Mutation of weak atrium/atrial myosin heavy chain disrupts atrial function and influences ventricular morphogenesis in zebrafish

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Summary

The embryonic vertebrate heart is composed of two major chambers, a ventricle and an atrium, each of which has a characteristic size, shape and functional capacity that contributes to efficient circulation. Chamber-specific gene expression programs are likely to regulate key aspects of chamber formation. Here, we demonstrate that epigenetic factors also have a significant influence on chamber morphogenesis. Specifically, we show that an atrium-specific contractility defect has a profound impact on ventricular development. We find that the zebrafish locus weak atrium encodes an atrium-specific myosin heavy chain that is required for atrial myofibrillar organization and contraction. Despite their atrial defects, weak atrium mutants can maintain circulation through ventricular contraction. However, the weak atrium mutant ventricle becomes unusually compact, exhibiting a thickened myocardial wall, a narrow lumen and changes in myocardial gene expression. As weak atrium/atrial myosin heavy chain is expressed only in the atrium, the ventricular phenotypes in weak atrium mutants represent a secondary response to atrial dysfunction. Thus, not only is cardiac form essential for cardiac function, but there also exists a reciprocal relationship in which function can influence form. These findings are relevant to our understanding of congenital defects in cardiac chamber morphogenesis.

Movies available online

Key words: Zebrafish, Ventricle, Atrium, Cardiac myosin heavy chain, Chamber formation, Atrial natriuretic factor

Introduction

The embryonic vertebrate heart is initially divided into two major chambers, a ventricle and an atrium, each with a characteristic morphology and contractile rhythm (Yelon and Stainier, 1999). Coordinated sequential contractions of the chambers drive circulation unidirectionally from the atrium, to the ventricle, through the vasculature and back to the origin of the circulatory loop. Proper chamber formation is essential for the maintenance of efficient circulation, and congenital heart defects often include abnormal chamber morphologies (Hoffman and Kaplan, 2002). Little is known about the mechanisms that regulate the size and shape of the cardiac chambers.

Intrinsic chamber-specific differentiation pathways clearly play a major role in the acquisition of chamber morphology. For example, in mice, the bHLH transcription factors Hand1 and Hand2 are required for normal ventricular growth and morphology (Firulli et al., 1998; Riley et al., 1998; Riley et al., 2000; Srivastava et al., 1997), and the T-box transcription factor Tbx5 is essential for normal atrial morphogenesis (Brunéau et al., 2001).

In addition to its genetic regulation, chamber morphology may also be susceptible to epigenetic influences. The morphology of the adult heart is known to be responsive to increased functional demand; for example, pressure overload can stimulate ventricular hypertrophy (Seidman and Seidman, 2001). The embryonic heart can also respond to hemodynamic changes; one striking example comes from a recent study in which a physical blockade of blood flow was shown to cause defects in valve formation, bulbus arteriosus formation and cardiac looping in the zebrafish embryo (Hove et al., 2003). Can embryonic hemodynamics also influence the morphology of the ventricle and the atrium – the number of cells in each chamber, the thickness of the chamber wall and the dimensions of the chamber lumen?

To understand the regulation of chamber morphogenesis, we have identified a number of zebrafish mutations that cause cardiac chamber defects (Alexander et al., 1998; Stainier et al., 1996). One of these mutations, weak atrium (wea), exhibits defects in both chambers: contractility defects in the atrium and morphological defects in the ventricle. Through candidate gene analysis, we demonstrate that wea mutations disrupt the zebrafish atrial myosin heavy chain (amhc) gene. Loss of amhc function can explain the atrial contractile defects in wea mutants. However, because expression of amhc is restricted to the atrium, the wea mutant ventricular phenotype, including defects in chamber circumference, wall thickness, lumen size and gene expression, represents a ventricular response to atrial dysfunction. Thus, our studies of wea mutants clearly indicate that function of one chamber can influence morphogenesis of...
the other, which implicates epigenetic factors in the regulation of chamber morphology.

Materials and methods

Zebrafish

All zebrafish and embryos were maintained at 28°C and staged as previously described (Westerfield, 1995). wea sk7 and wea m58 are recessive mutant alleles that segregate in a Mendelian fashion. Homozygous mutant embryos were generated by mating adult heterozygotes. No phenotypes are apparent in wea heterozygotes. The wea sk7 mutation was identified during a screen for ethynitrosourea-induced mutations that disrupt cardiogenesis in haploids (D.H.L., A. F. Schier and D.Y., unpublished). wea sk7 and wea m58 fail to complement each other: in crosses between heterozygotes, 25.3% (53/209) of the progeny exhibit the wea mutant phenotype. The wea sk7 and wea m58 mutant phenotypes are identical in all characterized aspects; all data shown are from wea sk7 mutants.

Immunofluorescence, in situ hybridization and photography

Whole-mount immunofluorescence experiments were performed as previously described (Yelon et al., 1999), using the monoclonal antibodies MF20 (Bader et al., 1982), S46 (generous gift from F. Stockdale) and CH1 (Lin et al., 1985). MF20 and CH1 were obtained from the Developmental Studies Hybridoma Bank, maintained by the Department of Biological Sciences, University of Iowa, under contract NO1-HD-2-3144 from the NICHD.

In situ hybridization experiments with amhc and cmhc cDNA antisense probes were performed as previously described (Yelon et al., 1999). An antisense amhc probe was synthesized from a 645 bp fragment of amhc cDNA (beginning at nucleotide 2802). An antisense anf probe was synthesized from a 422 bp fragment of anf cDNA (see below).

Stained embryos were examined with Zeiss Axiosplan and M2Bio microscopes, and photographed with a Zeiss Axioquant digital camera. Images were processed using Zeiss Axiovision and Adobe Photoshop software. Live embryo videos were recorded and processed using an Optronics DEI750 video camera and an Axiosplan microscope, and Pixelink, QuickTimePro and iMovie software.

Cloning of zebrafish amhc, vmhc and anf cDNAs

To identify a zebrafish amhc gene, we evaluated the expression patterns of available zebrafish ESTs (RZPD, Berlin) resembling myosin heavy chain genes, found one that was atrium-specific (fc52a03) (Clark et al., 2001), and cloned a corresponding full-length cDNA using previously described reverse transcription and RACE techniques (Keegan et al., 2002). To detect amhc mutations, we amplified fragments of amhc cDNA from mutant embryos using previously described RT-PCR strategies (Keegan et al., 2002). For these experiments, mutant embryos were generated by mating wea homoyzogotes that had survived to adulthood. All sequences were confirmed in at least two independent amplifications of each region of cDNA. Oligonucleotides used for amhc amplification were:

5’-CGCTCGCTGATGTCCCTCACAGTTCT-3’ with 5’-TCCACCT-GACGACACGGTTGCTCC-3’;

5’-AGTTTTGAAGCCACCTTTCCAGGCGC-3’ with 5’-CTCCA-ACGTCCTCGTCTCGAGGCTC-3’; and

5’-AGCACTACGCCCTCTTCTACG-3’ with 5’-GTTGGAAGTT-GGAGCAGCTTGGCC-3’.

We also cloned a full-length vmhc cDNA, using RACE initiated from our previously reported partial cDNA clone (AF114427) (Yelon et al., 1999). Oligonucleotides used for RACE were: 5’-TCTGACGCTCCCTCTTTCACTCTGTCG-3’ and 5’-GTCTTCTTCTC-ATCCTTCTCAGGGTGAC-3’.

The coding sequences of both amhc and vmhc appear polymorphic in wild-type zebrafish strains from our fish facility (E.B. and D.Y., unpublished); reported sequences represent the most common wild-type allele.

To identify a zebrafish anf gene, we assembled a consensus cDNA sequence from available ESTs (zeh1014, zeh1366, zah4805, zah5977, zeh11098 and bb02c03) and then amplified a 422 bp cDNA fragment using the oligonucleotides 5’-ACACGGTGGACAGCACGAC-3’ and 5’-TGTTAACAATATAGCCTATGTT-3’.

GenBank Accession Numbers are AY138982 (amhc), AY138983 (vmhc) and AY319419 (anf).

Radiation hybrid mapping and linkage analysis

Physical mapping of amhc with a radiation hybrid panel and meiotic mapping of wea with SSLP markers were performed using previously described protocols (Keegan et al., 2002; Yelon et al., 2000). Linkage of amhc and wea was also confirmed by demonstrating that the wea m58 mutant phenotype and the single-base deletion detected in wea m58 mutants are tightly linked (0 recombinants in 188 meioses). Additional radiation hybrid mapping placed amhc on LG2 near Zb517.

Morpholino microinjection

Wild-type embryos were injected at the one-cell or two-cell stage with 1-3 ng of anti-amhc morpholino (GeneTools). The anti-amhc morpholino (5’-ACTCTGCATTAAAGCAGACCATC -3’) is predicted to block translation of Amhc.

Transmission electron microscopy

Embryos were fixed at 48 hours postfertilization (hpf) with 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, postfixed with 1% osmium tetroxide followed by 1% uranyl acetate, dehydrated through a graded series of ethanol washes, and embedded in LX112 resin (LADD Research Industries, Burlington, VT). Ultrathin (80 nm) sections were cut on a Reichert Ultracut UCT, stained with uranyl acetate followed by lead citrate, and viewed on a JEOL 1200EX transmission electron microscope at 80 kV.

Histology

Prior to sectioning, embryos were fixed in 4% paraformaldehyde, dehydrated through an ethanol series, cleared in xylene and embedded in paraffin wax. 4 μm longitudinal sections were cut, dewaxed, dried, and stained with Hematoxylin and Eosin.

Results

The weak atrium locus is required for atrial contractility

Mutation of the zebrafish weak atrium (wea) locus causes atrial contractility defects that are easily observed in live embryos (see Movie 1 at http://dev.biologists.org/supplemental/). In wild-type embryos, the atrium exhibits vigorous, rhythmic contractions; in wea mutant embryos, the atrium exhibits vigorous, rhythmic contractility defects that are easily observed in live embryos (see Movie 1 at http://dev.biologists.org/supplemental/). Despite the lack of an atrial pump, circulation proceeds through the heart and vasculature of wea mutants (see Movie 1 at http://dev.biologists.org/supplemental/). However, a characteristic blood pool caudal to the wea mutant atrium indicates that blood flow is inefficient relative to wild type
Atrial function influences ventricular form

Nevertheless, *wea* mutants do not exhibit any general growth defects or obvious abnormalities in organs other than the heart (data not shown) (Chen et al., 1996; Stainier et al., 1996). In fact, some homozygous *wea* mutants can survive to become fertile adults. In *wea* mutant survivors, ventricular function continues and valve and vessel formation proceed; however, atrial defects remain throughout life (E.B. and D.Y., unpublished). Multiple *wea* alleles with these phenotypic characteristics have been identified in screens focused on mutations disrupting cardiac form and function (Alexander et al., 1998; Chen et al., 1996; Stainier et al., 1996). Here, we focus on two *wea* alleles: *wea*^m58^ (Stainier et al., 1996) and *wea*^sk7^ (see Materials and methods).

**Fig. 1.** Cardiac morphology in *wea* mutant embryos. (A-D) Lateral views of live embryos at 48 hpf, anterior to the left. (A,B) Atrial plane of focus. Compared with the wild-type (wt) atrium (A, arrow), the *wea* mutant atrium (B, arrow) appears dilated. (C,D) Ventricular plane of focus. Compared with the wild-type ventricle (C, arrow), the *wea* mutant ventricle (D, arrow) appears slightly compact. Mutant embryos shown are *wea*^m58^ homozygotes. The *wea*^m58^ and *wea*^sk7^ mutant phenotypes are identical in all characterized aspects; data in all figures are from *wea*^m58^ mutants.

(see Movie 1 at http://dev.biologists.org/supplemental/). Nevertheless, *wea* mutants do not exhibit any general growth defects or obvious abnormalities in organs other than the heart (data not shown) (Chen et al., 1996; Stainier et al., 1996). In fact, some homozygous *wea* mutants can survive to become fertile adults. In *wea* mutant survivors, ventricular function continues and valve and vessel formation proceed; however, atrial defects remain throughout life (E.B. and D.Y., unpublished). Multiple *wea* alleles with these phenotypic characteristics have been identified in screens focused on mutations disrupting cardiac form and function (Alexander et al., 1998; Chen et al., 1996; Stainier et al., 1996). Here, we focus on two *wea* alleles: *wea*^m58^ (Stainier et al., 1996) and *wea*^sk7^ (see Materials and methods).

**Fig. 2.** Myocardial ultrastructure is disrupted in the *wea* mutant atrium and intact in the *wea* mutant ventricle. (A-D) Longitudinal sections of myocardiocytes at 48 hpf viewed by transmission electron microscopy. (A,B) Atrial cells in wild-type embryos contain myofibrillar arrays (A), but myofibrils are rarely found in *wea* mutant atrial cells (B). Occasionally, *wea* mutant atrial cells contain a few disorganized myofilaments (B, arrow). (C,D) Ventricular cells in wild-type (C) and *wea* mutant embryos (D) contain normal myofibrillar arrays.
expression, which begins around the 13-somite stage (Yelon et al., 1999). From its onset, amhc expression is complementary to vmhc expression: the expression patterns of vmhc and amhc subdivide the myocardial precursors into two separate populations that are likely to represent the ventricular and atrial precursors (Fig. 4) (Yelon et al., 1999). For example, at the 21-somite stage, the inner portion of the cardiac myosin light chain 2 (cmlc2; mylc2a – Zebrafish Information Network)-expressing myocardial cone expresses vmhc, and the outer portion expresses amhc (Fig. 4A-C). The cardiac cone then elongates to form a heart tube with vmhc-expressing cells at one end and amhc-expressing cells at the other (Fig. 4E-G). By 48 hpf, the vmhc-expressing ventricle and amhc-expressing atrium are morphologically distinct within the looped heart (Fig. 4I-K). We have never observed amhc expression in the ventricle, nor have we observed vmhc expression in the atrium. Neither wea58 nor wea57 mutants exhibit robust expression of amhc (Fig. 4D,H,L, and data not shown). Initial amhc expression is detectable in wea mutants (Fig. 4D), but there is no evident expression at later stages (Fig. 4H,L). By contrast, other cardiac genes, such as cmlc2, cmlc1, vmhc, nkx2.5, tbx20 and tbx5, are expressed at normal levels in the wea mutant heart (stages examined range from 24-48 hpf, data not shown).

Fig. 4. amhc is expressed only in atrial myocardium. (A-L) Whole-mount in situ hybridization compares expression of cmlc2 (A,E,I), vmhc (B,F,J) and amhc (C,D,G,H,K,L). (A-D) Dorsal views at the 21-somite stage, anterior to the top. In wild-type embryos (A-C), cmlc2 expression (A) is observed throughout the cardiac cone. Presumed ventricular precursors express vmhc (B) and are found in the central portion of the cardiac cone. At this stage, presumed atrial precursors are beginning to express amhc (C) and are found in the outer portion of the cardiac cone. (E-H) Dorsal views through the head at 24 hpf, anterior to the bottom. In wild-type embryos (E-G), cmlc2 (E) is expressed throughout the heart tube, vmhc (F) is expressed in the ventricular precursors, and amhc (G) is expressed in the atrial precursors. (I-L) Frontal views at 48 hpf, head to the top. In wild-type embryos, cmlc2 (I) is expressed throughout the heart, vmhc (J) is expressed in the ventricle, and amhc (K) is expressed in the atrium. wea mutant embryos express amhc in atrial precursors initially (D), but do not maintain amhc expression (H,L). At all stages examined, ranging from the 19-somite stage through adulthood, there appears to be little, if any, overlap between vmhc and amhc expression. We have not observed amhc expression anywhere outside of the atrium.
The wea locus encodes Amhc

Hypothesizing that wea could encode Amhc or a regulator of amhc expression, we chose to map both amhc and wea. Using radiation hybrid panels (Geisler et al., 1999; Hukriede et al., 2001), we mapped amhc to zebrafish LG20 near the SSLP marker Z4329 (Shimoda et al., 1999). Through meiotic mapping, we mapped wea to the same region of LG20 (near Z7568). The concordance of these map positions made amhc a strong candidate gene for the wea locus.

We proceeded to look for amhc mutations in cDNA isolated from weam58 and wea sk7 mutants. cDNA from weam58 mutants has a deletion of a single T at position 4024 of the amhc orf (Fig. 5A), creating a frame-shift that would produce 66 missense codons followed by a stop codon. cDNA from wea sk7 mutants contains a T to A substitution at position 4577 of the amhc orf, creating a stop codon (Fig. 5B). The premature stop codons found in both weam58 and wea sk7 suggest that the decreased stability of the mutant amhc RNA (Fig. 4H,L) could be the result of nonsense-mediated decay (Culbertson, 1999; Hentze and Kulozik, 1999). Based on the lack of MF20 and S46 immunoreactivity (Fig. 3B), and the low levels of amhc mRNA (Fig. 4H,L), in the wea mutant atrium, it is unlikely that wea mutants contain much Amhc protein. Even so, both mutant amhc cDNAs would be predicted to encode truncated Amhc proteins that, if stable, could be deficient in dimerization and/or aggregation (Fig. 5C). Together, our data suggest that weam58 and wea sk7 are strong hypomorphic, if not null, alleles of amhc.

To confirm that wea mutations cause a strong loss-of-function of amhc, we compared the phenotypes of wea mutant embryos and embryos injected with an antisense morpholino (Nasevicius and Ekker, 2000; Summerton and Weller, 1997) designed to inhibit Amhc translation. Injection of the anti-amhc morpholino phenocopies the wea mutation (Fig. 5D-F).

Morpholino-injected embryos lack atrial contractility and do not display S46 or MF20 atrial immunoreactivity. Together, our analyses of S46 immunoreactivity, amhc expression, genetic linkage, amhc mutations and an anti-amhc morpholino indicate that wea encodes Amhc.

Ventricular morphology responds to atrial dysfunction

As myosin heavy chains are essential for myofibrillogenesis, the lack of functional Amhc in wea mutants can account for the observed defects in atrial myofibrillar organization and contractility. In addition to their atrial defects, wea mutants exhibit significant ventricular defects. As amhc expression is restricted to the atrium (Fig. 4), any ventricular phenotypes in wea mutants are likely to represent secondary consequences of atrial dysfunction.

During the first 36 hours of development, ventricular form and function appear normal in wea mutants (Fig. 6A-C, and data not shown). By 48 hpf, although the rhythm of ventricular contractions remains normal, the wea mutant ventricle acquires an unusual and variable morphology (Fig. 6D-F; also see Movie 1 at http://dev.biologists.org/supplemental/, Fig. 1C,D, Fig. 3 and Fig. 5D-F). Specifically, the wea mutant ventricle becomes more compact, with a smaller circumference than the wild-type ventricle. Sections through the wea mutant heart reveal significant thickening of the ventricular wall and narrowing of the ventricular lumen (Fig. 7). The thickness of the wea ventricular wall varies between, and within, individual embryos, which is in contrast to the consistent and uniform structure of the wild-type ventricular wall (Fig. 7C,D). Increased ventricular thickness in wea mutants does not seem to be caused by excess proliferation. The number of ventricular myocardial cells varies between wea mutant embryos, but is
not greater than the number found in wild-type embryos [at 72 hpf, the total number of ventricular myocardial nuclei in serial sections from wild-type embryos was 350±15 (n=2) compared with 274±70 (n=3) in sections from wea mutant embryos]. All characterized features of the wea ventricular phenotype are found in both wea m58 and wea sk7 mutants, and in embryos injected with the anti-amhc morpholino. Overall, the wea ventricular phenotype indicates that a loss of atrial function indirectly stimulates reorganization of the ventricular myocardium, producing significant changes in ventricular form.

**Myocardial gene expression responds to atrial dysfunction**

Changes in ventricular morphology are likely to be accompanied by changes in myocardial gene expression. In particular, we expected that the ventricular response in wea mutants could involve gene expression changes similar to those observed in mammalian cardiomyopathies. To confirm this, we chose to examine the atrial natriuretic factor (anf) gene because of its established responsiveness to a variety of physiological stimuli, including conditions causing hypertrophic or dilated cardiomyopathy in mammals (e.g. Aronow et al., 2001; Barrans et al., 2002; Hwang et al., 2002; Cameron and Ellmers, 2003). We also assessed expression of the cmlc2 gene (Yelon et al., 1999), as genes encoding sarcomere components are often upregulated in response to pathologic conditions (e.g. Aronow et al., 2001; Barrans et al., 2002; Hwang et al., 2002).

As in other species (Zeller et al., 1987; Small and Krieg, 2000; Houweling et al., 2002), the zebrafish anf gene is expressed in both the ventricle and atrium before becoming restricted to the atrium (Fig. 8A, and data not shown). In comparison with wild-type embryos, wea mutants exhibit striking upregulation of anf in both chambers (Fig. 8A,B).

Fig. 6. Defects in ventricular morphology emerge by 48 hpf in wea mutants. (A-F) Lateral views, anterior to the left, of whole-mount immunofluorescence with the anti-tropomyosin antibody CH1 (FITC). V entricle (V) and atrium (A) are indicated. (A-C) At 36 hpf, the wild-type ventricle (A) and the wea mutant ventricle (B,C) have similar morphology. The wea mutant atrium is slightly dilated at this stage. (D-F) At 48 hpf, the wea mutant ventricle (E,F) is noticeably smaller than the wild-type ventricle (D). The morphology of the wea mutant ventricle varies between individuals (E,F), as does the degree of dilation of the wea mutant atrium. Distortion of cardiac looping is also apparent in wea mutants at this stage.

Fig. 7. Ventricular morphology responds to atrial dysfunction in wea mutants. (A-D) Longitudinal sections through the heart at 72 hpf, stained with Hematoxylin and Eosin, anterior to the top. (A,B) Sections through the wild-type (A, arrow) and wea mutant atrium (B, arrow) demonstrate similar thickness of the atrial wall. The wea mutant atrium is dilated in comparison with the wild-type atrium and contains less blood. (C,D) Comparison of sections through the wild-type (C, arrow) and wea mutant ventricle (D, arrow) demonstrates that the ventricular wall is thicker, and that the ventricular lumen is narrower, in wea mutants. The morphology of the wea mutant ventricle varies between individuals (D); insets show two additional examples of ventricular sections from other wea mutant embryos. Variability is also apparent within individual embryos, as the wea mutant ventricular wall does not exhibit a uniform thickness. (D) Thickening of the wea mutant ventricular wall is apparent by 48 hpf, and increases between 48 and 72 hpf (data not shown). All sections shown are the central section from serial sectioning through the respective chamber; results are representative of the examination of more than 15 embryos of each genotype.
which is the same thickness in wild-type and increased on a per cell basis; this is especially clear for the atrium, with mammalian cardiomyopathies. The mutant heart exhibits upregulation of \( \text{amhc} \) expression throughout the ventricle. Although it is difficult to address precisely whether there is overlap of \( \text{amhc} \) and \( \text{vmhc} \) expression at the atventricular boundary, it is clear that there are two separate zones of \( \text{amhc} \) and \( \text{vmhc} \) expression that are compatible with the expected locations of atrial and ventricular myocardiocytes throughout development (Yelon et al., 1999).

Although atrial contractility is aberrant in \( \text{wea} \) mutants from the initiation of the embryonic heartbeat (around 24 hpf), the \( \text{wea} \) mutant ventricular defects first become apparent around 48 hpf. This sequence of events fits a model in which the ventricular myocardium responds to a physiological stimulus that is the result of atrial dysfunction. There are several possibilities for the nature of the signal received by the \( \text{wea} \) mutant ventricle. For example, ventricular mechanosensation of hemodynamic changes produced by atrial failure could trigger changes in ventricular morphology and gene expression. Alternatively, oxygen sensors could provide feedback to the ventricle regarding inefficient circulation, or the nonfunctional atrial myocardium could emit stress signals that are perceived by the ventricle.

A comparison of \( \text{wea} \) mutants with other zebrafish mutants with sarcomere defects yields insights regarding potential triggers of the \( \text{wea} \) ventricular phenotype. The zebrafish \( \text{silent heart/tnt2 (sih)} \) locus encodes cardiac troponin T (Sehnert et al., 2002), and the \( \text{pickwick/ttn (pik)} \) locus encodes titin (Xu et al., 2002). In \( \text{sih} \) and \( \text{pik} \) mutants, neither the atrium nor the ventricle contract or assemble sarcomeres normally (Sehnert et al., 2002; Xu et al., 2002). Additionally, neither the \( \text{sih} \) ventricle nor the \( \text{pik} \) ventricle acquire the compact and thick morphology typical of the \( \text{wea} \) ventricle (Sehnert et al., 2002; Xu et al., 2002). Indeed, \( \text{pik} \) mutants feature a contrasting phenotype, a dilated cardiomyopathy in which the embryonic ventricle becomes unusually thin (Xu et al., 2002). We have also examined the roles of two other zebrafish loci, both of which are required for ventricular sarcomere formation and are dispensable for atrial sarcomere formation (H.C., C. Fabricant and D.Y., unpublished). Like \( \text{sih} \) and \( \text{pik} \), neither of these ventricular mutants exhibit ventricular thickening. These comparisons indicate that reduced blood flow is not necessarily sufficient to provoke the ventricular phenotypes observed in \( \text{wea} \) mutants. Perhaps a specific type of hemodynamic alteration elicits the \( \text{wea} \) ventricular response, and this physiological circumstance is not replicated when the ventricle, or the entire heart, fails to contract. Alternatively, ventricular contractility and/or sarcomere assembly might provide a degree of cellular integrity that is a prerequisite for the type of chamber morphogenesis observed in \( \text{wea} \) mutants.

### Conservation of chamber responsiveness and relevance to congenital heart disease

Zebrafish \( \text{wea} \) mutants demonstrate that epigenetic
parameters can play key roles in regulating ventricular morphogenesis. A recent study suggests that the relationship between atrial function and ventricular development may be conserved among vertebrate species. Specifically, analysis of mice lacking MLC2a, an atrial regulatory myosin light chain gene essential for atrial myofibrillogenesis, indicates that loss of atrial function affects ventricular morphology in the mouse embryo (Huang et al., 2003). The conserved influence of function on form is likely to be relevant to the causes of congenital abnormalities in cardiac chamber formation.

When considering the relationship of zebrafish wea mutations with human disease, it is important to note that autosomal dominant mutations in human cardiac myosin heavy chain genes can cause either hypertrophic or dilated cardiomyopathy, depending on the specific gene, mutation and individual (Seidman and Seidman, 2001). In affected individuals, missense mutations are thought to be responsible for the production of malfunctioning myosin heavy chain proteins that act cell-autonomously to evoke cardiomyopathy. By contrast, loss-of-function wea mutations appear entirely recessive, as heterozygotes are phenotypically wild-type. Thus, wea mutants demonstrate an alternate method by which a sarcomere defect can trigger a morphogenetic response — not only by directly affecting the cells expressing the mutant gene, but also by indirectly affecting another chamber.

Alterations in circulation, as observed in wea mutants, can trigger significant changes in chamber shape, size, cellular organization and gene expression. Our data suggest that similar scenarios could be responsible for congenital heart defects. For example, our studies lend credence to the proposal that hypoplastic left heart syndrome, which includes defects in left ventricular morphogenesis, can be caused by reduced blood flow through the left ventricle (Harh et al., 1973; Grossfeld, 1999; Sedmera et al., 1999; Sedmera et al., 2002). Just as reduced ventricular loading, caused by mitral atresia, is suggested to trigger hypoplastic left heart syndrome (Grossfeld, 1999), reduced ventricular loading in wea mutants, caused by atrial failure, is associated with changes in ventricular morphology. Altogether, because of their striking ventricular phenotypes and relevance to congenital heart defects, zebrafish wea mutants provide a valuable genetic model for the analysis of the epigenetic mechanisms that influence cardiac chamber formation.

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