias: Mesoderm movement and fate during avian gastrulation and neurulation. Dev Dyn 193, 235-248 (1992)

41. T.M. Schultheiss, S. Xydas and A.B. Lassar: Induction of avian cardiac myogenesis by anterior endoderm. *Development* 121, 4203-4214 (1995)

42. S. Yuan and G.C Schoenwolf: Reconstitution of the organizer is both sufficient and required to re-establish a fully patterned body plan in avian embryos. *Development* 126, 2461-2473 (1999)

43. Y. Wei and T. Nikawa. Fate diversity of primitive streak cells during heart field formation in ovo. *Dev Dyn* 219, 505-513 (2000)

44. C.X. Deng, A. Wynshaw-Boris, M.M. Shen, C. Daugherty, D.M. Ornitz and P. Leder: Murine FGFR-1 is required for early postimplantation growth and axial organization. *Genes Dev* 8, 3045-3057 (1995)

45. T.P. Yamaguchi, K. Harpal, M. Henkemeyer and J. Rossant: Fgfr-1 is required for embryonic growth and mesodermal patterning during mouse gastrulation. *Genes Dev* 8, 3032-3044 (1994)

46 B.G. Ciruna, L. Schwartz, K. Harpal, T.P.Yamaguchi and J. Rossant. Chimeric analysis of fibroblast growth factor receptor-1 (Fgfr1) function: a role for FGFR1 in morphogenic movement through the primitive streak. *Development* 124, 2829-2841 (1997)

47. B. Ciruna and J. Rossant: FGF Signaling Regulates Mesoderm Cell Fate Specification and Morphogenetic Movement at the Primitive Streak. *Dev Cell* 1, 37-49 (2001)

48. C.A. Burdsal, C.H. Damsky and R.A. Pedersen: The role of E-cadherin and integrins in mesoderm differentiation and migration at the mammalian primitive streak. *Development* 118, 829-844 (1993)

49. M. Chuai, W. Zeng, X. Yang, V. Boychenko, J. Glazier and C. Weijer: Cell movement during chick primitive streak formation. *Dev Biol* 296, 137-149 (2006)

50. X. Yang, H. Chrisman and C.J. Weijer: PDGF signalling controls the migration of mesoderm cells during chick gastrulation by regulating N-cadherin expression. *Development* 135, 3521-3530 (2008)

51. C.P. Heisenberg, M. Tada, G.J. Rauch, L. Saude, M.L. Concha, R. Geisler, D.L. Stemple, J.C. Smith and S.W. Wilson: Silberblick/Wnt11 mediates convergent extension movements during zebrafish gastrulation. *Nature* 405, 76-81 (2000)

52. M. Park and R.T Moon: The planar cell-polarity gene stbm regulates cell behaviour and cell fate in vertebrate embryos. *Nat Cell Biol* 4, 20-52 (2002)

53. M.T. Veeman, D.C. Slusarski, A. Kaykas, S.H. Louie and R.T. Moon: Zebrafish prickle, a modulator of noncanonical Wnt/Fz signaling, regulates gastrulation movements. *Curr Biol* 13, 680-685 (2003)

54. G.J. Rauch, M. Hammerschmidt, P. Blader, H.E. Schauerte, U. Strähle, P.W. Ingham, A.P. McMahon and P. Haffter: Wnt5 is required for tail formation in the zebrafish embryo. *Cold Spring Harb Symp Quant Biol* 62, 227-234 (1997)

55. B. Kilian, H. Mansukoski, F.C. Barbosa, F. Ulrich, M. Tada and C.P. Heisenberg:The role of Ppt/Wnt5 in regulating cell shape and movement during zebrafish gastrulation. *Mech Dev* 120, 467-76 (2003)

56. S.C. Chapman, R. Brown, L. Lees, G.C. Schoenwolf and A. Lumsden: Expression analysis of chick Wnt and frizzled genes and selected inhibitors in early chick patterning. *Dev Dyn* 229, 668-676 (2004)

57. K.M. Hardy, R.J. Garriok, T.A. Yatskievych, S.L, D'Agostino, P.B. Antin and P.A. Krieg: Non-canonical Wnt signaling through Wnt5a/b and novel Wnt11 gene, Wnt11b, regulates cell migration during avian gastrulation. *Dev Biol* 320, 391-401 (2008)

58. B. Feldman, W. Poueymirou, V.E. Papaioannour, T.M. DeChiara and M. Goldfarb: Requirements of FGF-4 for postimplantation mouse development. *Science* 267, 246-249 (1995)

59. X. Sun, E.N. Meyers, M. Lewandoski and G.R. Martin: Targeted disruption of Fgf8 causes failure of cell migration in the gastrulating mouse embryo: *Genes Dev* 13, 1834-1846 (1999)

60. N. Nakatsuji, M.H. Snow and C.C. Wylie: Cinemicrographic study of cell movement in the primitive-streak-stage mouse embryo. *J Embryol Exp Morphol* 96, 99-109 (1986)

61. K.A. Lawson, J.J. Menesis and R.A. Pedersen: Clonal analysis of epiblast fate during germ layer formation in the mouse embryo. *Developmen*t 113, 8891-911 (1991)

62. J.S. Lunn, K.J. Fishwick, P.A. Halley and K.G. Storey; A spatial and temporal map of FGF/Erk1/2 activity and response repertoires in the early chick embryo. *Dev Biol* 302, 536-552 (2007)

63. A. Gonzalez-Sanchez and D. Bader: *In vitro* analysis of cardiac progenitor cell differentiation. *Dev Biol* 139, 197-209 (1990)

64. P.B. Antin, R.G. Taylor and T. Yatskievych: Precardiac mesoderm is specified during gastrulation in quail. *Dev Dyn* 200, 144-154 (1994)

65. L. Lin, L. Cui, W. Zhou, D. Dufort, X. Zhang, C.L Cai, L.Bu, L. Yang, J. Martin, R. Kemler, M.G. Rosenfeld, J. Chen and S.M Evans: β-Catenin directly regulates Islet1 expression in cardiovascular progenitors and is required for multiple aspects of cardiogenesis. *Proc Natl Acad Sci USA* 104, 9313-9318 (2007)

66. Y. Tian, E.D. Cohen and E.E Morrisey: The Importance of Wnt Signaling in Cardiovascular Development. *Pediatr Cardiol* 31, 342-348 (2010)

67. K.E. Yutzey and K.L. Kirby: Wherefore Heart Thou? Embryonic Origins of Cardiogenic Mesoderm. *Dev Dyn* 223, 307-320 (2002)

68. L.A. Dyer and M.L. Kirby: The role of secondary heart field in cardiac development. *Dev Biol* 336, 137-144 (2009)

69. J. Lough and Y. Sugi: Endoderm and heart development. *Dev Dyn* 217, 327-242 (2000)

70. T.M. Schultheiss, S. Xydas and A.B. Lassar: Induction of avian cardiac myogenesis by anterior endoderm. *Development* 121, 4203-4214 (1995)

71. Y. Sugi and J. Lough: Activin-A and FGF-2 mimic the inductive effects of anterior endoderm on terminal cardiac myogenesis *in vitro*. *Dev Biol* 168, 567-574 (1995)

72. Y. Sugi and R.R. Markwald: Formation and early morphogenesis of endocardial endothelial precursor cells and the role of the endoderm. *Dev Biol* 175, 66-83 (1996)

73. K.M. Cadigan and R. Nusse: Wnt signaling: a common theme in animal development. *Genes Dev 11*, 3286-3305 (1997)

74. M.T. Veeman, J.D. Axelrod and R.T. Moon: A Second Canon: Functions and Mechanisms of β-Catenin-Independent Wnt Signaling. *Dev Cell* 5, 367-377 (2003)

75. C.Y. Logan and R. Nusse: The Wnt signaling pathway in development and disease. *Annu Rev Cell Dev Biol* 20, 781-810 (2004)

76. R.T. Moon, A.D. Kohn, G.V. De Ferrari and A. Raykas: WNT and β-catenin signalling: diseases and therapies. *Nat Rev Genet* 5, 691-701 (2004)

77. Y. Komiya and R. Habas: Wnt signal transduction pathways. *Organogenesis* 2, 68-75 (2008)

78. M. Kühl. Non-canonical Wnt signaling in Xenopus: regulation of axis formation and gastrulation. *Semin Cell Dev Biol* 3, 243-249 (2002)

79. L.M. Eisenberg and C.A. Eisenberg: Evaluating the role of Wnt signal transduction in promoting the development of the heart. *Scientific World Journal* 2, 161-176 (2007)

80. M.J. Solloway and R.P. Harvey: Molecular pathways in myocardial development: a stem cell perspective. *Cardiovasc Res* 58, 264-277 (2003)

81. M. Kaarbø, D.I. Crane and W.G. Murrell: RhoA is highly up-regulated in the process of early heart development of the chick and important for normal embryogenesis. *Dev Dyn* 227, 35-47 (2003)

82. C.R. Hume and J. Dodd: Cwnt-8C: a novel Wnt gene with a potential role in primitive streak formation and hindbrain organization. *Development* 119, 1147-60 (1993)

83. H. Pöpperl, C. Schmidt, V. Wilson, C.R. Hume, J. Dodd, R. Krumlauf and R.S. Beddington: Misexpression of Cwnt8C in the mouse induces an ectopic embryonic axis and causes a truncation of the anterior neuroectoderm. *Development* 124, 2997-3005 (1997)

84. B. Andree, D. Duprez, B. Vorbusch, H.H. Arnold and T. Brand: BMP-2 induces ectopic expression of cardiac lineage markers and interferes with somite formation in chicken embryos. *Mech Dev* 70, 119-131 (1998)

85. M.J. Walters, G.A. Wayman and J.L. Christian: Bone morphogenetic protein function is required for terminal differentiation of the heart tube but not for early expression of cardiac marker genes. *Mech Dev* 100, 263-273 (2001)

86. Y. Nakajima, T. Yamagishi, K. Ando and H. Nakamura: Significance of bone morphogenetic protein-4 function in the initial myofibrillogenesis of chick cardiogenesis. *Dev Biol* 245, 291-303 (2002)

87. B.H. Alsan and T.M. Schultheiss: Regulation of avian cardiogenesis by Fgf8 signaling. *Development* 129, 1935-1943 (2002)

88. J. Lough, M. Barron, M. Brogley, Y. Sugi, D.L. Bolender and X. Zhu: Combined BMP-2 and FGF-4, but neither factor alone, induces cardiogenesis in non-precardiac embryonic mesoderm. *Dev Biol* 178, 198-202 (1996)

89. S. Ueno, G. Weidinger, T. Osugi, A.D. Kohn, J.L. Golob, L. Pabon, H. Reinecke, R.T. Moon and C.E. Murry: Biphasic role for Wnt/beta-catenin signaling in cardiac specification in zebrafish and embryonic stem cells. *Proc Natl Acad Sci U S A*. 104, 9685-9690 (2007)

90. A.T Naito, I. Shiojima, H. Akazawa, K. Hidaka, T. Morisaki, A. Kikuchi and I. Komuro: Developmental stage-specific biphasic roles of Wnt/beta-catenin signaling in cardiomyogenesis and hematopoiesis. *Proc Natl Acad Sci USA* 103, 19812-19817 (2006)

91. A.V. Sánchez-Sánchez, E. Camp, A. Leal-Tassias and J.L. Mullor: Wnt signaling has different temporal roles during retinal development. *Dev Dyn* 239, 297-310 (2010)

92. Y. Nakajima, T. Yamagishi, K. Ando and H. Nakamura: Significance of bone morphogenetic protein-4 function in the initial myofibrillogenesis of chick cardiogenesis. *Dev Biol* 245, 291-303 (2002)

93. P.B. Antin, M.A. Bales, W. Zhang, R.J. Garriock, T.A. Yatskievych and M.A. Bates: Precocious expression of cardiac troponin T in early chick embryos is independent of bone morphogenetic protein signaling. *Dev Dyn* 225, 135-141 (2002)

Abbreviations: CPCs: cardiac progenitor cells; CE: convergence and extension; PS: primitive streak; EMT: epithelial to mesenchymal transition; HH: Hamburger Hamilton; BMP: bone morphogenic protein; FGF: fibroblast growth factor; PDGF: platelet growth factor; GFP: green fluorescent protein; ao: area opaca; ap: area pellucida; TGF-beta: transforming growth factor-beta; Fgfr1: FGF receptor 1; Dvl: dishevelled; pk: pickle; slb: silberblick; JNK: c-Jun N-terminal kinase; siRNA: small interfering RNA; Dkk1: dickopf

Key Words : Chicken embryo, Gastrulation, Cell migration, Cardiac progenitor cells, Wnt, BMP, FGF, Review

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