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REVIEW ARTICLE

Heart Development in *Drosophila* and Vertebrates: Conservation of Molecular Mechanisms

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ABSTRACT Vertebrate and insect (*Drosophila*) hearts look and function quite differently from each other. Nevertheless, during embryogenesis their mesodermal origin and initial assembly into a linear heart tube are comparable in many respects. In the past few years, numerous gene functions have been identified that are utilized by both vertebrates and *Drosophila* for the specification and differentiation of the heart progenitor cells. These studies have begun with the discovery of the homeobox gene tinman in *Drosophila* and its vertebrate counterparts. By now, there is also evidence that MEF2 transcription factors and TGF-β signaling have cardiogenic functions in both these systems. Perhaps in a few years, the GATA and HAND transcription factors and Wnt signaling, which currently only have a demonstrated cardiogenic function in one of the systems, may also be part of this group. One of the pressing but still wide open questions is if the spectrum of targets for these transcription factors and signaling pathways is also conserved. Dev. Genet. 22:181–186, 1998. © 1998 Wiley-Liss, Inc.

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INTRODUCTION

It is becoming increasingly evident that, despite the variety in morphological differences among vertebrate and invertebrate species, many of the molecular mechanisms that orchestrate individual developmental processes are remarkably conserved. For example, the discovery of the hox genes over ten years ago has revolutionized the developmental biology field by providing first insights into the common way by which regional identities along a body axis are specified in both vertebrates and invertebrates (e.g. Krumlauf, 1994). Since then many other gene products implicated in pattern formation or the specification of body parts have been shown to be conserved in structure as well as in function. Specifically, our progress in the understanding of heart development in recent years has been greatly facilitated by parallel studies in *Drosophila* and vertebrates, as illustrated in this special issue of *Developmental Genetics*.

The *Drosophila* and the vertebrate heart

The heart of *Drosophila* is a simple linear tubular structure that forms at the dorsal midline of the embryo and pumps the haemolymph through the larval body cavity in an open circulatory system. The heart in *Drosophila* consists of two major cell types: the inner contractile muscle cells (cardial cells) are flanked on each side by pericardial cells, which do not express muscle-specific structural proteins.

The early events of mesodermal specification and subdivision in *Drosophila* that are prerequisite for the formation of the major mesodermal derivatives, including heart, have been studied in considerable detail at the genetic, molecular and morphological levels (reviewed in Bodmer et al., 1997; Bodmer and Frasch, 1998; see also in this issue Michelson et al., 1998; Yin and Frasch, 1998; Park et al., 1998). The *Drosophila* heart primordia form bilaterally at the dorsal-most portions of the trunk mesoderm, which then fuse into a linear heart tube at the dorsal midline. Superficially, the *Drosophila* heart looks very different from a vertebrate heart: unlike invertebrates, the vertebrate heart is looped, consists of multiple chambers with numerous specialized cell types and is connected to an elaborate

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circulatory system. Despite these anatomical differences, there are striking embryological and molecular similarities by which vertebrate and *Drosophila* heart development is initiated (Bodmer, 1995). Consequently, the *Drosophila* heart has become a very fruitful model for cardiogenesis (Harvey, 1996; Olson and Srivastava, 1996). For example, in both systems the heart assembles at the midline from bilaterally symmetrical mesodermal progenitors that have migrated most distally from the point of invagination during gastrulation. Because of the reversal of the dorsal-ventral axis between vertebrates and invertebrates (Francois and Bier, 1995; DeRobertis and Sasai, 1996), the *Drosophila* heart forms dorsally whereas the vertebrate heart forms ventrally (from anterior lateral plate mesoderm).

In the following, we will summarize the emerging body of evidence that heart development initiates through molecular mechanisms that have been largely conserved during the evolution of invertebrates and vertebrates (also reviewed in Scott, 1994; Bodmer, 1995; Harvey, 1996; Olson and Srivastava, 1996). Because of the additional complexities of vertebrate heart morphology and physiology, a comparison of cardiogenesis between *Drosophila* and vertebrates is naturally limited to cardiac precursor determination and differentiation, and to the initial events of heart tube morphogenesis. Along these lines, it has been suggested that the present day vertebrate heart probably evolved from a simple tubular heart (similar to that of *Drosophila*) present in primitive chordates (Harvey, 1996; Fishman and Chien, 1997; Fishman and Olson, 1998). Interestingly, the mutant phenotypes of vertebrate homologs of *Drosophila* cardiogenic genes studied thus far (see below) suggest that their essential functions are most apparent in vertebrate-specific morphogenesis of the heart, such as looping and chamber specification (e.g. Fishman and Olson, 1998). Therefore, we propose that in addition to their anticipated earlier function during cardiac induction, these *Drosophila*-related genes have taken on additional, i.e. vertebrate-specific functions during the course of evolution.

tinman and *tinman*-related *Nkx* genes

The formation of the heart in *Drosophila* is dependent on *tinman*, a homeodomain containing transcription factor (Bodmer et al., 1990; Bodmer, 1993; Azpiazu and Frasch, 1993). *tinman* is first expressed uniformly in the presumptive mesoderm. After gastrulation, *tinman* expression is restricted to the dorsal portion of the mesoderm which gives rise to the heart, the gut muscles and some dorsal skeletal muscles. Later, *tinman* is expressed transiently in a portion of the visceral mesoderm and permanently in the forming heart. *tinman* mutants lack not only heart, but also other dorsal mesodermal derivatives, i.e. visceral and dorsal skeletal muscles. Thus, *tinman* is crucial for subdividing the mesoderm and endowing the dorsal mesoderm with the competence to form heart (Bodmer et al.,1997; Bodmer and Frasch, 1998; Yin and Frasch, 1998, this issue).

In vertebrates, an understanding of the molecular basis of cardiac differentiation was hampered due to lack of suitable in vitro systems (Olson and Srivastava, 1996). The demonstrated importance of *tinman* in *Drosophila* prompted several groups to identify homologs of *tinman* in vertebrates. The first *tinman*-related gene has been isolated in mouse, *Nkx2–5* or *Csx* (Komuro and Izumu,1993; Lints et al., 1993; Harvey 1996; in this issue: Tanaka et al., 1998; Newman and Krieg, 1998). *Nkx2–5* is initially expressed in the bilateral cardiac progenitors of the anterior lateral plate mesoderm and in part of the pharyngeal endoderm. It is the earliest known marker for the cardiac lineage in vertebrates. In mice homozygous for a *Nkx2.5* knockout mutation (Lyons et al., 1995), the early heart tube does form and most contractile proteins are expressed, except for a ventricular-specific myosin light chain gene. Later however, the heart tube fails to undergo normal looping and two transcription factors fail to be expressed in *Nkx2–5* mutant hearts: the bHLH protein eHAND (Biben and Harvey, 1997) and the ankyrin-repeat protein CARP (Zou et al., 1997). Although this phenotype demonstrates that *Nkx2–5* is required for heart development, the mutant defect manifests itself later than is expected from the complete absence of heart formation in *tinman* mutants of *Drososphila*. Interestingly however, when vertebrate *tinman*-like genes are expressed in transgenic *Drosophila*, they are capable of substituting functionally for the *Drosophila tinman* gene and rescue partially the defects of *tinman* mutants (M. Park and R. Bodmer, unpublished observations). Thus, it has been suggested that several *tinman*-related genes may have partially redundant function during cardiogenesis.

In addition to *Nkx2.5* , several other *tinman*-related genes have been isolated in a number of species and shown to be expressed during early heart development. So far only two members, *Nkx2–3* and *Nkx2–5* have been reported in multiple species, while *Nkx2–6/Tix*, *Nkx2–7*, *Nkx2–8* and *Nkx2–9* have been only described in single species (Lee et al., 1996; Reecy et al., 1997; in this issue: Newman and Krieg, 1998; Tanaka et al., 1998). Aside from the homeodomain, significant sequence similarities are also present in a N-terminal 11 amino acid region, the TN domain, and a C-terminal 20 amino acid stretch of hydrophobic residues, the NK2-specific domain. A summary of the different *tinman*-related genes and their expression pattern is provided in this issue by Newman and Krieg (1998) and Tanaka et al. (1998).

MADS box genes: *Mef2*

Another class of transcription factors that appear to play a role in cardiogenesis in both *Drosophila* and

vertebrates are the *MEF2* genes (myocyte enhancer factor-2), which belong to the MADS box family (Olson et al., 1995). The four *MEF2* genes in mice are expressed in precursors of cardiac, skeletal and smooth muscle lineages as well as in other cell types. A targeted mutation in the *MEF2C* gene causes defects in heart morphogenesis (looping) and cardiac-specific gene expression to a similar extend as is observed in *dHAND* (see below) or *Nkx2–5* mutants (Lin et al., 1997).

In *Drosophila*, only a single mef2 gene seems to exist: *Dmef2*. Like its vertebrate counterparts, it is also expressed in the precursors of myogenic lineages and their descendants. In *Dmef2* null mutants, the founder cells of individual muscles seem to be positioned and specified as in wildtype, but correct myogenic differentiation, also of the heart, does not occur (Bour et al., 1995; Lilly et al., 1995). This phenotype demonstrates that *Dmef2* controls a relatively late step in muscle development and indicates that different muscle types, including the cardiac muscle, share common aspects of a myogenic regulatory program under the control of MEF2 factors. *Dmef2* is likely a direct target of *tinman*, because two *tinman* binding sites found in the cardiac enhancer of *Dmef2* are required for promoting expression in the heart (Gajewski et al., 1997).

dpp and BMPs

In addition to transcription factors, signaling pathways in cardiac development seem to be also conserved during evolution. In *Drosophila*, the product of *decapentaplegic* (*dpp*), a secreted factor of the TGF-β superfamily, acts as an inductive signal originating from the dorsal ectoderm to maintain *tinman* expression in the underlying dorsal mesoderm, which is a prerequisite for heart specification (Frasch, 1995; Staehling-Hampton et al., 1994; Yin and Frasch, 1998 (this issue), reviewed in Venkatesh and Bodmer, 1995). As in *tinman* mutants, heart and visceral mesoderm formation is abolished in *dpp* mutant embryos. In contrast, when the Dpp pathway is activated in more ventral regions, the *tinman* expression domain is also expanded ventrally (Frasch, 1995), which can lead to the ectopic induction of cardiac-specific fates in the ventral mesoderm (Yin and Frasch, 1998, this issue; W.B. Lockwood and R. Bodmer, unpublished observations).

Similarly in vertebrates, expression of BMP-2, -4 and -7 is present in endodermal or ectodermal tissues that are closely apposed to *Nkx2–5* expressing precardiac mesoderm. In chick, transplantation of BMP-2 expressing endoderm to the anterior paraxial mesoderm or implantation of BMP-2 or -4 laden beads in that tissue can specifically induce *Nkx2–5* expression (Schultheiss et al., 1995; 1997). In addition, culturing anterior paraxial mesoderm in vitro with either BMP-2 or BMP-4 induces robust cardiac differentiation. In contrast, incubation of precardiac mesoderm with Noggin, an antagonist of BMP signalling inhibits cardiac myogenesis.

wingless and *heartless*

In *Drosophila*, another signaling molecule encoded by *wingless*, homolog of vertebrate *Wnt-1*, is absolutely required for the induction of the cardiac but not the visceral mesoderm (Wu et al., 1995; Park et al., 1996; 1998, this issue). *wingless* is expressed orthogonally to *dpp* in the early embryo and thereby seems to provide positional information to further subdivide the dorsal mesoderm. The intersect of *wingless* and *dpp* in the context of *tinman*-expressing mesoderm apparently is not only necessary but also sufficient for inducing cardiac cell fates (W.B. Lockwood and R. Bodmer, unpublished observations). One of the known components of the Wingless signaling pathway, the GSK3β-kinase encoded by the *shagg/zeste-white3* gene, does not only have a cardiac-specific function downstream of *wingless*, but is also required for specifying the entire dorsal mesoderm (Park et al., 1998, this issue). None of the vertebrate Wnt genes have yet been shown to be directly involved in heart development.

The *heartless* gene of *Drosophila* encodes a FGF-receptor that is necessary for cardiac development (Gisselbrecht et al., 1996; Michelson et al., 1998, this issue). *heartless* is expressed as early as *tinman* in all mesoderm. Unlike *tinman*, however, its major role does not seem to be in specifying cardiac competence or cell fates directly, but rather by allowing early mesodermal cells to migrate dorsally to come in contact with *dpp*-expressing (dorsal) ectoderm, which is a prerequisite for cardiac induction (see above). Although FGF (and Wnt) signaling is crucial during mesoderm induction in vertebrates (as demonstrated by gain-of-function experiments carried out primarily in frogs), a direct role in determining the cardiac lineage has not been evident.

bHLH proteins

The MyoD family of basic-Helix-Loop-Helix proteins, which regulate skeletal myogenesis, are not expressed in the heart. Recently, two new bHLH genes, *dHAND* and *eHAND*, have been isolated that show prominent expression in the early cardiac progenitors, the looping heart tube, as well as in cardiac neural crest-derived cells (Srivastava and Olson, 1996). Incubation of chick embryos with antisense oligonucleotides to both of these genes simultaneously results in arrest of heart development at the looping stage (Srivastava et al., 1995). Mice that are deficient in *dHAND* display prominent defects in the morphology of the aortic sac and ventricular chambers, and in looping morphogenesis (Srivastava et al., 1997). Because of the similarities in phenotype of *Nkx2–5*, *MEF2C*, and *dHAND* knock-out mice it is suggested that these genes may cooperate in the specification of the cardiac lineage and/or differen-

tiation of the primitive heart tube. In *Drosophila*, no *HAND*-type bHLH factors are known.

GATA factors

Zinc-finger-containing GATA transcription factors are involved in regulating lineage-restricted patterns of gene expression (Simon, 1995; Evans, 1997). Three members of this family, *GATA4/5/6*, are expressed in the developing heart and various endoderm-derived tissues in overlapping patterns, and they bind to similar DNA sequences (in this issue: Durocher and Nemer, 1998; Jiang et al., 1998). *GATA4* expression in mouse, frog, and chick is detected in the early precardiac mesoderm, as *Nkx2–5*, and persists throughout development (Heikinheimo et al., 1994; Laverriere et al., 1994; Jiang and Evans, 1996). Binding sites for GATA4 are found in the promoter regions of several cardiac muscle genes, and inhibition of *GATA4* expression with antisense RNA blocks the differentiation of cardiac myocytes from pluripotent P19 embryonal carcinoma cell lines (Greppin et al., 1995; 1997).

Recent studies on mice carrying a homozygous null mutation for *GATA4* revealed that they die early and the bilateral precardiac mesodem does not fuse into a heart tube (Kuo et al., 1997; Molkentin et al., 1997). Nevertheless, cardiac precursor differentiation and cardiac-specific gene expression are not significantly affected, suggesting that ventral morphogenesis, and not cardiac specification, is the primary defect. Jiang et al. (1998, this issue) have addressed the role of *GATA4/5/6* during cardiogenesis in chick using antisense oligonucleotides. The results suggest an involvement of all three factors in cardiac morphogenesis. Moreover, the GATA factors are likely to function in combination with *Nkx2–5* in regulating cardiac transcription (Durocher and Nemer, 1998, this issue). In *Drosophila*, no GATA4–6-related factors have been identified. Another vertebrate transcription factor with an essential role in later cardiac development is TEF-1: disruption of this gene by a gene trap insertion caused impaired cardiac growth (Chen et al., 1994).

Left-right asymmetry

An important aspect of vertebrate heart development is an asymmetry along the left-right axis, which becomes evident early during embryogenesis. The heart loops to the right in all vertebrates. When considering the mechanisms for cardiac looping it is important to distinguish between the process of looping and the directionality of looping (Yost 1995 ; Olson and Srivastava, 1996; Fishman and Chien, 1997). The directionality of looping reflects the overall asymmetry of the embryo. Genetic functions which initiate the rightward looping of the heart have been identified, which includes those of *nodal, sonic hedgehog,situs invertus, activin, lefty* and *Vg1* (Levin et al., 1995; Meno et al., 1996; Hyatt et al., 1996; reviewed in Olson and Srivastava 1996, Fishman and Chien, 1997; Goldstein et al., 1998, this issue). The direction of looping is randomized in ectopic overexpression experiments, which bilaterally equalizes the presence of these factors (Levin et al., 1995; Issac et al., 1997). Expression of these genes is also reversed or randomized in mice carrying mutations that affect laterality, such as *lefty* and *situs invertus* (Lowe et al., 1996; Meno et al 1996). However, none of these genes are expressed in precardiac cells themselves, suggesting that their role is rather in establishing the overall embryonic left-right asymmetry. How the cardiac progenitors interpret this information remains speculative (see Goldstein et al., 1998, this issue).

Genetic dissection of heart development

Large scale genetic screens in *Drosophila* and *C. elegans* have provided a wealth of information regarding the patterning of the body plan (St. Johnson and Nüsslein-Volhard, 1992; Horvitz, 1998). These invertebrate models have their limitations for studies of vertebrate organanogenesis for obvious reasons. With the advent of zebrafish as a vertebrate model system, classical genetic approaches to vertebrate development have now become feasible. Because of the transparence of zebrafish embryos, its developing heart is easily amenable to such studies (Stainier and Fishman, 1994). To date, many mutants which exhibit defects in cardiac development and function have been isolated in large scale mutagenesis screens (Driever et al., 1996, Haffter et al., 1996). Several of these mutants affect individual elements of heart morphogenesis and, based on the analysis of these mutants, a genetic model for the assembly of the heart has been proposed (Fishman and Chien, 1997). Many of these mutations resemble the targeted mutations of known genes in mice. Alexander et al. (1998) in this issue describe a new genetic screen for heart induction and patterning in zebrafish, which is expected to yield new genes involved in early cardiogenesis.

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