Current Biology, Vol. 15, 441–446, March 8, 2005, ©2005 Elsevier Ltd All rights reserved. DOI 10.1016/j.cub.2004.12.083

Hand2 Regulates Epithelial Formation during Myocardial Differentiation

Le A. Trinh,[1] Deborah Yelon,[2] and Didier Y.R. Stainier[1,*]
[1]Department of Biochemistry and Biophysics
Programs in Developmental Biology, Genetics, and Human Genetics
University of California, San Francisco
San Francisco, California 94143
[2]Developmental Genetics Program
Department of Cell Biology
Skirball Institute of Biomolecular Medicine
New York University School of Medicine
New York, New York 10016

Summary

Myocardial differentiation is initiated by the activation of terminal-differentiation gene expression within a subset of cells in the anterior lateral plate mesoderm. We have previously shown that shortly after this activation, myocardial cells undergo epithelial maturation [1], suggesting that myocardial differentiation encompasses both molecular and cellular changes. To address the question of how the molecular programs driving myocardial gene expression and the formation of the myocardial epithelium are integrated, we analyzed the role of two essential myocardial terminal-differentiation factors, Hand2 and Gata5, in myocardial epithelia formation. *hand2* and *gata5* mutants exhibit a much-reduced number of myocardial cells and defects in myocardial gene expression [2, 3]. We find that the few myocardial precursors that are present in *hand2* mutants do not polarize. In contrast, embryos with reduced Gata5 function exhibit polarized myocardial epithelia despite a similar reduction in myocardial precursor number, indicating that proper cell number is not required for epithelial formation. Taken together, these results indicate that Hand2 is uniquely required for myocardial polarization, a previously unappreciated role for this critical transcription factor. Furthermore, these results demonstrate that two independent processes, the polarization of myocardial precursors and the allocation of proper cell number, contribute to myocardial development.

Results and Discussion

Hand2 Regulates the Formation of the Myocardial Epithelia

One of the earliest steps in heart development is the activation of myocardial gene expression within a subset of cells in the anterior lateral plate mesoderm (ALPM). Myocardial differentiation is regulated by a number of transcription-factor genes, including *nkx2.5* and *gata4/5/6*, which contribute to the activation of genes encoding components of the cardiac contractile machinery [4]. Although the transcriptional regulation of myocardial gene expression has been well characterized, the cellular basis of myocardial differentiation is not well understood. Recently, we demonstrated that myocardial precursors undergo epithelial maturation during their migration to the midline in zebrafish [1]. Myocardial differentiation appears to be critical for migration because mutants with defective myocardial differentiation exhibit migration defects [2, 3]. Furthermore, the maturation of the myocardial epithelium coincides with the onset of myocardium-specific gene expression [1, 5]. Therefore, we asked whether the molecules that regulate myocardial differentiation also control epithelial formation of the myocardial precursors.

A number of transcription-factor genes expressed in overlapping patterns in the ALPM appear to be important for the differentiation of this tissue [4, 6]. These genes include the basic helix-loop-helix (bHLH) family member *hand2* (also known as *dHand*), which is expressed broadly within the lateral plate mesoderm (LPM) in zebrafish [3, 7]. Loss-of-function analyses indicate that *hand2* plays a vital role in cardiac development [3, 8]. Analyses of the zebrafish locus *hands off*, which encodes Hand2, reveal an early role for the function of *hand2* in the morphogenesis and differentiation of the ALPM [3]. Specifically, *hand2* mutants display a severe reduction in the number of cells that express myocardium-specific genes, and they also exhibit defects in ALPM morphogenesis (Figure 1B) [3]. Molecular evidence indicates a significant block in the progression of myocardial differentiation in *hand2* mutants; *nkx2.5* expression is initiated, but few *nkx2.5*-expressing cells go on to express myocardium-specific genes such as *cmlc2* [3].

In mouse, Hand2 appears to play later roles in myocardial differentiation; *Hand2* null mice lack a right ventricle and die between the embryonic days 9.5 and 10.5 [8]. Expression analysis in mouse is consistent with this loss-of-function phenotype because *Hand2* expression is restricted to the right ventricle after the linear heart tube forms [9]. Before ventricular restriction occurs, *hand2* is expressed more broadly in the cardiac crescent. Coexpression of a second *Hand* gene, *Hand1* (also known as *eHand*), in the cardiac crescent in mouse and chick has been suggested to provide redundant function during early heart formation [9], a hypothesis supported by recent genetic evidence [10]. Despite this essential role in cardiac development, little is known as to how Hand2 regulates the morphogenesis and differentiation of the myocardial precursors.

In addition to its role in cardiac development, Hand2 is involved in the development of the branchial arches, limb, and vasculature [11–15]. Due to its essential role in the development of a number of tissues, there has been much interest in understanding the molecular mechanism(s) by which Hand2 functions. Many studies have focused on the mechanisms by which Hand2 regulates transcription [16–19]. Here, we sought to understand the cellular requirements for Hand2 function during myocardial differentiation.

*Correspondence: didier_stainier@biochem.ucsf.edu

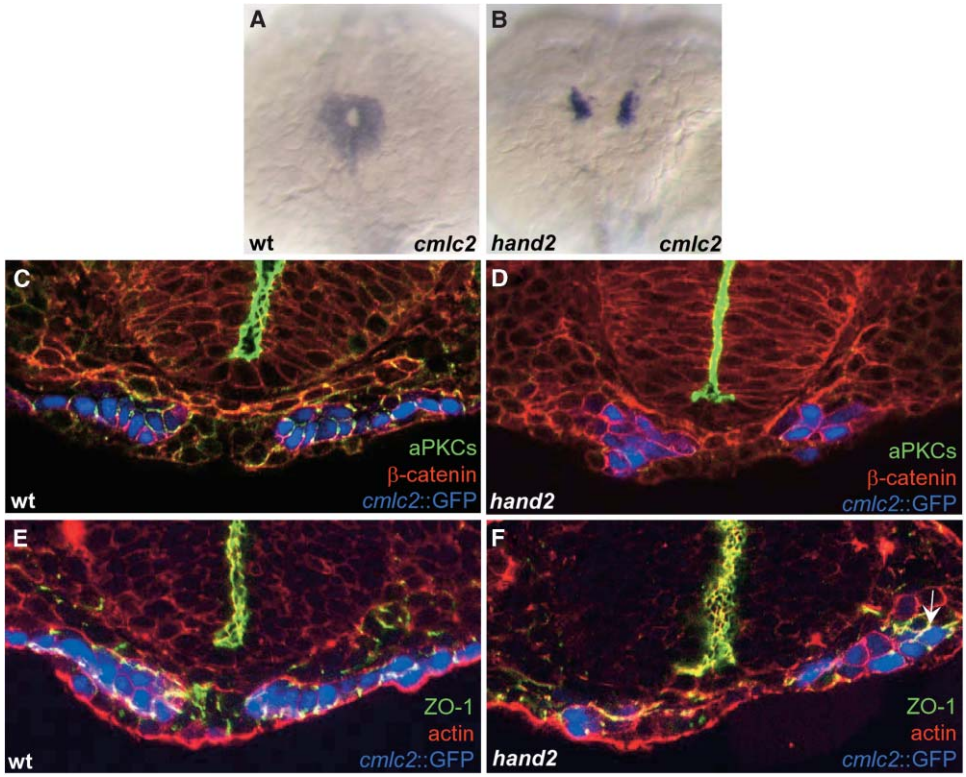

Figure 1. Hand2 Regulates the Formation of the Myocardial Epithelia

Wild-type (A, C, and E) and *hand2* (B, D, and F) mutants at the 20-somite stage. (A) and (B) show *cmlc2* expression; dorsal views, anterior is to the top.

(A) In wild-type embryos, the myocardial precursors have fused to form the cardiac cone.

(B) In *hand2* mutants, the myocardial precursors are reduced in number and fail to migrate to the midline and fuse.

(C–F) Transverse confocal images of *cmlc2*::GFP (false colored blue) transgenic embryos immunostained with antibodies against β-catenin (red) and aPKCs (green) (C and D) or against ZO-1 (green) and filamentous actin (rhodamine-phalloidin) (E and F); dorsal is to the top. (C and E) In wild-type embryos, the myocardial precursors form clear epithelia: Within the myocardial precursors, aPKCs are restricted to the apicolateral membranes, whereas ZO-1 is localized basally along the lateral membranes. Additionally, β-catenin and actin are enriched at the basolateral membranes. (D and F) In *hand2* mutants, the myocardial precursors do not localize junctional proteins asymmetrically. aPKC staining is absent from the myocardial precursors, and ZO-1 is localized cortically in some myocardial precursors. β-catenin and filamentous actin fail to restrict to the basolateral membranes and instead remain cortical in their localization.

To assess the myocardial epithelia in *hand2* mutants, we used a transgenic line that expresses green fluorescent protein (GFP) under the control of the *cmlc2* regulatory elements to mark the myocardial precursors [20]. In conjunction with this transgenic line, we used antibodies against junctional proteins to assess epithelial formation. In wild-type embryos, the myocardial epithelia consist of two rows of cells with their apical surfaces facing each other (Figure 1C) [1]. At the 20-somite stage, an antibody that recognizes both atypical protein kinase C λ and ζ (aPKCs) stains the apical domain of the lateral membranes (Figure 1C, green) [1]. The basolateral membranes are marked by the asymmetric localization of β-catenin (Figure 1C, red) [1]. In addition to its asymmetric localization in the myocardial precursor, β-catenin also localizes to the cortex of all cells in the zebrafish embryo and thus labels cell boundaries [21]. In *hand2* mutants, the few myocardial precursors that are present cluster together and do not form an epithelium as they do in wild-type embryos (Figure 1D, blue). aPKC staining is absent from the myocardial cells in *hand2* mutants (Figure 1D, green). Additionally, β-catenin localizes to the entire cortex of myocardial cells and thus fails to restrict asymmetrically as in wild-type embryos (Figure 1D, red).

To further assess the polarity of the myocardial precursors in *hand2* mutants, we examined the localization of actin and the junctional protein zonula occludens-1 (ZO-1). Both molecules exhibit asymmetric localization in the myocardial precursors [1]: ZO-1 is localized basally along the lateral membranes (Figure 1E, green), and actin is enriched along the basolateral membranes (Figure 1E, red). In *hand2* mutants, ZO-1 is mislocalized (Figure 1F, green). Some myocardial precursors show cortical ZO-1 localization (Figure 1F, arrow), whereas others show either an absence or a punctate localization of ZO-1. Additionally, actin fails to restrict to the basolateral membranes but remains cortical in its localization in *hand2* mutant myocardial precursors (Figure 1F, red). The lack of basolateral restriction of β-catenin and actin, the absence of aPKCs, and the mislocalization of ZO-1 indicate that Hand2 is essential for myocardial epithelia formation. Taken together, these results suggest that myocardial precursors in *hand2* mutants lack apico-basal polarity.

The absence of myocardial epithelia formation in

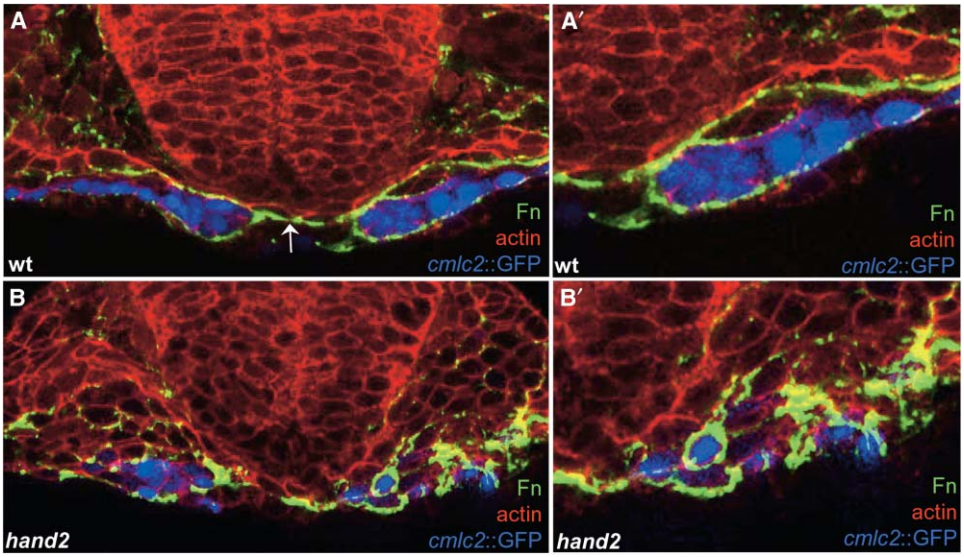

Figure 2. Defective Fibronectin Deposition in *hand2* Mutants

(A and B) transverse sections of *cmlc2*::GFP (false colored blue) transgenic embryos immunostained for β-catenin (red) and for Fibronectin (green) in wild-type (A and A′) and *hand2* (B and B′) mutant embryos at the 20-somite stage; dorsal is to the top. (A′) and (B′) show magnified views of the lower right corners of (A) and (B), respectively. In wild-type embryos (A and A′), Fibronectin is deposited around the basal surface of the myocardial epithelia (blue) and at the midline between the endoderm and endocardial precursors (arrow). (B and B′) In *hand2* mutants, Fibronectin deposition is disorganized and no longer restricted to the basal surface of the myocardial precursors.

hand2 mutants appears to be specific to the myocardial precursors; the localization of junctional proteins (aPKCs and ZO-1) to the hindbrain ventricular surface appears unaffected (Figures 1D and 1F). Additionally, *hand2* mutants do not appear to exhibit defects in other epithelial tissues within the embryo (data not shown). *hand2* is expressed broadly within the LPM [3, 7], yet the posterior LPM does not exhibit polarity defects (S. Horne-Badovinac and D.Y.R.S., unpublished data). Furthermore, several lines of evidence indicate that the lack of aPKCs in the myocardial precursors of *hand2* mutants is not due to Hand2 directly regulating the expression of aPKCs. First, expression analyses indicate that the expression of *aPKC*λ is not reduced in *hand2* mutants (data not shown). Second, overexpression of *aPKC*λ in *hand2* mutants cannot rescue adherens junction formation (data not shown). Third, the absence of aPKCs in the myocardial precursors of *hand2* mutants is not due to an overall lack of adherens junction formation in the embryo because aPKCs localization in the hindbrain ventricular surface is unaffected (Figures 1D and 1F). Taken together, these data suggest that the polarity defects in *hand2* mutants are specific to the myocardial precursors. On a cellular level, our results indicate that Hand2 is essential for the progression of the myocardial precursors to a polarized state.

Defective Fibronectin Deposition in *hand2* Mutants

We have previously reported that Fibronectin is deposited on the basal surface of the myocardial epithelia [1]. Cell-substratum interactions have been implicated in establishing the polarity axis in epithelial cells [22, 23]. In the developing myocardial epithelia, analyses of mutants for the *natter* (*nat*) gene, which encodes Fibronectin, indicate that the basal deposition of Fibronectin is required for maintaining the epithelial organization of the myocardial precursors [1]. The absence of apico-basal identity within the myocardial precursors of *hand2* mutants prompted us to assess the requirement of Hand2 for the deposition of Fibronectin. In wild-type embryos, Fibronectin is deposited around the basal surface of the myocardial epithelia (Figures 2A and 2A′, blue) and at the midline between the endoderm and endocardial precursors (Figure 2A, arrow). In *hand2* mutants, Fibronectin deposition is disorganized and no longer restricted to a single surface of the myocardial precursors (Figures 2B and 2B′). This result is consistent with our findings that junctional proteins do not exhibit asymmetric localization in the myocardial precursors of *hand2* mutants and further demonstrates that the myocardial precursors lack apico-basal polarity.

The presence of Fibronectin deposition around some myocardial precursors in *hand2* mutants indicates that the myocardial precursors have the ability to assemble Fibronectin. However, it is the assembly of Fibronectin to a single surface of the myocardial precursors that appears to be disrupted in *hand2* mutants. This phenotype is clearly distinct from that seen in *nat* mutants, which completely lack Fibronectin. In the complete absence of Fibronectin, adherens junctions formation is defective; however, β-catenin localization appears relatively unaffected along the basolateral membrane [1], suggesting that loss of cell-substratum adhesion does not lead to the complete loss of epithelial polarity. In contrast, the myocardial precursors in *hand2* mutants lack junctional proteins and a basal substratum and therefore appear to be completely devoid of epithelial identity. These results indicate that the role of Hand2 for myocardial epithelia formation is independent of the requirement for Fibronectin in epithelial maintenance.

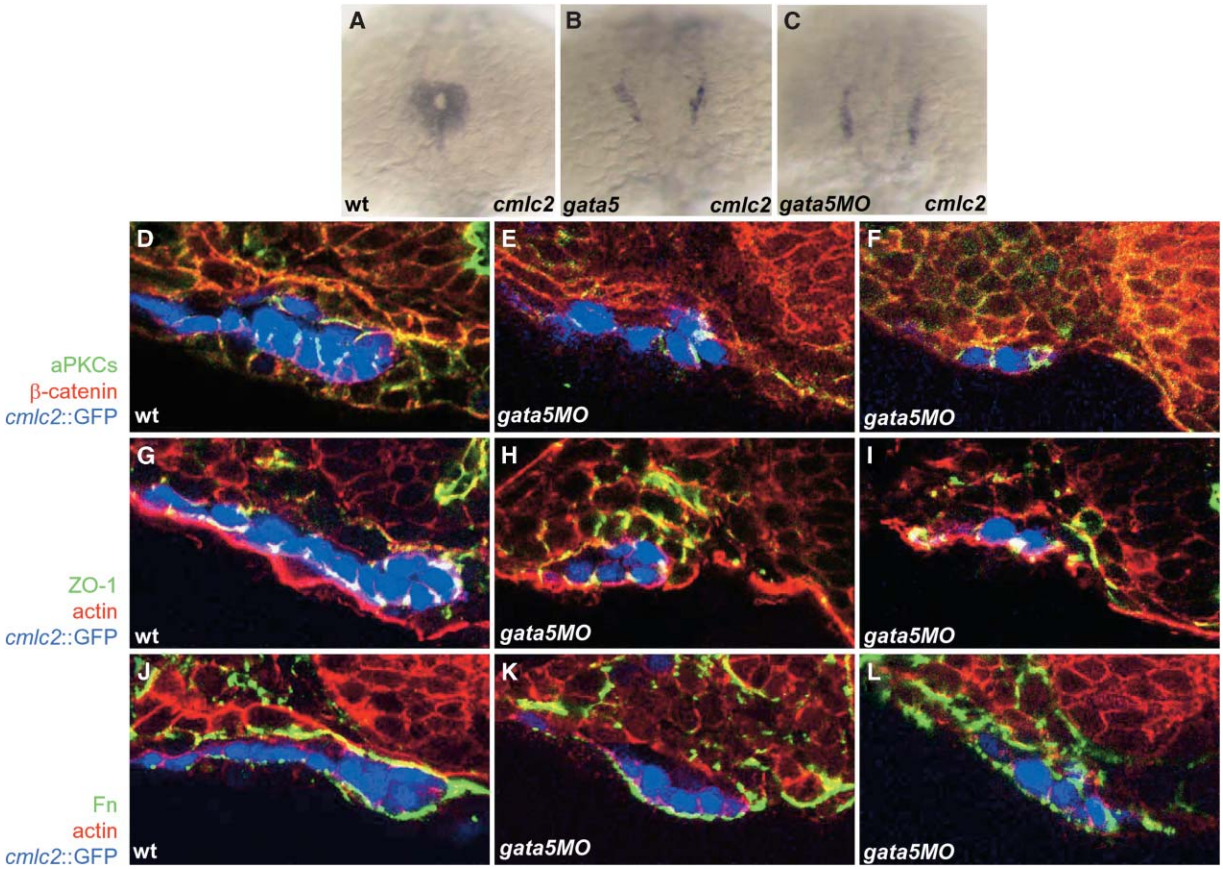

Figure 3. Myocardial Epithelia Organization in Embryos with Reduced Gata5 Function

Wild-type (A, D, F, and H), *gata5* mutants (*fau*) (B), and *gata5MO*-injected (C, E, F, H, I, K, and L) embryos at the 20-somite stage.

(A–C) *cmlc2* expression; dorsal views, anterior is to the top. (A) In wild-type embryos, the myocardial precursors have fused to form the cardiac cone. (B) In *gata5* mutants, the myocardial precursors are reduced in number and remain lateral. (C) In *gata5MO*-injected embryos, the myocardial precursors are reduced in number and remain lateral as in *gata5* mutants.

(D–L) Transverse confocal images of *cmlc2*::GFP (false colored blue) transgenic embryos immunostained with antibodies against β-catenin (red) and aPKCs (green) (D–F), ZO-1 (green) and filamentous actin (rhodamine-phalloidin) (G–I), or β-catenin (red) and Fibronectin (green) (J–L); the left side of the myocardium is shown; dorsal is to the top. (D and G) In wild-type embryos, the myocardial epithelia localize aPKCs to the apicolateral membranes and ZO-1 to the basal portion of the lateral membranes, and they localize β-catenin and filamentous actin basolaterally. (J) Additionally, Fibronectin is deposited around the basal surface of the myocardial precursors. (E–H) In *gata5MO*-injected embryos, fewer myocardial precursors are present, but they form epithelia with aPKCs, ZO-1, β-catenin, and actin localized as in wild-type embryos. The asymmetric localizations of aPKCs, ZO-1, β-catenin, and actin are independent of the number of myocardial precursors; *gata5MO*-injected embryos with as few as two myocardial precursors (F and I) show asymmetric localization of these junctional markers. (K and L) Additionally, Fibronectin is deposited around the myocardial epithelia of *gata5MO*-injected embryos as in wild-type embryos (J).

Myocardial Epithelia Formation in Embryos Deficient for Gata5 Function

Because cell-cell adhesion is necessary for establishing apico-basal polarity [23, 24, 25], it is possible that a critical mass of myocardial precursors is required for establishing cell-cell adhesion that in turn would be required for establishing apico-basal polarity. To test this hypothesis, we examined epithelial formation in embryos lacking Gata5 function. Like Hand2, Gata5 plays an essential role in cardiac development. Zebrafish Gata5 is encoded by the *faust* (*fau*) locus [2]. Similar to *hand2* mutants, *gata5* mutants exhibit a dramatic reduction in myocardial cell number and also show defects in myocardial gene expression (Figure 3B) [2]. To facilitate our analyses of myocardial epithelia formation in the absence of Gata5, an antisense morpholino (MO) designed against a splice donor site upstream of the zinc-finger-encoding region was used to knockdown Gata5 function (see Experimental Procedures). Greater than 95% of the embryos injected with 20 ng of the *gata5MO* appear morphologically similar to *gata5* mutants, in that they exhibit pericardial edema and two separate hearts, a phenotype referred to as cardia bifida ($n > 500$, data not shown). Based on *cmlc2* expression, the myocardial precursors are reduced in number and fail to migrate to the midline in *gata5MO*-injected embryos (Figure 3C), phenotypes identical to those seen in *gata5* mutants (Figure 3B) [2]. Confocal microscopy shows that the myocardial precursors in *gata5MO*-injected embryos are polarized: The few myocardial precursors that are present form epithelia with aPKCs localized to the apicolateral membranes (Figure 3E), β-catenin and actin restricting basolaterally (Figures 3E and 3H), and ZO-1 localizing to the basal portion of the

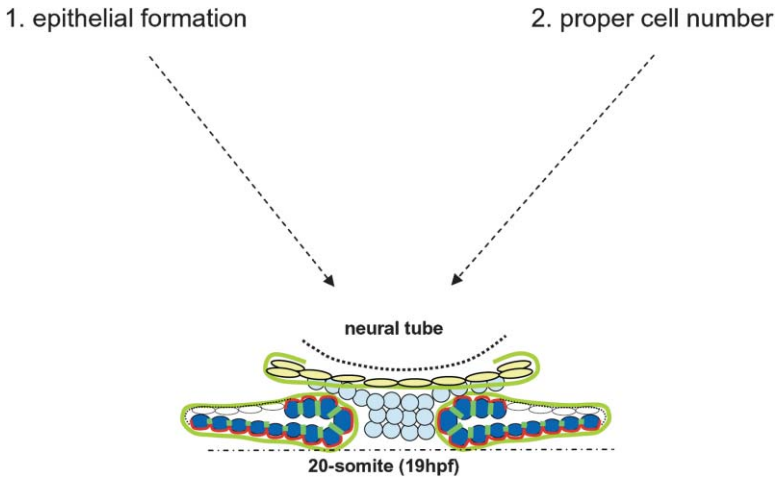

Figure 4. Processes in Myocardial Development

From our analyses of the myocardial epithelia in *hand2* mutants and *gata5MO*-injected embryos, it appears that Hand2 independently regulates two processes in myocardial development: myocardial epithelia identity and the allocation of proper cell number. The myocardial epithelia are diagrammed in transverse sections showing the arrangement of the myocardial precursors (dark blue cells), endocardial precursors (light blue), β-catenin (red), aPKCs and ZO-1 (green) in the myocardial precursors, and Fibronectin (lime green) deposition.

lateral membranes (Figure 3H). To our surprise, *gata5MO*-injected embryos with as few as two myocardial precursors per side of each confocal section appear to exhibit polarized localization of aPKCs, β-catenin, ZO-1, and actin (Figures 3F and 3I). Furthermore, in *gata5MO*-injected embryos, Fibronectin is deposited around the basal surface of the myocardial epithelia despite a substantial reduction in myocardial cell number (Figures 3K and 3L). These results demonstrate that in embryos with reduced Gata5 function, the few myocardial precursors that form are able to establish epithelial identity.

It is possible that the reduction in myocardial precursors in *gata5MO*-injected embryos is less severe than that seen in *hand2* mutants and that this difference may account for the lack of polarity defects in *gata5MO*-injected embryos. To compare the reduction of myocardial precursors in *hand2* mutants and *gata5MO*-injected embryos, we counted the number of myocardial precursors observed in confocal sections of *cmlc2*::GFP transgenic embryos. In wild-type embryos, each side of the myocardial epithelia contains 12.5 ± 1.5 myocardial precursors per confocal section (n = 43). In *hand2* mutants and *gata5MO*-injected embryos, there appears to be a 3-fold reduction in the number of myocardial precursors. More importantly, *hand2* mutants and *gata5MO*-injected embryos show a similar number of myocardial precursors on each side of the embryo. On average, *hand2* mutants have 3.5 ± 2.0 myocardial cells per side of each confocal section (n = 39). Similarly, *gata5MO*-injected embryos have 3.9 ± 1.8 myocardial cells per side of each confocal section (n = 64). Additionally, no significant difference in cell number was observed between the left and right side of either *hand2* mutants or *gata5MO*-injected embryos. These data demonstrate that the reduction in myocardial cell number in *gata5MO*-injected embryos appears to be equivalent to that seen in *hand2* mutants. Therefore, the polarization of the myocardial precursors in *gata5MO*-injected embryos cannot be explained by an increase in myocardial cell number relative to that in *hand2* mutants.

Comparison of the *hand2* and *gata5MO* phenotypes suggests a unique role for Hand2 in the polarization of myocardial precursors. One possibility for the difference that we observed between the *hand2* and *gata5MO* phenotypes may be a difference in the efficiency of our experimental approaches to eliminate each gene function. Although the *hand2* allele that we used is a null, it is possible that residual Gata5 function in the *gata5MO*-injected embryos is sufficient for the polarization of the myocardial precursors. It is important to note that the existing *gata5* mutations (*tm236* and *s26*) do not represent null alleles; wild-type transcript can be detected in both of these mutants [2]. However, few myocardial precursors form in these mutants, and this is also the case in the *gata5MO*-injected embryos. Therefore, and as observed in severely affected mutants, it is likely that a complete reduction of Gata5 function leads to the absence of myocardial precursors [2], thereby precluding the assessment of myocardial epithelia formation. The ability to downregulate Gata5 function temporally will be required to determine whether Gata5 also plays a role in myocardial epithelia polarity.

Conclusions

In this paper, we investigated the relationship between the molecular program driving the formation of the myocardial epithelium and that driving myocardial gene expression. Specifically, we analyzed the role of the myocardial differentiation gene, *hand2*, in myocardial epithelia formation. Our results indicate that there are two distinct cellular processes involved in making a mature myocardium: establishing myocardial epithelia identity and establishing proper cell number (Figure 4). On the molecular level, the establishment of myocardial epithelia identity appears to be distinctly regulated by Hand2, whereas myocardial cell number is regulated by both Hand2 and Gata5. These results predict that Hand2 and Gata5 activate distinct as well as overlapping transcriptional targets involved in myocardial development. More importantly, these results indicate a new and unique role for Hand2 in myocardial development.

Experimental Procedures

Zebrafish Strains and Morpholino Injections

Adult fish and embryos were maintained as described [26]. The following mutant alleles were used: *hand2*^{s6} (which is a null allele) [3] and *gata5*^{tm236a} [2]. We used an antisense morpholino (MO) against the splice donor site 5′-TCTTAAGATTTTTACCTATACTGGA-3′ of

the second intron of the *gata5* gene to knockdown Gata5 function. The high efficiency of the *gata5MO* facilitated our cellular analyses by confocal microscopy; greater than 95% of the embryos examined were informative compared to embryos from a heterozygous cross, where only 25% were informative.

Whole-mount In Situ Hybridization and Antibody Staining

Whole-mount in situ hybridization (ISH) was performed as described [27]. For antibody staining, embryos were fixed overnight at 4°C in 2% paraformaldehyde (PFA). Fixed embryos were embedded in 4% NuSieve GTG low-melting agarose and cut into 250 μm sections with a Leica VT1000S vibratome before antibody staining. Antibody staining was performed in PBDT (1% bovine serum albumin [BSA], 1% dimethyl sulfoxide [DMSO], 0.1% Triton X-100 in phosphate-buffered saline [PBS] pH 7.3).

We used the following antibodies: rabbit polyclonal anti-Fn (Sigma) at 1:200, mouse IgG_1 anti-β-catenin (Sigma) at 1:500, mouse IgG anti-ZO-1 (Zymed) at 1:200, and rabbit polyclonal anti-aPKCs at 1:1000 (Santa Cruz Biotechnology; [28]). Polymerized actin was detected by phalloidin (Molecular Probe) staining (1:50) via a protocol similar to the antibody-staining protocol.

Fluorescence images were acquired with a Zeiss LSM5 Pascal confocal microscope.

Acknowledgments

We thank members of the Stainier lab for helpful discussions. The sequence for the *gata5* splice MO was provided by Roger Patient. L.A.T. was supported by predoctoral fellowships from the National Science Foundation and American Heart Association. This work was supported in part by grants from the National Heart, Lung, and Blood Institute of the National Institutes of Health, from the American Heart Association, and from the Packard Foundation to D.Y.R.S.

Received: September 28, 2004
Revised: December 9, 2004
Accepted: December 17, 2004
Published: March 8, 2005

References

1. Trinh, L.A., and Stainier, D.Y.R. (2004). Fibronectin regulates epithelial organization during myocardial migration in zebrafish. Dev. Cell *3*, 371–382.
2. Reiter, J.F., Alexander, J., Rodaway, A., Yelon, D., Patient, R., Holder, N., and Stainier, D.Y.R. (1999). *Gata5* is required for the development of the heart and endoderm in zebrafish. Genes Dev. *13*, 2983–2995.
3. Yelon, D., Ticho, B., Halpern, M.E., Ruvinsky, I., Ho, R.K., Silver, L.M., and Stainier, D.Y.R. (2000). The bHLH transcription factor Hand2 plays parallel roles in zebrafish heart and pectoral fin development. Development *127*, 2573–2582.
4. Bruneau, B.G. (2002). Transcriptional regulation of vertebrate cardiac morphogenesis. Circ. Res. *90*, 509–519.
5. Yelon, D., Horne, S.A., and Stainier, D.Y.R. (1999). Restricted expression of cardiac myosin genes reveals regulated aspects of heart tube assembly in zebrafish. Dev. Biol. *214*, 23–37.
6. Cripps, R.M., and Olson, E.N. (2002). Control of cardiac development by an evolutionary conserved transcriptional network. Dev. Biol. *246*, 14–28.
7. Angelo, S., Lohr, J., Lee, K.H., Ticho, B., Breitbart, R., Hill, S., Yost, H.J., and Srivastava, D. (2000). Conservation of sequence and expression of *Xenopus* and zebrafish dHAND during cardiac, branchial arch, and lateral mesoderm development. Mech. Dev. *95*, 231–237.
8. Srivastava, D., Thomas, T., Lin, Q., Kirby, M.L., Brown, D., and Olson, E.N. (1997). Regulation of cardiac mesodermal and neural crest development by the bHLH transcription factor, dHAND. Nat. Genet. *16*, 154–160.
9. Srivastava, D., Cserjesi, P., and Olson, E.N. (1995). A subclass of bHLH proteins required for cardiac morphogenesis. Science *270*, 1995–1999.
10. McFadden, D.G., Barbosa, A.C., Richardson, J.A., Schneider, M.D., Srivastava, D., and Olson, E.N. (2004). The Hand1 and Hand2 transcription factors regulate expansion of the embryonic cardiac ventricles in a gene dosage-dependent manner. Development *132*, 189–201.
11. Charite, J., McFadden, D., and Olson, E.N. (2000). The bHLH transcription factor dHAND controls *Sonic hedgehog* expression and establishment of the zone of polarizing activity during limb development. Development *127*, 2461–2470.
12. Fernandez-Teran, M., Piedra, M.E., Kathiriya, I.S., Srivastava, D., Rodriguez-Rey, J.C., and Ros, M.A. (2000). Role of dHAND in the anterior-posterior polarization of the limb bud: Implications for the Sonic hedgehog pathway. Development *127*, 2133–2142.
13. Yamagishi, H., Olson, E.N., and Srivastava, D. (2000). The basic helix-loop-helix transcription factor, dHAND, is required for vascular development. J. Clin. Invest. *105*, 261–270.
14. te Welscher, P., Fernandez-Teran, M., Ros, M.A., and Zeller, R. (2002). Mutual genetic antagonism involving GLI3 and dHAND prepatterns the vertebrate limb bud mesenchyme prior to SHH signaling. Genes Dev. *16*, 421–426.
15. Yanagisawa, H., Clouthier, D.E., Richardson, J.A., Charite, J., and Olson, E.N. (2003). Targeted deletion of a branchial arch-specific enhancer reveals a role of dHAND in craniofacial development. Development *130*, 1069–1078.
16. Dai, Y.S., and Cserjesi, P. (2002). The basic helix-loop-helix factor, HAND2, functions as a transcriptional activator by binding to E-boxes as a heterodimer. J. Biol. Chem. *277*, 12606–12612.
17. McFadden, D.G., McAnally, J., Richardson, J.A., Charite, J., and Olson, E.N. (2002). Misexpression of dHAND induces ectopic digits in the developing limb bud in the absence of direct DNA binding. Development *129*, 3077–3088.
18. Firulli, B.A., Howard, M.J., McDaid, J.R., McIlreavey, L., Dionne, K.M., Centonze, V.E., Cserjesi, P., Virshup, D.M., and Firulli, A.B. (2003). PKA, PKC, and the Protein Phosphatase 2A influence HAND factor function: A mechanism for tissue-specific transcriptional regulation. Mol. Cell *12*, 1225–1237.
19. Murakami, M., Kataoka, K., Fukuhara, S., Nakagawa, O., and Kurihara, H. (2004). Akt-dependent phosphorylation negatively regulates the transcriptional activity of dHAND by inhibiting the DNA binding activity. Eur. J. Biochem. *271*, 3330–3339.
20. Huang, C.J., Tu, C.T., Hsiao, C.D., Hsich, F.J., and Tsai, H.J. (2003). Germ-line transmission of a myocardium specific GFP transgene reveals critical regulatory elements in the cardiac myosin light chain 2 promoter in zebrafish. Dev. Dyn. *228*, 30–40.
21. Topczewska, J.M., Topczewski, J., Shostak, A., Kume, T., Solnica-Krezel, L., and Hogan, B.L. (2001). The winged helix transcription factor Foxc1a is essential for somitogenesis in zebrafish. Genes Dev. *15*, 2483–2493.
22. Wang, A.Z., Ojakian, G.K., and Nelson, W.J. (1990). Steps in the morphogenesis of a polarized epithelium. I. Uncoupling the roles of cell-cell and cell-substratum contact in establishing plasma membrane polarity in multicellular epithelial (MDCK) cysts. J. Cell Sci. *95*, 137–151.
23. Yeaman, C., Grindstaff, K.K., and Nelson, W.J. (1999). New perspectives on mechanisms involved in generating epithelial cell polarity. Physiol. Rev. *79*, 73–98.
24. Vega-Salas, D.E., Salas, P.J., Gundersen, D., and Rodriguez-Boulan, E. (1987). Formation of the apical pole of epithelial (Madin-Darby Canine Kidney) cells: Polarity of an apical protein is independent of tight junctions while segregation of a basolateral marker requires cell-cell interactions. J. Cell Biol. *104*, 905–916.
25. Nelson, W.J., and Veshnock, P.J. (1987). Modulation of fodrin (membrane skeleton) stability of cell-cell contact in Madin-Darby Canine Kidney epithelial cells. J. Cell Biol. *104*, 1527–1537.
26. Westerfield, M. (1994). The Zebrafish Book (Eugene, OR: University of Oregon Press).
27. Alexander, J., Stainier, D.Y., and Yelon, D. (1998). Screening mosaic F1 females for mutations affecting zebrafish heart induction and patterning. Dev. Genet. *22*, 288–299.
28. Horne-Badovinac, S., Lin, D., Waldron, S., Schwarz, M., Mbamalu, G., Pawson, T., Jan, J., Stainier, D.Y.R., and Abdelilah-Seyfried, S. (2001). Positional cloning of *heart and soul* reveals multiple roles for PKC lambda in zebrafish organogenesis. Curr. Biol. *11*, 1492–1502.

Update

Current Biology
Volume 15, Issue 9, 10 May 2005, Page 879–881

DOI: https://doi.org/10.1016/j.cub.2005.04.045

Current Biology, Vol. 15, 879–881, May 10, 2005, ©2005 Elsevier Ltd All rights reserved. DOI 10.1016/j.cub.2005.04.045

Erratum

Hand2 Regulates Epithelial Formation during Myocardial Differentiation

Le A. Trinh,[1] Deborah Yelon,[2] and Didier Y.R. Stainier[1,*]
[1]Department of Biochemistry and Biophysics
Programs in Developmental Biology, Genetics, and Human Genetics
University of California, San Francisco
San Francisco, California 94143
[2]Developmental Genetics Program
Department of Cell Biology
Skirball Institute of Biomolecular Medicine
New York University School of Medicine
New York, New York 10016

*Correspondence: didier_stainier@biochem.ucsf.edu

As a result of a printing error in the March 8, 2005 issue of *Current Biology*, pp. 441–446, the data in Figures 1, 2, and 3 in this paper were compromised. Reprinted figures appear below. *Current Biology* regrets the error.

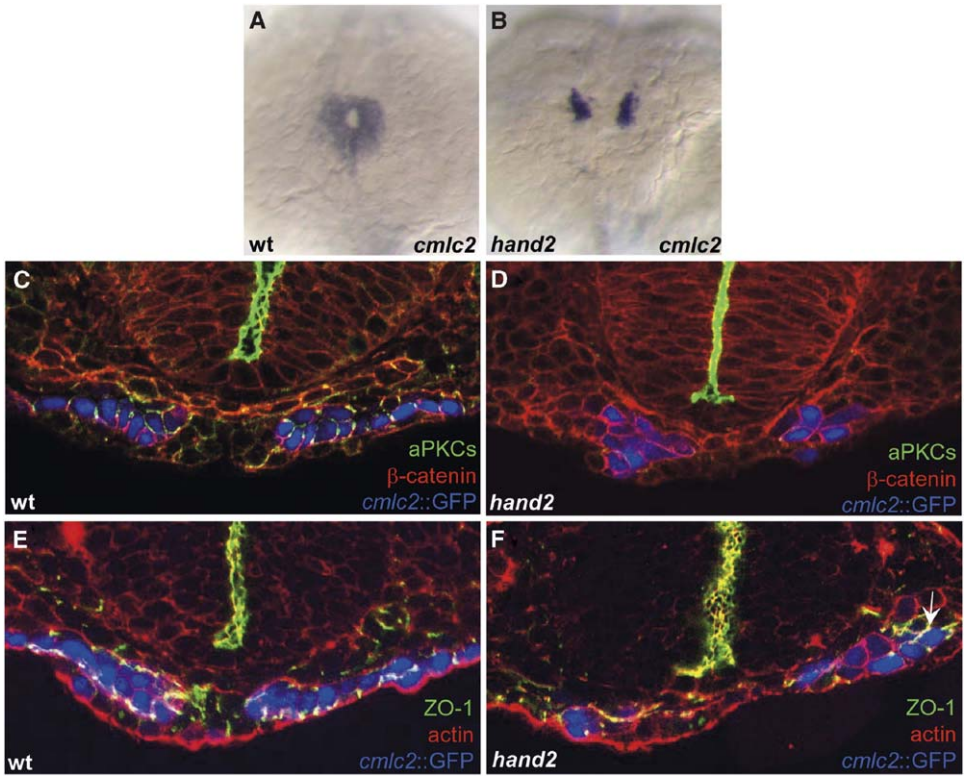

Figure 1. Hand2 Regulates the Formation of the Myocardial Epithelia

Wild-type (A, C, and E) and *hand2* (B, D, and F) mutants at the 20-somite stage. (A) and (B) show *cmlc2* expression; dorsal views, anterior is to the top.

(A) In wild-type embryos, the myocardial precursors have fused to form the cardiac cone.

(B) In *hand2* mutants, the myocardial precursors are reduced in number and fail to migrate to the midline and fuse.

(C–F) Transverse confocal images of *cmlc2*::GFP (false colored blue) transgenic embryos immunostained with antibodies against β-catenin (red) and aPKCs (green) (C and D) or against ZO-1 (green) and filamentous actin (rhodamine-phalloidin) (E and F); dorsal is to the top. (C and E) In wild-type embryos, the myocardial precursors form clear epithelia: Within the myocardial precursors, aPKCs are restricted to the apicolateral membranes, whereas ZO-1 is localized basally along the lateral membranes. Additionally, β-catenin and actin are enriched at the basolateral membranes. (D and F) In *hand2* mutants, the myocardial precursors do not localize junctional proteins asymmetrically. aPKC staining is absent from the myocardial precursors, and ZO-1 is localized cortically in some myocardial precursors. β-catenin and filamentous actin fail to restrict to the basolateral membranes and instead remain cortical in their localization.

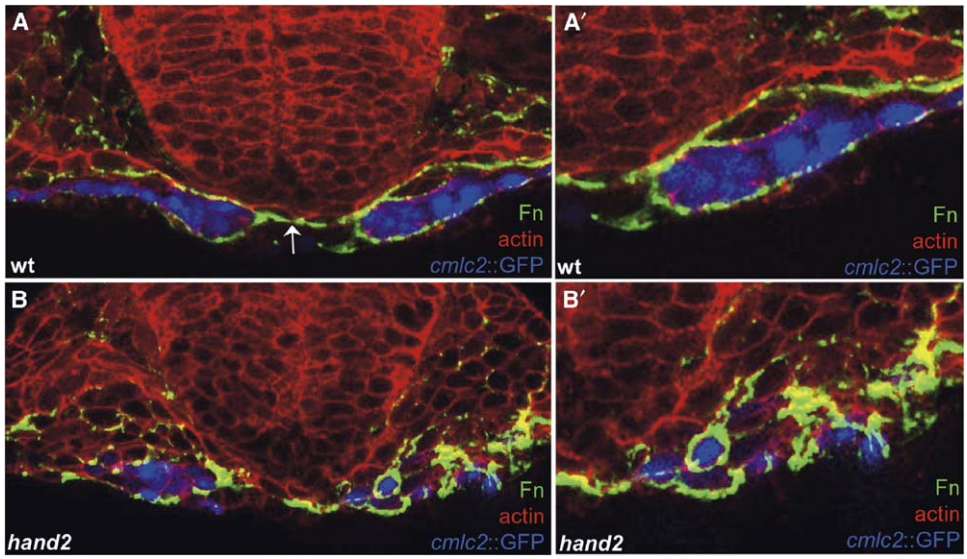

Figure 2. Defective Fibronectin Deposition in *hand2* Mutants

(A and B) transverse sections of *cmlc2*::GFP (false colored blue) transgenic embryos immunostained for β-catenin (red) and for Fibronectin (green) in wild-type (A and A′) and *hand2* (B and B′) mutant embryos at the 20-somite stage; dorsal is to the top. (A′) and (B′) show magnified views of the lower right corners of (A) and (B), respectively. In wild-type embryos (A and A′), Fibronectin is deposited around the basal surface of the myocardial epithelia (blue) and at the midline between the endoderm and endocardial precursors (arrow). (B and B′) In *hand2* mutants, Fibronectin deposition is disorganized and no longer restricted to the basal surface of the myocardial precursors.

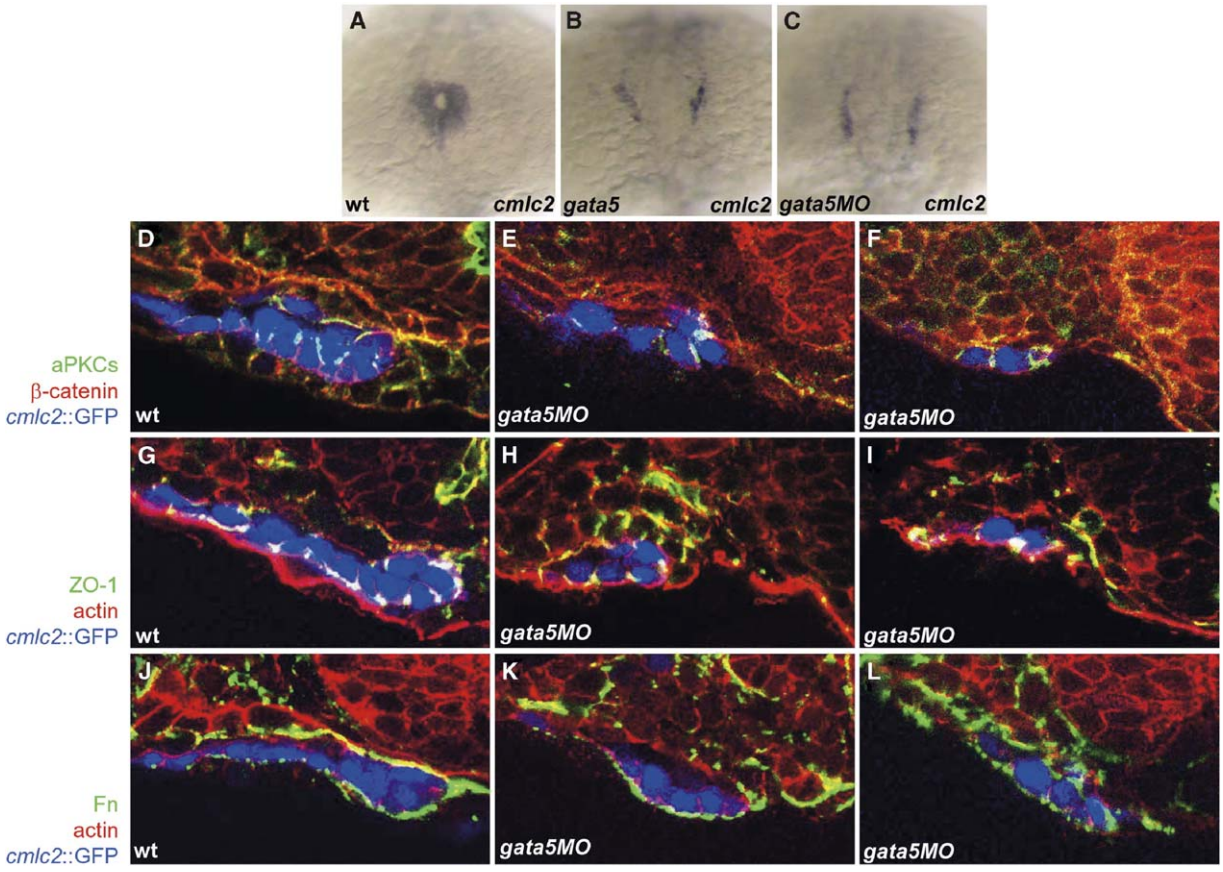

Figure 3. Myocardial Epithelia Organization in Embryos with Reduced Gata5 Function

Wild-type (A, D, F, and H), *gata5* mutants (*fau*) (B), and *gata5MO*-injected (C, E, F, H, I, K, and L) embryos at the 20-somite stage.

(A–C) *cmlc2* expression; dorsal views, anterior is to the top. (A) In wild-type embryos, the myocardial precursors have fused to form the cardiac cone. (B) In *gata5* mutants, the myocardial precursors are reduced in number and remain lateral. (C) In *gata5MO*-injected embryos, the myocardial precursors are reduced in number and remain lateral as in *gata5* mutants.

(D–L) Transverse confocal images of *cmlc2*::GFP (false colored blue) transgenic embryos immunostained with antibodies against β-catenin (red) and aPKCs (green) (D–F), ZO-1 (green) and filamentous actin (rhodamine-phalloidin) (G–I), or β-catenin (red) and Fibronectin (green) (J–L); the left side of the myocardium is shown; dorsal is to the top. (D and G) In wild-type embryos, the myocardial epithelia localize aPKCs to the apicolateral membranes and ZO-1 to the basal portion of the lateral membranes, and they localize β-catenin and filamentous actin basolaterally.

(J) Additionally, Fibronectin is deposited around the basal surface of the myocardial precursors. (E–H) In *gata5MO*-injected embryos, fewer myocardial precursors are present, but they form epithelia with aPKCs, ZO-1, β-catenin, and actin localized as in wild-type embryos. The asymmetric localizations of aPKCs, ZO-1, β-catenin, and actin are independent of the number of myocardial precursors; *gata5MO*-injected embryos with as few as two myocardial precursors (F and I) show asymmetric localization of these junctional markers. (K and L) Additionally, Fibronectin is deposited around the myocardial epithelia of *gata5MO*-injected embryos as in wild-type embryos (J).