

# Heart development before beating

Yuji Nakajima · Masahide Sakabe ·  
Hiroko Matsui · Hirokazu Sakata ·  
Nariaki Yanagawa · Toshiyuki Yamagishi

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**Abstract** During heart development at the pregastrula stage, prospective heart cells reside in the posterior lateral region of the epiblast layer. Interaction of tissues between the posterior epiblast and hypoblast is necessary to generate the future heart mesoderm. Signaling regulating the interaction involves fibroblast growth factor (FGF)-8, Nodal, bone morphogenetic protein (BMP)-antagonist, and canonical Wnt and acts on the posterior epiblast to induce the expression of genes specific for the anterior lateral mesoderm. At the early gastrula stage, prospective heart cells accumulate at the posterior midline and migrate to the anterior region of the primitive streak. During gastrulation, future heart cells leave the primitive streak and migrate anterolaterally to form the left and right anterior lateral plate mesoderm including the precardiac mesoderm. At this stage, prospective heart cells receive endoderm-derived signals, including BMP, FGF, and Wnt-antagonist, and thereby become committed to the heart lineage. At the neurula stage, the left and right precardiac mesoderm move to the ventral midline and fuse, resulting in the formation of a single primitive heart tube.

Therefore, a two-step signaling cascade, which includes tissue interaction between epiblast and hypoblast at the blastula stage and endoderm-derived signals during gastrulation, is required to generate a beating heart.

**Keywords** Blastula · Gastrula · Heart development · Heart mesoderm

## Developmental anatomy of the heart before beating

In a fate map analysis using a fluorescence dye in chick embryos, Hatada and Stern (1994) showed that during heart development in the pre-gastrula stage embryo (blastula), prospective heart cells reside in the posterior half of the blastoderm (green in Fig. 1a), which consists of two concentric germ layers, the epiblast (upper layer) and hypoblast (lower layer). Subsequent explantation experiments of the chick blastoderm showed that prospective heart cells are located in the posterior lateral region of the epiblast layer (Yatskievych et al. 1997; Ladd et al. 1998; Matsui et al. 2005). Shortly after the hypoblast is completed, a new layer, called the endoblast (sickle endoblast or secondary hypoblast), develops from the posterior marginal zone and displaces the hypoblast. The hypoblast expresses several marker genes, including *Gooseoid*, *Hex*, *Hesx1/Rpx*, *Cerberus* and *Otx2*, but the endoblast does not. These observations show that the hypoblast and endoblast are different and that the hypoblast is similar to the anterior visceral endoderm (AVE) of the mouse embryo (Stern 2004).

Soon after the hypoblast is completed, posterior epiblast cells move into the midline (arrows in Fig. 1a) and form a triangular-shaped initial primitive streak (PS) at the posterior midline of the epiblast layer, from which future mesendoderm cells ingress into the subepiblast space,

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Y. Nakajima (✉) · M. Sakabe · H. Matsui · H. Sakata ·  
N. Yanagawa  
Department of Anatomy and Cell Biology,  
Graduate School of Medicine,  
Osaka City University, 1-4-3 Asahimachi,  
Abenoku, Osaka 545-8585, Japan  
e-mail: yuji@med.osaka-cu.ac.jp

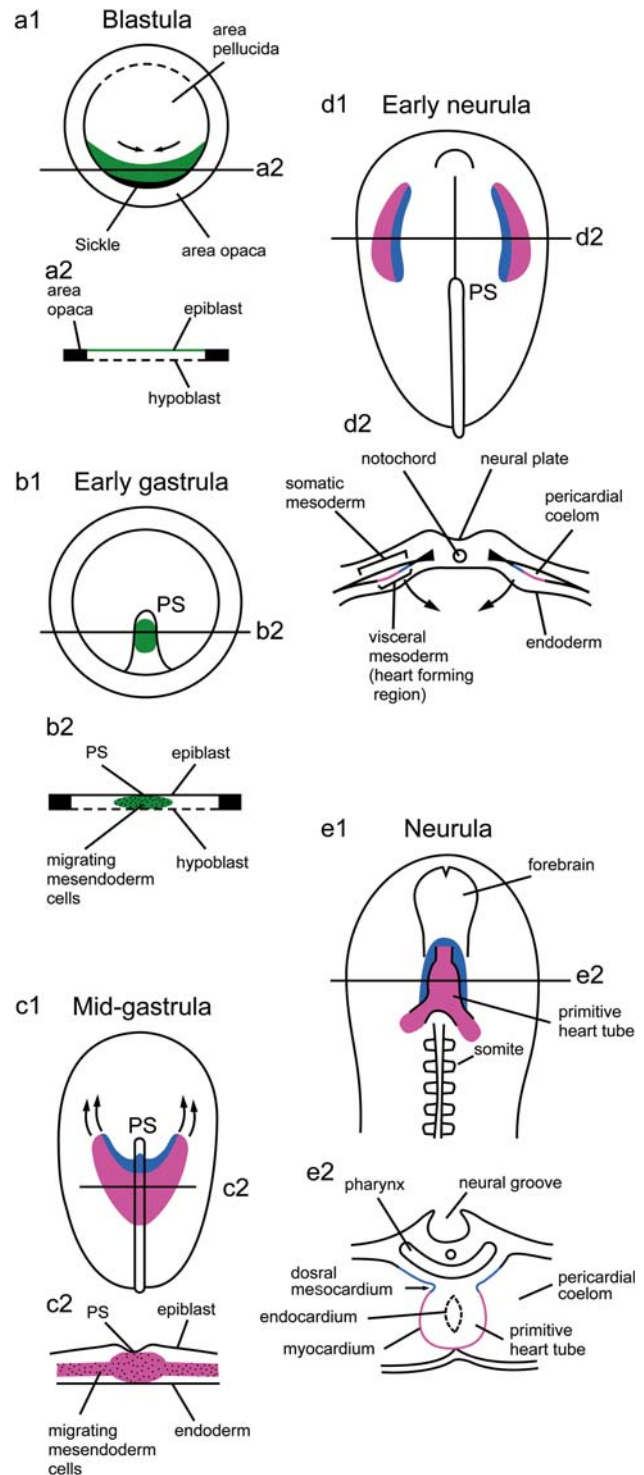
### Present Address:

M. Sakabe  
Division of Mammalian Development,  
National Institute of Genetics, Shizuoka, Japan

T. Yamagishi  
Department of Anatomy, School of Medicine,  
Saitama Medical University, Saitama, Japan

**Fig. 1** Developmental anatomy of heart before beating. At the blastula stage, prospective heart cells reside in the posterior region of the epiblast layer anterior to the sickle (green in **a**). As development proceeds, cells in the posterior lateral region migrate (arrows in **a**) and accumulate into the midline region, then undergo epithelial–mesenchymal transition (gastrulation), resulting in the formation of a primitive streak (PS in **b**, **c**). At the mid-gastrula stage, prospective heart cells reside in the anterior half of the PS with an anterior–posterior sequence (green in **b** and pink/blue in **c**). During gastrulation, future mesoderm cells that include prospective heart cells leave the PS and migrate anterolaterally (arrows in **c1**) to form the left and right lateral plate mesoderm. At the late gastrula to early neurula stage, the anterior lateral plate mesoderm divides dorsoventrally to form the somatic mesoderm and visceral mesoderm (heart-forming mesoderm, pink/blue in **d**), between which the pericardial coelom is established (**d2**). During the embryonic folding (arrows in **d2**), the right and left heart-forming regions move toward the ventral midline of the intestinal portal and fuse together to form the primitive heart tube (pink in **e**). The secondary/anterior heart field develops from the medial limb of the lateral plate (blue in **d**). The secondary/anterior heart field that gives rise to the outflow tract, right ventricle, and atrium is mapped in the dorsal coelomic epithelium of the pericardial coelom, dorsal mesocardium, and pharyngeal mesoderm (blue in **e**). **a1**, **b1**, **c1**, **d1** Schematic representations of the dorsal view of the embryos, **e1** ventral view. **a2–e2** Sections indicated by black lines in the panels immediately above

resulting in the formation of the PS (PS in stage 3 gastrula embryo in Fig. 1b) <sup>1</sup>. A fate map analysis of the cardiovascular system from the primitive streak using a quail–chick chimera technique showed that the prospective heart cells, including future endocardial, myocardial, and pericardial cells, occupy the anterior half of the PS in an anterior–posterior sequence at the early PS stage (the prospective bulbus cordis arises more anteriorly in the streak than does the prospective ventricle, which in turn arises more anteriorly than the sinus venosus; Fig. 1b, c; Garcia-Martinez and Schoenwolf 1993). Recent work has shown that the anterior–posterior polarity of the heart field is not established definitively until stage 8 (early neurula), just before fusion of the bilateral heart mesoderm in the chick embryo (Redkar et al. 2001). Prospective heart cells leave the PS, migrate anterolaterally, and accumulate in the left and right anterior lateral plate mesoderm (arrows in Fig. 1c), resulting in the formation of the precardiac mesoderm (blue/pink in Fig. 1d). By the early neurula stage (stage 6), the prospective heart cells have come to occupy a crescent in the anterior lateral region of the embryo (Rosenquist and DeHaan 1966). The precardiac mesoderm of the gastrula embryo is defined molecularly using various expression markers, such as *Nkx2.5* and *GATA4*. A recent study using dye marking and molecular



expression in the same embryos demonstrated that the two types of labeling do not correspond, suggesting a relatively high level of complexity of the heart field in the chick gastrula embryo (Redkar et al. 2001).

The anterior lateral mesoderm is initially a mesenchymal population of cells that includes precursors of the somatic and visceral mesoderm, and the visceral mesoderm includes

<sup>1</sup> In the description of chick developmental stages, Roman numerals from I to XIV are used to classify stages before the formation of the PS (Eyal-Giladi and Kochav 1976), and numbers from 2 onward are used for post-streak embryos (Hamburger and Hamilton 1992, originally published in *J Morphology* 88:49–92, 1951)

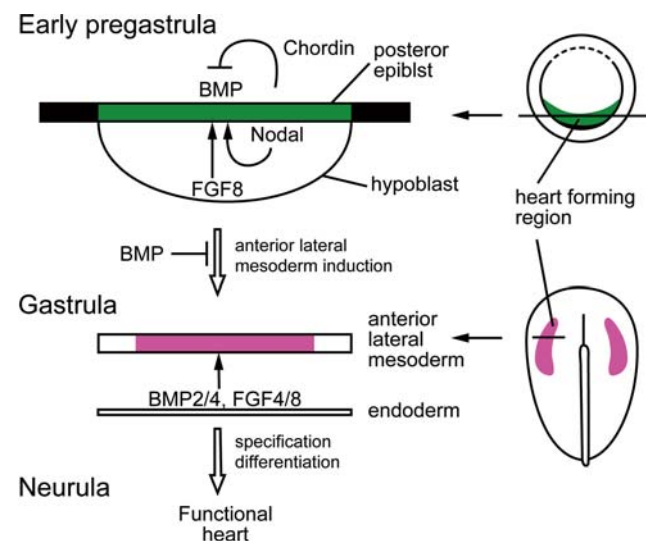
precardiac cells (pre-cardiomyocytes, pre-endocardocytes, and pre-epicardocytes). The anterior lateral mesoderm soon divides dorsoventrally to form the somatic mesoderm and visceral mesoderm, between which the pericardial coelom is established (Fig. 1d; Linask et al. 1997). During embryonic folding (arrows in Fig. 1d2), the right and left heart-forming mesoderm move toward the ventral midline of the intestinal portal and fuse together, resulting in the formation of the primitive heart tube in the pericardial coelom (Fig. 1e) and demonstrating that the cardiomyocyte mantle originates from the epithelialized visceral mesoderm of the pericardial coelom. During this process, the endocardial cell population is developed or sorted out from the visceral mesoderm into the space between the visceral mesoderm (developing myocardium) and pharyngeal endoderm (Linask and Lash 1993). Immediately after the formation of the primitive heart tube, the heart starts to beat spontaneously, indicating that myofibrillogenesis occurs rapidly (Hiruma and Hirakow 1985).

The primitive heart tube hangs in the pericardial coelom via the dorsal mesocardium as well as via the arterial and venous poles of the myocardium. Lineage tracing experiments have shown that the primitive heart tube at the neurula stage is composed of only two primitive cardiac segments (the future apical trabecular regions and the atrium), which originate from the outer region of the precardiac mesoderm (primary heart field; pink in Fig. 1d). Thus, the current view is that the heart is built in a sequential and segmental manner by the continued addition of future cardiomyocytes, which originate from the coelomic epithelium of the dorsomedial region of the pericardial cavity and pharyngeal arch mesoderm (these regions are known as the secondary heart field or anterior heart field; blue in Fig. 1d, e; Kelly et al. 2001; Mjaatvedt et al. 2001; Waldo et al. 2001; Cai et al. 2003; Abu-Issa et al. 2004; Moreno-Rodriguez et al. 2006). The secondary/anterior heart field contains undifferentiated multi-potent heart cells and contributes to the elongation of the heart tube at both the arterial and venous poles by adding myocytes to the right ventricle and outflow tract as well as to the atrioventricular canal and atria (Buckingham et al. 2005). Recent work has shown that the secondary heart field surrounding the venous pole also gives rise to the dorsal part of the atrioventricular septum as well as the primary septum, called the spina vestibuli (Snarr et al. 2007).

### Signaling regulates heart specification and differentiation at the gastrula to neurula stages

Early heart development at the blastula to neurula stages has been investigated extensively using chick embryos, as the early chick embryo consists of disk-like germ layers

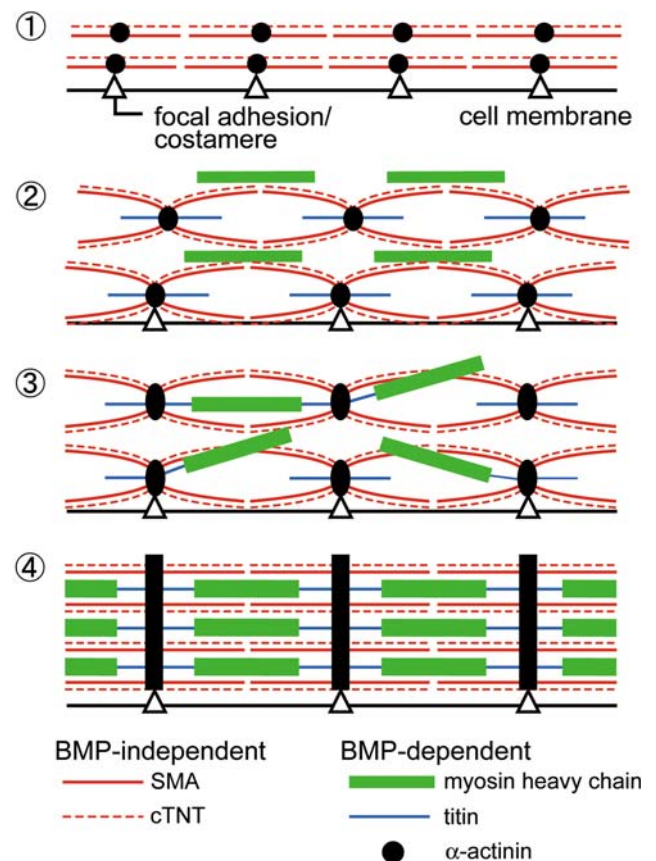
similar to those found in the human embryo and is easy material to work with. Furthermore, the whole-embryo culture model established by New [1955] and the tissue culture system using a serum-free defined medium are useful for analyzing cellular/tissue interactions regulated by growth factors as well as by the extracellular matrix (New 1955; Ladd et al. 1998; Matsui et al. 2006). Developmental biology studies have revealed that the interaction between the anterior lateral mesoderm and its adjacent endoderm is necessary to generate beating tissue from the excited anterior lateral mesoderm (Fig. 2). Therefore, an endoderm-emitting inductive signal(s) during gastrulation is required for the genesis of functional cardiomyocytes and the heart (Orts-Llorca 1963; Lough and Sugi 2000; Brand 2003). At the gastrula stages, the anterior endoderm subjacent to the anterior lateral plate mesoderm (containing prospective cardiomyocytes) expresses bone morphogenetic proteins (BMP)2 and -4 (Schultheiss et al. 1997). Loss-of-function and gain-of-function experiments have revealed that BMP2 and -4 are necessary—but not sufficient on their own—to induce the expression of both the



**Fig. 2** Signaling regulating early heart mesoderm/cardiomyogenesis. At the pregastrula stage, tissue interaction between the posterior epiblast and hypoblast, which is regulated by hypoblast-derived fibroblast growth factor (*FGF*)-8 and epiblast-derived Nodal, is necessary to generate the anterior lateral mesoderm (containing the heart-forming mesoderm) as well as its adjacent endoderm. FGF8 and Nodal, but neither factor alone, is capable of inducing the expression of Chordin to inhibit bone morphogenetic protein (*BMP*) activity (BMP has an antagonistic effect on cardiogenesis at the pregastrula stage). The heart mesoderm is induced to express SMA, Tbx5, and cTNT independently of BMP activity. During gastrulation, endoderm-emitted growth factors BMP2/4 and FGF4/8 are necessary for heart specification and subsequent terminal differentiation. Wnt/ $\beta$ -catenin signaling also involves cardiogenesis in a stage-dependent fashion (see Fig 4). *Right panels* Schematic representations of the dorsal view of the pregastrula and gastrula embryos, *left panels* sectional diagrams indicated by black lines seen in the corresponding right panel



heart-specific transcription factors Nkx2.5 and GATA4 and the cardiac contractile proteins sarcomeric  $\alpha$ -actinin and sarcomeric myosin (Schultheiss et al. 1995, 1997; Lough et al. 1996; Walters et al. 2001; Nakajima et al. 2002). In *Drosophila*, DPP, a member of the BMP family, induces the expression of the heart-specific *tinman* gene, a homologue of the vertebrate Nkx2.5 (Harvey 1997). BMP2-null mutant mice show abnormal heart development, ranging from the complete absence of a heart to an ectopic heart, and the BMP4-null or type I BMP receptor null-mutant dies before gastrulation (Mishina et al. 1995; Winnier et al. 1995; Zhang and Bradley 1996). Non-cardiogenic mesoderm from the early neurula embryo cultured in BMP2 together with fibroblast growth factor (FGF)-4 differentiates into beating tissue and expresses cardiac-specific genes (Lough et al. 1996). The anterior endoderm expresses FGF8 at the gastrula stage, and the ectopic administration of both BMP2/4 and FGF8 in non-cardiogenic mesoderm is able to induce the expression of Nkx2.5. Therefore, cardiogenesis appears to occur in the anterior lateral plate mesoderm, where both BMP and FGF signaling are present, indicating that BMP signaling cooperates with FGF signaling to regulate cardiogenesis during the gastrula stages (Fig. 2; Alsan and Schultheiss 2002). However, loss-of-function experiments using anti-FGF8 antibody, truncated FGF receptor, or SU5402 (FGFR1 antagonist) fails to suppress cardiogenesis in whole embryos at gastrula stages. The *Fgfr1*-null embryo stem cells also fail to differentiate into cardiomyocytes without affecting hematopoiesis, suggesting that FGF signaling at the blastula and/or early gastrula stage is necessary for heart mesoderm induction/cardiogenesis (Dell'era et al. 2003). After the precardiac mesoderm is committed to the heart lineage, terminal differentiation (expression of contractile proteins and generation of the sarcomeric apparatus) occurs. Loss-of-function experiments have shown that BMP2/4 are necessary to induce the expression of sarcomeric  $\alpha$ -actinin, titin, and sarcomeric myosin. However, the expression of smooth muscle  $\alpha$ -actin (SMA), which is first expressed in the precardiac mesoderm and recruited into the nascent myofibrils as an initial  $\alpha$ -actin, is not regulated by endoderm or BMP (Nakajima et al. 2002). Other experiments have shown that cardiac troponin T (cTNT), Tbx3, and Tbx5 are also expressed in the anterior lateral plate mesoderm and that their expression is not endoderm-dependent (Schlange et al. 2000; Yamada et al. 2000; Antin et al. 2002). These observations suggest that (1) endoderm-emitted BMP together with FGF regulates heart specification as well as terminal differentiation and that (2) in addition to endoderm-derived signaling, an additional regulatory pathway(s) appears to exist for the activation of cardiac genes (Fig. 2 and following section).



**Fig. 3** Schematic drawing of a model for myofibrillogenesis. ① The first step involves a stress fiber-like structure acting as a scaffold for myofibrillogenesis. Sarcomeric  $\alpha$ -actinin is associated with a nascent focal-adhesion/costamere that contains several focal-adhesion molecules, including vinculin, talin, and integrin  $\alpha 6\beta 1$ . ②–③ I-Z-I components (containing sarcomeric  $\alpha$ -actinin,  $\alpha$ -actin, and Z-line titin) and the A-band (thick filament consisting mainly of myosin) are assembled independently. ③–④ Sarcomeric  $\alpha$ -actinin components are aligned and A-bands are recruited into the I-Z-I components to later become involved with striated myofibrils. During heart myofibrillogenesis, endoderm-derived BMP is involved in the expression of sarcomeric myosin,  $\alpha$ -actinin, and titin, but smooth muscle  $\alpha$ -actin (SMA) and cardiac troponin T (cTNT) are BMP-independent

Soon after the cardiac specification is completed, the left and right precardiac regions migrate to the anterior midline and fuse to form the primitive heart tube. As the heart immediately starts to beat spontaneously, myofibrillogenesis occurs rapidly after the specification. The processes of de novo myofibrillogenesis are highly conserved in vertebrate striated muscle. Several models have been proposed to explain how myofibrillogenesis is conducted during cardiomyogenesis using fetal and neonatal cardiomyocyte culture models (Fig. 3). In one model, the first step involves a stress fiber-like structure acting as a scaffold; this is followed by the independent assembly of I-Z-I components (sarcomeric  $\alpha$ -actinin,  $\alpha$ -actin, and Z-line titin) and the A-band (thick filament consisting mainly of myosin), which subsequently become involved with striated

myofibrils (Holtzer et al. 1997). A different (but similar) model is the premyofibril model (Turnacioglu et al. 1997) in which the premyofibril, a primordium of the mature myofibril, consists of mini-sarcomeres containing thin filaments,  $\alpha$ -actinin, and non-muscle myosin IIb. The distance between the dense materials of the sarcomeric  $\alpha$ -actinin initially increases, thereafter the N-terminal region of titin is incorporated into the Z-band of the nascent myofibril, and non-muscle myosin is replaced by muscle-specific myosin. During this process, nebulin, a ruler protein, plays a role in the determination of the thin filament's length (McElhinny et al. 2005; Bang et al. 2006). The myosin light chain(s) as well as the M-line epitope of titin is essential for thick-filament assembly and myofibrillogenesis (Xu et al. 2002; Rottbauer et al. 2006). The structural linkage between the cytoskeletal architecture, sarcomeres, and the cell-substratum adhesion apparatus, costameres, is important for the generation of striated cardiomyocyte. During heart myofibrillogenesis, endoderm-derived BMP is required for the expression of sarcomeric proteins, such as sarcomeric  $\alpha$ -actinin, titin, troponin C, myosin light chain, and the sarcomeric myosin heavy chain (Walters et al. 2001; Nakajima et al. 2002), while the expression of  $\alpha$ -actin (initially smooth muscle  $\alpha$ -actin) and cTNT are BMP-independent (Fig. 3). An intracellular signaling pathway, the Rho–Rock pathway, is also important in the regulation of myofibrillogenesis via actin–myosin assembly, focal adhesion/costamere, and N-cadherin-based cell–cell adhesion (Sakata et al. 2007). However, the mechanisms that regulate the genesis of accurate striation as well as those that mediate the differentiation of specific cardiomyocytes (conduction myocytes) are still unclear.

### Signaling regulating heart mesoderm formation at the blastula stage

Numerous studies have detailed in the role of BMP and FGF signals provided by the anterior lateral endoderm in the heart specification and its terminal differentiation (Fig. 2; see the review of Lough and Sugi 2000; Brand 2003). The specification of premyocardial cells to the heart lineage occurs while the cells are within the streak and during their migration to the anterior lateral mesoderm (precardiac mesoderm). On the other hand, BMP2 or BMP4 inhibits cardiac myogenesis before stage 3 (early gastrula stage), indicating multiple or opposing roles for BMP in heart mesoderm/cardiomyocyte induction in a stage-dependent fashion (Fig. 2; Ladd et al. 1998). Explantation experiments using chick blastoderm explants showed that signaling from the hypoblast is required to induce cardiac myogenesis in the early epiblast and that the

hypoblast-derived signal(s) appears to act upstream of the heart-inducing signals emitted from the anterior lateral endoderm (Yatskievych et al. 1997). Several genes expressed in the anterior lateral mesoderm, such as those for SMA, cTNT, and Tbx5, are regulated independently of endoderm-emitted signaling, including BMP (Schlange et al. 2000; Yamada et al. 2000; Antin et al. 2002; Nakajima et al. 2002). Genetic studies in the mouse and zebrafish have shown that transforming growth factor beta (TGF $\beta$ ) signals of the Nodal family are essential for the formation of mesoderm in vertebrates (Schier and Shen 2000). Mouse *nodal* mutants lack a PS and most of the mesoderm (Conlon et al. 1994). In chick blastoderm, loss-of-function and gain-of-function experiments have revealed that Nodal, which is expressed in the posterior epiblast, plays a role in the expression of BMP-independent heart mesoderm genes (including SMA, Tbx5, and cTNT) as well as heart mesoderm formation/cardiomyogenesis. However, Nodal itself fails to induce the expression of SMA, while Nodal in association with a BMP-antagonist, such as Chordin, Noggin, or Follistatin, is capable of inducing the expression of SMA (Matsui et al. 2005). Therefore, not only Nodal but also anti-BMP activity at the pregastrula stage is necessary for heart mesoderm induction/cardiogenesis (Fig. 2).

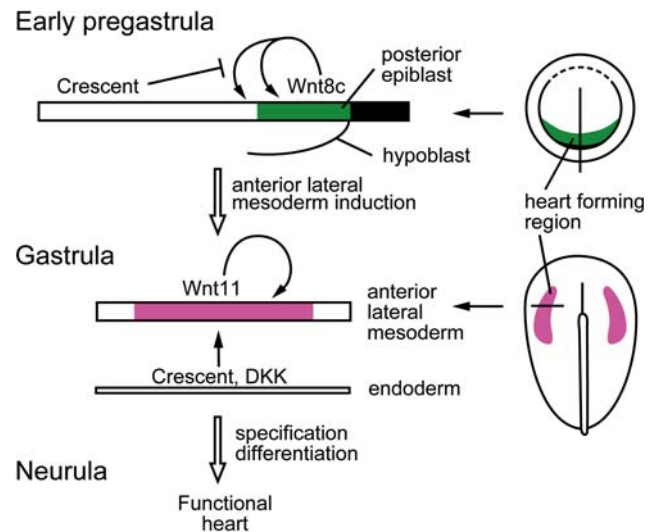
Fibroblast growth factor has been reported to have a potent inductive signaling role by inducing mesoderm induction in the *Xenopus* animal cap (Kimelman and Kirschner 1987; Slack et al. 1987). Experiments by Cornell and Kimelman (1994) using a dominant negative form of the type I FGF receptor revealed that the FGF signal is required for mesoderm induction by Activin. In mouse, nascent mesoderm of the *fgfr1*-mutant differentiates into the mesoderm subtype without patterning, suggesting that FGFR1 transduces signals which specify correct mesoderm cell fates and proper mesoderm patterning during gastrulation (Deng et al. 1994; Yamaguchi et al. 1994; Ciruna et al. 1997). *Fgfr1* mutant embryos fail to express *Snail* and to downregulate the expression of *E-cadherin*, suggesting that FGF signaling downregulates the expression of *E-cadherin* through *Snail* to promote epithelial–mesenchymal transition during gastrulation (Ciruna and Rossant 2001). Sun et al. (1999) demonstrated that targeted disruption of mouse *Fgf8* resulted in the failure of epiblast cells to migrate away from the PS, thereby impairing the formation of the mesoderm and endoderm. Therefore, FGF signaling affects mesoderm specification as well morphogenetic cell movements during gastrulation. Vertebrate gastrulation requires the orchestration of mesoderm specification with morphogenetic movements, and both of these processes require FGF signaling. During FGF-dependent gastrulation, Sprouty (receptor tyrosine kinase inhibitor) inhibits morphogenesis/pattern formation and Ca<sup>2+</sup> and PKC $\delta$  signaling, leaving

MAPK activation and mesoderm specification intact, while Spreads inhibits MAPK activation and mesoderm specification, with little effect on  $\text{Ca}^{2+}$  or  $\text{PKC}\delta$  signaling, leaving morphogenetic movement intact (Sivak et al. 2005). It is also reported that p38 MAP kinase and a p38-interacting protein are critical for the downregulation of E-cadherin expression during gastrulation (Zohn et al. 2006).

In early chick embryos, FGF8 is expressed in the posterior hypoblast before gastrulation and in the anterior PS after gastrulation; therefore, expression is restricted to the regions where prospective heart cells reside. In chick blastoderm culture, anti-FGF8 properties were found to inhibit the expression of BMP-antagonist as well as BMP-independent heart mesoderm genes (SMA, Tbx5, and cTNT), but not the pan-mesoderm marker Brachyury (Matsui et al. 2008). Gain-of-function experiments show that FGF8 and Nodal, but neither factor alone, induce cardiomyogenesis in the non-cardiogenic anterior epiblast. Therefore, FGF8 acts synergistically with Nodal on the posterior epiblast to induce the expression of the BMP antagonist Chordin and to specifically induce the expression of endoderm-independent heart mesoderm genes, thereby inducing heart mesoderm/cardiomyogenesis (Fig. 2). The results suggest that a two-step signaling cascade regulates cardiomyogenesis in a stage-dependent fashion: hypoblast-derived FGF8 plus epiblast-derived Nodal act on the posterior epiblast at the blastula stage for heart mesoderm induction, and endoderm-derived BMP2/4 plus FGF4/8 act on the anterior lateral mesoderm for heart specification and terminal differentiation (Fig. 2).

### Wnt signaling early in cardiogenesis

Wnts are a family of secreted signaling proteins encoded by 19 distinct genes in the vertebrate genome. They initiate several signal transduction pathways, including the canonical Wnt/ $\beta$ -catenin, Wnt/ $\text{Ca}^{2+}$ , and the Wnt/planar cell polarity pathways (Logan and Nusse 2004; Moon et al. 2004; Reya and Clevers 2005). There are two Wnt groups among which a divergence of functional activity is evident (Foley et al. 2006; Eisenberg and Eisenberg 2007; Tzahor 2007). The Wnt1 group (Wnt1, -3a, and -8) transmits signals exclusively via the canonical Wnt/ $\beta$ -catenin pathway (Logan and Nusse 2004). In contrast, the Wnt5a group (Wnt4a, -5a, and -11) has more complex signaling properties. The Wnt5a subgroup does not normally trigger canonical Wnt/ $\beta$ -catenin signaling and, therefore, acts as dominant-negative inhibitors of Wnt1 class factors (Topol et al. 2003; Weidinger and Moon 2003; Maye et al. 2004). There are two distinct soluble extracellular factors that can inhibit canonical Wnt/ $\beta$ -catenin signaling. Secreted frizzled-related proteins (sFRPs and Crescent) have a



**Fig. 4** Distinct and biphasic roles mediated by Wnt/ $\beta$ -catenin regulate cardiogenesis in a stage-dependent fashion. Before gastrulation, the canonical Wnt/ $\beta$ -catenin pathway (*Wnt8c*) regulates the induction of anterior lateral mesoderm/cardiac progenitor cells. At this stage, Crescent (canonical Wnt/ $\beta$ -catenin antagonist) is expressed in the anterior region of the epiblast, therefore resulting in predominant Wnt signaling in the posterior epiblast, in which prospective heart cells reside. At the gastrula stages, Wnt/ $\beta$ -catenin signaling acts on the anterior lateral mesoderm to suppress cardiogenesis. Therefore, inhibition of Wnt/ $\beta$ -catenin signaling by Crescent, Dickkopf (*DKK*), and Wnt11 is necessary for heart specification and terminal differentiation. *Right panels* Schematic representations of the dorsal view of the pregastrula and gastrula embryos, *left panels* sectional diagrams indicated by black lines seen in the corresponding *right panels*

Wnt-binding domain that is conserved among sFRP and the frizzled cell membrane receptor protein. Thus, sFRP affects canonical Wnt/ $\beta$ -catenin signaling by competitively inhibiting the binding of Wnts to their cell membrane receptor, frizzled (Rattner et al. 1997). Dickkopf (*DKK*) binds to the cell membrane receptors LRP5 and LRP6 and competitively inhibits canonical Wnt activity (Kawano and Kypta 2003). Intracellular signaling cascades regulated by canonical and non-canonical Wnt pathways have been reviewed elsewhere (Logan and Nusse 2004; Gordon and Nusse 2006; Eisenberg and Eisenberg 2007). The latest information on Wnt is available on the Wnt Home page (<http://www.stanford.edu/~rnusse/wntwindow.html>).

There are three distinct antagonists of the canonical Wnt/ $\beta$ -catenin pathway that act as a mediator of cardiogenesis at the gastrula stages—*DKK1*, Crescent, and Wnt11 (Fig. 4; Eisenberg and Eisenberg 1999; Marvin et al. 2001; Schneider and Mercola 2001; Pandur et al. 2002). In chick gastrula, Crescent (Frizzled-related protein) is expressed in the anterior endoderm just beneath the heart mesoderm. Inhibition of the canonical Wnt pathway synergistically acts with BMP and FGF to promote heart formation in the anterior lateral mesoderm, whereas canonical Wnt signaling



in the posterior lateral mesoderm induces hematopoiesis (Marvin et al. 2001). Another experiment showed that Wnt11, which is expressed in the anterior lateral mesoderm, is capable of inducing cardiac differentiation in the non-cardiogenic posterior mesoderm (Eisenberg et al. 1997; Eisenberg and Eisenberg 1999). In *Xenopus*, cardiogenesis in mesoderm depends on Cerberus (mediated by nodal), which functions parallel to homeodomain protein Hex mediated by Dkk1. Therefore, both pathways in the endoderm appear to initiate cardiogenesis in the overlying mesoderm (Foley et al. 2007). Interestingly, mice lacking Hex showed a number of congenital heart defects, including hypoplasia of the right ventricle, ventricular septal defects, outflow tract abnormalities, atrio-ventricular (AV) valve dysplasia, and aberrant development of the compact myocardium (Hallaq et al. 2004).

The above descriptions indicate that the Wnt/ $\beta$ -catenin pathway negatively regulates cardiac differentiation (Marvin et al. 2001; Eisenberg and Eisenberg 2007; Foley et al. 2007); however, studies with embryonic carcinoma cells suggest that the canonical Wnt pathway, Wnt3A and -8A, promotes cardiogenesis (Nakamura et al. 2003). In zebrafish embryos, Wnt/ $\beta$ -catenin signaling before gastrulation promotes cardiac differentiation, whereas signaling during gastrulation inhibits heart formation (Ueno et al. 2007). In an in vitro system comprising mouse embryonic stem (ES) cells, activation of the Wnt/ $\beta$ -catenin pathway early during the embryoid body formation was seen to enhance the differentiation of ES cells into cardiomyocytes, whereas Wnt/ $\beta$ -catenin signaling in the late phase after embryoid body had formed inhibited the differentiation and enhanced the hematopoiesis (Naito et al. 2006). In vivo, a tissue-specific manipulation strategy showed that  $\beta$ -catenin is required for the development and proliferative expansion of cardiac progenitor cells (Kwon et al. 2007). Furthermore, Wnt/ $\beta$ -catenin signaling is required for *isll* expression in cardiac progenitors and is also necessary upstream of genes regulating the secondary/anterior heart field (Ai et al. 2007; Lin et al. 2007). Current views suggest that Wnt/ $\beta$ -catenin plays biphasic and distinct—even opposing—roles in cardiogenesis depending on the stage (Fig. 4): before gastrulation, the canonical Wnt/ $\beta$ -catenin pathway regulates positively the induction of anterior lateral mesoderm/cardiac progenitor cells and subsequently acts on the precardiic mesoderm to regulate negatively the cardiac specification and differentiation during gastrulation.

### Micro-RNA in cardiogenesis

MicroRNAs (miRNAs) are endogenous non-coding RNAs that mediate post-transcriptional gene silencing and control protein expression in various cellular pathways (Ambros

2004). More than 400 mammalian miRNAs are transcribed in the nucleus and subsequently processed to ultimately yield mature miRNA of approximately 20–22 nucleotides by the enzymes Drosha and Dicer (Berezikov et al. 2006). Mature miRNAs bind to target mRNAs by partial sequence matching in the 3′ untranslated region of target mRNAs, and the complex is then incorporated into the RNA-induced silencing complex (RISC), resulting in degradation of the mRNA transcript and/or translational inhibition (Kloosterman and Plasterk 2006). It has been reported that miRNAs are involved in the regulation of development as well as oncogenesis because of their tissue-specific or developmental stage-dependent expression and their evolutionary conservation (Alvarez-Garcia and Miska 2005). However, the in vivo requirement for specific miRNA in mammalian organogenesis, including that of the heart, is largely unknown. Several recent reports have described a role for miRNAs in the regulation of in vivo cardiac functions, such as the conduction of electrical signals, heart muscle contraction, heart growth and morphogenesis (Chien 2007; Mishima et al. 2007). Zhao et al. (2007) reported that mice lacking the miRNA processing enzyme Dicer in the heart show cardiac failure associated with pericardial effusion and a thin-walled myocardium. These phenotypes are consistent with the defects observed in zebrafish embryonic heart lacking a functional Dicer (Giraldez et al. 2005). Two members of the miR-1 class of miRNA, miR-1-1 and miR-1-2, are specifically expressed in the developing mouse heart. Mice lacking miR-1-2 have a spectrum of cardiac abnormalities, including ventricular septal defect, cardiac rhythm disturbances and cardiomyocyte cell-cycle abnormality that leads to hyperplasia of the heart (Zhao et al. 2007). Yang et al. (2007) reported that overexpression of miR-1 in the hearts of mice can induce cardiac arrhythmia, indicating a crucial role in normal cardiac conduction. In vivo inhibition of miR-133 causes marked and sustained cardiac hypertrophy (Car et al. 2007). In the mouse heart, a cardiac-specific miR-208 encoded by an intron of the  $\alpha$ -myosin heavy chain gene is required for cardiomyocyte hypertrophy and the expression of the  $\beta$ -myosin heavy chain during stress as well as thyroid hormone-induced cardiac growth (van Rooij et al. 2007). These observations suggest that a subset of miRNAs can modulate a diverse spectrum of cardiac functions and morphogenesis in vivo. Although miRNAs appear to control cardiac muscle cell lineages, such as ventricular muscle, isoforms of myosin, and conduction myocardium, the roles of miRNAs during early cardiogenesis remain uncertain.

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## References

- Abu-Issa R, Waldo K, Kirby ML (2004) Heart fields: one, two or more? *Dev Biol* 272:281–285
- Ai D, Fu X, Wang J, Lu MF, Chen L, Baldini A, Klein WH, Martin JF (2007) Canonical Wnt signaling functions in second heart field to promote right ventricular growth. *Proc Natl Acad Sci USA* 104:9319–9324
- Alsan BH, Schultheiss TM (2002) Regulation of avian cardiogenesis by Fgf8 signaling. *Development* 129:1935–1943
- Alvarez-Garcia I, Miska EA (2005) MicroRNA functions in animal development and human disease. *Development* 132:4653–4662
- Ambros V (2004) The functions of animal microRNAs. *Nature* 431:350–355
- Antin PB, Bales MA, Zhang W, Garriock RJ, Yatskievych TA, Bates MA (2002) Precocious expression of cardiac troponin T in early chick embryos is independent of bone morphogenetic protein signaling. *Dev Dyn* 225:135–141
- Bang ML, Li X, Littlefield R, Bremner S, Thor A, Knowlton KU, Lieber RL, Chen J (2006) Nebulin-deficient mice exhibit shorter thin filament lengths and reduced contractile function in skeletal muscle. *J Cell Biol* 173:905–916
- Berezikov E, Thuemmler F, van Laake LW, Kondova I, Bontrop R, Cuppen E, Plasterk RHA (2006) Diversity of microRNAs in human and chimpanzee brain. *Nat Genet* 38:1375–1377
- Brand T (2003) Heart development: molecular insights into cardiac specification and early morphogenesis. *Dev Biol* 258:1–19
- Buckingham M, Meilhac S, Zaffran S (2005) Building the mammalian heart from two sources of myocardial cells. *Nat Rev Genet* 6:826–835
- Cai CL, Liang X, Shi Y, Chu PH, Pfaff SL, Chen J, Evans S (2003) Isl1 identifies a cardiac progenitor population that proliferates prior to differentiation and contributes a majority of cells to the heart. *Dev Cell* 5:877–889
- Car A, Catalucci D, Felicetti F, Bonci D, Addario A, Gallo P, Bang ML, Segnalini P, Gu Y, Dalton ND, Elia L, Latronico MVG, Høydal M, Autore C, Russo MA GW, Dorn II, Ellingsen Ø, Ruiz-Lozano P, Peterson KL, Croce CM, Peschle C, Condorelli G (2007) MicroRNA-133 controls cardiac hypertrophy. *Nat Med* 13:613–618
- Chien KR (2007) Molecular medicine: MicroRNAs and the tell-tale heart. *Nature* 447:389–390
- Ciruna B, Rossant J (2001) FGF signaling regulates mesoderm cell fate specification and morphogenetic movement at the primitive streak. *Dev Cell* 1:37–49
- Ciruna BG, Schwartz L, Harpal K, Yamaguchi TP, Rossant J (1997) Chimeric analysis of fibroblast growth factor receptor-1 (Fgfr1) function: a role for FGFR1 in morphogenetic movement through the primitive streak. *Development* 124:2829–2841
- Conlon FL, Lyons KM, Takaesu N, Barth KS, Kispert A, Herrmann B, Robertson EJ (1994) A primary requirement for nodal in the formation and maintenance of the primitive streak in the mouse. *Development* 120:1919–1928
- Cornell RA, Kimelman D (1994) Activin-mediated mesoderm induction requires FGF. *Development* 120:435–462
- Dell'Era P, Ronca R, Coco L, Nicoli S, Metra M, Presta M (2003) Fibroblast growth factor receptor-1 is essential for in vitro cardiomyocyte development. *Circ Res* 93:414–420
- Deng CX, Wynshaw-Boris A, Shen MM, Daugherty C, Ornitz DM, Leder P (1994) Murine FGFR-1 is required for early postimplantation growth and axial organization. *Genes Dev* 8:3045–3057
- Eisenberg CA, Eisenberg LM (1999) WNT11 promotes cardiac tissue formation of early mesoderm. *Dev Dyn* 216:45–58
- Eisenberg LM, Eisenberg CA (2007) Evaluating the role of Wnt signal transduction in promoting the development of the heart. *TWS Dev Embryol (Scientific World Journal)* 7:161–176
- Eisenberg CA, Gourdie RG, Eisenberg LM (1997) Wnt-11 is expressed in early avian mesoderm and required for the differentiation of the quail mesoderm cell line QCE-6. *Development* 124:525–536
- Eyal-Giladi H, Kochav S (1976) From cleavage to primitive streak formation: a complementary normal table and a new look at the first stages of the development of the chick. I. General morphology. *Dev Biol* 49:321–337
- Foley AC, Gupta RW, Guzzo RM, Korol O, Mercola M (2006) Embryonic heart induction. *Ann NY Acad Sci* 1080:85–96
- Foley AC, Korol O, Timmer AM, Mercola M (2007) Multiple functions of Cerberus cooperate to induce heart downstream of Nodal. *Dev Biol* 303:57–65
- Garcia-Martinez V, Schoenwolf G (1993) Primitive-streak origin of the cardiovascular system in avian embryos. *Dev Biol* 159:706–719
- Giraldez AJ, Cinali RM, Glasner ME, Enright AJ, Thomson JM, Baskerville S, Hammond SM, Bartel DP, Schier AF (2005) MicroRNAs regulate brain morphogenesis in zebrafish. *Science* 308:833–838
- Gordon MD, Nusse R (2006) Wnt signaling: multiple pathways, multiple receptors, and multiple transcription factor. *J Biol Chem* 281:22429–22433
- Hallaq H, Pinter E, Enciso J, McGrath J, Zeiss C, Brueckner M, Madri J, Jacobs HC, Wilson CM, Vasavada H, Jiang X, Bogue CW (2004) A null mutation of Hhex results in abnormal cardiac development, defective vasculogenesis and elevated Vegfa levels. *Development* 131:5197–5209
- Hamburger V, Hamilton HL (1992) A series of normal stages in the development of the chick embryo. *Dev Dyn* 195:231–272
- Harvey RP (1997) NK-2 homeobox genes and heart development. *Dev Biol* 178:203–216
- Hatada Y, Stern CD (1994) A fate map of the epiblast of the early chick embryo. *Development* 120:2879–2899
- Hiruma T, Hirakow R (1985) An ultrastructural topographical study on myofibrillogenesis in the heart of the chick embryo during pulsation onset period. *Anat Embryol* 172:325–329
- Holtzer H, Hijikata T, Lin ZX, Zhang ZQ, Holtzer S, Protasi F, Franzini-Armstrong C, Sweeney HL (1997) Independent assembly of 1.6 µm long bipolar MHC filaments and I-Z-I bodies. *Cell Struct Funct* 22:83–93
- Kawano Y, Kypta R (2003) Secreted antagonists of the Wnt signalling pathway. *J Cell Sci* 116:2627–2634
- Kelly R, Brown N, Buckingham M (2001) The arterial pole of the mouse heart forms from *Fgf10*-expressing cells in pharyngeal mesoderm. *Dev Cell* 1:435–440
- Kimelman D, Kirschner M (1987) Synergistic induction of mesoderm by FGF and TGF-β and the identification of an mRNA coding for FGF in the early *Xenopus* embryo. *Cell* 51:869–877
- Kloosterman WP, Plasterk RHA (2006) The diverse functions of microRNAs in animal development and disease. *Dev Cell* 11:441–450
- Kwon C, Arnold J, Hsiao EC, Taketo MM, Conklin BR, Srivastava D (2007) Canonical Wnt signaling is a positive regulator of mammalian cardiac progenitors. *Proc Natl Acad Sci USA* 104:10894–10899
- Ladd AN, Yatskievych TA, Antin PB (1998) Regulation of avian cardiac myogenesis by Activin/TGFβ and bone morphogenetic proteins. *Dev Biol* 204:407–419



- Lin L, Cui L, Zhou W, Dufort D, Zhang X, Cai CL, Bu L, Yang L, Martin J, Kemler R, Rosenfeld MG, Chen J, Evans SM (2007)  $\beta$ -Catenin directly regulates *Islet1* expression in cardiovascular progenitors and is required for multiple aspects of cardiogenesis. *Proc Natl Acad Sci USA* 104:9313–9318
- Linask KK, Lash JW (1993) Early heart development: dynamics of endothelial cell sorting suggests a common origin with cardiomyocytes. *Dev Dyn* 195:62–69
- Linask KK, Knudsen KA, Gui YH (1997) N-Cadherin-catenin interactions: necessary component of early heart development and cardiac myofibrillogenesis. *Dev Biol* 185:148–164
- Logan CY, Nusse R (2004) The Wnt signaling pathway in development and disease. *Annu Rev Cell Dev Biol* 20:781–810
- Lough J, Sugi Y (2000) Endoderm and heart development. *Dev Dyn* 217:327–342
- Lough J, Barron M, Brogley M, Sugi Y, Bolender DL, Zhu X (1996) Combined BMP-2 and FGF-4, but neither factor alone, induces cardiogenesis in non-precardiac embryonic mesoderm. *Dev Biol* 178:198–202
- Marvin MJ, Rocco GD, Gardiner A, Bush SM, Lassar AB (2001) Inhibition of Wnt activity induces heart formation from posterior mesoderm. *Genes Dev* 15:316–327
- Matsui H, Ikeda K, Nakatani K, Sakabe M, Yamagishi T, Nakanishi T, Nakajima Y (2005) Induction of initial cardiomyocyte  $\alpha$ -actin—smooth muscle  $\alpha$ -actin—in cultured avian pregastrula epiblast: a role for Nodal and BMP antagonist. *Dev Dyn* 233:1419–1429
- Matsui H, Sakabe M, Sakata H, Nakatani K, Ikeda K, Fukui M, Ando K, Yamagishi T, Nakajima Y (2006) Heart myofibrillogenesis occurs in isolated chick posterior blastoderm: a culture model. *Acta Histochem Cytochem* 39:139–144
- Matsui H, Sakabe M, Sakata H, Yanagawa N, Ikeda K, Yamagishi T, Nakajima Y (2008) Induction of initial heart  $\alpha$ -actin, smooth muscle  $\alpha$ -actin, in chick pregastrula epiblast: the role of hypoblast and FGF8. *Dev Growth Differ* 50:143–157
- Maye P, Zheng J, Li L, Wu D (2004) Multiple mechanisms for Wnt11-mediated repression of the canonical Wnt signaling pathway. *J Biol Chem* 279:24659–24665
- McElhinny AS, Schwach C, Valichnac M, Mount-Patrick S, Gregorio CC (2005) Nebulin regulates the assembly and lengths of the thin filaments in striated muscle. *J Cell Biol* 170:947–957
- Mishima Y, Stahlhut C, Giraldez AJ (2007) Mir-1–2 gets to the heart of the matter. *Cell* 129:247–249
- Mishina Y, Suzuki A, Ueno N, Behringer RR (1995) *Bmpr* encodes a type I bone morphogenetic protein receptor that is essential for gastrulation during mouse embryogenesis. *Genes Dev* 9:3027–3037
- Mjaatvedt CH, Nakaoka T, Moreno-Rodriguez R, Norris RA, Kern MJ, Eisenberg CA, Turner D, Markwald RR (2001) The outflow tract of the heart is recruited from a novel heart-forming field. *Dev Biol* 238:97–109
- Moon RT, Kohn AD, De Ferrari GV, Kaykas A (2004) WNT and  $\beta$ -catenin signalling: diseases and therapies. *Nat Rev Genet* 5:691–701
- Moreno-Rodriguez RA, Krug EL, Reyes L, Villavicencio L, Mjaatvedt CH, Markwald RR (2006) Bidirectional fusion of the heart-forming fields in the developing chick embryo. *Dev Dyn* 235:191–202
- Naito AT, Shiojima I, Akazawa H, Hidaka K, Morisaki T, Kikuchi A, Komuro I (2006) Developmental stage-specific biphasic roles of Wnt/ $\beta$ -catenin signaling in cardiomyogenesis and hematopoiesis. *Proc Natl Acad Sci USA* 103:19812–19817
- Nakajima Y, Yamagishi T, Ando K, Nakamura H (2002) Significance of bone morphogenetic protein-4 function in the initial myofibrillogenesis of chick cardiogenesis. *Dev Biol* 245:291–303
- Nakamura T, Sano M, Songyang Z, Schneider MD (2003) A Wnt- and  $\beta$ -catenin-dependent pathway for mammalian cardiac myogenesis. *Proc Natl Acad Sci USA* 100:5834–5839
- New DAT (1955) A new technique for the cultivation of the chick embryo in vitro. *J Embryol Exp Morphol* 3:326–331
- Orts-Llorca F (1963) Influence of the endoderm on heart differentiation during the early stages of development of the chick embryo. *Wilhelm Roux Arch Entw Mech Org* 154:533–551
- Pandur P, Läsche M, Eisenberg LM, Kühl M (2002) Wnt-11 activation of a non-canonical Wnt signalling pathway is required for cardiogenesis. *Nature* 418:636–641
- Rattner A, Hsieh JC, Smallwood PM, Gilbert DJ, Copeland NG, Jenkins NA, Nathans J (1997) A family of secreted proteins contains homology to the cysteine-rich ligand-binding domain of frizzled receptors. *Proc Natl Acad Sci USA* 94:2859–2863
- Redkar A, Montgomery M, Litvin J (2001) Fate map of early avian cardiac progenitor cells. *Development* 128:2269–2279
- Reya T, Clevers H (2005) Wnt signalling in stem cells and cancer. *Nature* 434:843–850
- Rosenquist GC, DeHaan RL (1966) Migration of precardiac cells in the chick embryo: a radioautographic study. *Contribut Embryol* 38:111–121
- Rottbauer W, Wessels G, Dahme T, Just S, Trano N, Hassel D, Burns CG, Katus HA, Fishman MC (2006) Cardiac myosin light chain-2: a novel essential component of thick-myofilament assembly and contractility of the heart. *Circ Res* 99:323–331
- Sakata H, Sakabe M, Matsui H, Kawada N, Nakatani K, Ikeda K, Yamagishi T, Nakajima Y (2007) Rho kinase inhibitor Y27632 affects initial heart myofibrillogenesis in cultured chick blastoderm. *Dev Dyn* 236:461–472
- Schier AF, Shen MM (2000) Nodal signalling in vertebrate development. *Nature* 403:385–389
- Schlangé T, André B, Arnold HH, Brand T (2000) BMP2 is required for early heart development during a distinct time period. *Mech Dev* 91:259–270
- Schneider VA, Mercola M (2001) Wnt antagonism initiates cardiogenesis in *Xenopus laevis*. *Genes Dev* 15:304–315
- Schultheiss TM, Xydias S, Lassar AB (1995) Induction of avian cardiac myogenesis by anterior endoderm. *Development* 121:4203–4214
- Schultheiss TM, Burch JBE, Lassar AB (1997) A role for bone morphogenetic proteins in the induction of cardiac myogenesis. *Genes Dev* 11:451–462
- Sivak JM, Petersen LF, Amaya E (2005) FGF signal interpretation is directed by Sprouty and Spred proteins during mesoderm formation. *Dev Cell* 8:689–701
- Slack JMW, Darlington BG, Heath JK, Godsave SF (1987) Mesoderm induction in early *Xenopus* embryos by heparin-binding growth factors. *Nature* 326:197–200
- Snarr BS, O'Neal JL, Chintalapudi MR, Wirrig EE, Phelps AL, Kubalak SW, Wessels A (2007) *Isl1* expression at the venous pole identifies a novel role for the second heart field in cardiac development. *Circ Res* 101:971–974
- Stern CD (2004) Gastrulation in the chick. In: *Gastrulation: from cell to embryo*. Stern CD (ed) Cold Spring Harbor Laboratory Press, New York, pp 219–232
- Sun X, Meyers EN, Lewandoski M, Martin GR (1999) Targeted disruption of *Fgf8* causes failure of cell migration in the gastrulating mouse embryo. *Genes Dev* 13:1834–1846
- Topol L, Jiang X, Choi H, Garrett-Beal L, Carolan PJ, Yang Y (2003) Wnt-5a inhibits the canonical Wnt pathway by promoting GSK-3-independent  $\beta$ -catenin degradation. *J Cell Biol* 162:899–908
- Turnacioglu KK, Mittal B, Dabiri GA, Sanger JM, Sanger JW (1997) Zeugmatin is part of the Z-band targeting region of titin. *Cell Struct Funct* 22:73–82

- Tzahor E (2007) Wnt/ $\beta$ -catenin signaling and cardiogenesis: timing does matter. *Dev Cell* 13:10–13
- Ueno S, Gilbert Weidinger G, Osugi T, Kohn AD, Golob JL, Pabon L, Reinecke H, Moon RT, Murry CE (2007) Biphasic role for Wnt/ $\beta$ -catenin signaling in cardiac specification in zebrafish and embryonic stem cells. *Proc Natl Acad Sci USA* 104:9685–9690
- van Rooij E, Sutherland LB, Qi X, Richardson JA, Hill J, Olson EN (2007) Control of stress-dependent cardiac growth and gene expression by a microRNA. *Science* 316:575–579
- Waldo KL, Kumiski DH, Wallis KT, Stadt HA, Hutson MR, Platt DH, Kirby ML (2001) Conotruncal myocardium arises from a secondary heart field. *Development* 128:3179–3188
- Walters MJ, Wayman GA, Christian JL (2001) 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
- Weidinger G, Moon RT (2003) When Wnts antagonize Wnts. *J Cell Biol* 162:753–755
- Winnier G, Blessing M, Labosky PA, Hogan BL (1995) Bone morphogenetic protein-4 is required for mesoderm formation and patterning in the mouse. *Genes Dev* 9:2105–2116
- Xu X, Meiler SE, Zhong TP, Mohideen M, Crossley DA, Burggren WW, Fishman MC (2002) Cardiomyopathy in zebrafish due to mutation in an alternatively spliced exon of *titin*. *Nat Genet* 30:205–209
- Yamada M, Revelli JP, Eichele G, Barron M, Schwartz RJ (2000) Expression of chick *Tbx-2*, *Tbx-3*, and *Tbx-5* genes during early heart development: evidence for BMP2 induction of *Tbx2*. *Dev Biol* 228:95–105
- Yamaguchi TP, Harpal K, Henkemeyer M, Rossant J (1994) fgfr-1 Is required for embryonic growth and mesodermal patterning during mouse gastrulation. *Gene Dev* 8:3032–3044
- Yang B, Lin H, Xiao J, Lu Y, Luo X, Li B, Zhang Y, Xu C, Bai Y, Wang H, Chen G, Wang Z (2007) The muscle-specific microRNA *Mir-1* regulates cardiac arrhythmogenic potential by targeting *GJA1* and *KCNJ2*. *Nat Med* 13:486–491
- Yatskievych TA, Ladd AN, Antin PB (1997) Induction of cardiac myogenesis in avian pregastrula epiblast: the role of the hypoblast and activin. *Development* 124:2561–2570
- Zhang H, Bradley A (1996) Mice deficient for BMP2 are nonviable and have defects in amnion/chorion and cardiac development. *Development* 122:2977–2986
- Zhao Y, Ransom JF, Li A, Vedantham V, von Drehle M, Muth AN, Tsuchihashi T, McManus MT, Schwartz RJ, Srivastava D (2007) Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking miRNA-1-2. *Cell* 129:303–317
- Zohn IE, Li Y, Skolnik EY, Anderson KV, Han J, Niswander L (2006) P38 and a p38-Interacting protein are critical for downregulation of E-cadherin during mouse gastrulation. *Cell* 125:957–969