

### Coordinating Morphogenesis: Epithelial Integrity during Heart Tube Assembly

Formation of the embryonic heart tube requires the medial migration and merger of bilateral precursor populations. A new study of zebrafish cardiogenesis published in this issue of *Developmental Cell* indicates that precursor migration involves formation of a coherent epithelium and that fibronectin plays an important role in maintaining cardiac epithelial integrity.

Organogenesis requires the precise arrangement of multiple populations of precursor cells into a specific three-dimensional configuration. How can many individual cell movements be coordinated to insure production of the organ's proper form? Two basic paradigms for coordinated morphogenesis are easy to imagine: cells could respond independently to directional cues recruiting them to the proper location, or cells could be led to their destination as an integrated group, maintaining contact with each other as they travel. The same strategies can be observed in larger-scale migrations: for example, both independent tourists and organized tour groups find their way from the entrance of the Louvre to the Mona Lisa, but the routes they take, and their experiences on the way, can be very different. To understand the mechanisms governing the architecture of a particular organ, it is important to analyze the cell behaviors that underlie its construction.

During heart formation, bilateral populations of cardiac precursors migrate toward the embryonic midline, where they merge to assemble a heart tube. How are cardiac precursors recruited medially—through independent movements or integrated group activity? In this issue of *Developmental Cell*, Trinh and Stainier (2004) address this topic in the context of the zebrafish heart. By analyzing the cellular features of zebrafish cardiomyocytes, they make a key observation: during medial migration, cardiomyocytes become intimately connected, forming coherent epithelia. Molecular markers support this conclusion: for instance, as their migration proceeds, the cardiomyocyte populations adopt hallmarks of polarized epithelia, including basolateral localization of  $\beta$ -catenin and apicolateral localization of atypical protein kinase C molecules. Furthermore, the most medial cardiomyocytes in each population begin to exhibit an elongated morphology, distinct from the cuboidal shapes of the other cardiomyocytes, suggesting that these medial cells could act as a leading edge. The coincident timing of migration and epithelialization support the notion that cardiac precursors move toward the midline as integrated groups.

Which factors coordinate the coherent migration of cardiomyocytes? In this regard, Trinh and Stainier focus their attention on the zebrafish *natter* (*nat*) mutation, a

valuable tool for the analysis of the requirements for cardiac epithelialization. The *nat* mutation was originally identified because it causes *cardia bifida*, a condition in which the bilateral populations of cardiomyocytes fail to travel to the midline, causing two separate hearts to form in lateral positions (Chen et al., 1996). Two specific aspects of the *nat* mutant phenotype suggest that *nat* activity is essential for the coordination of coherent cardiomyocyte migration: *nat* mutant cardiomyocytes do not exhibit normal epithelial maturation and, in some *nat* mutants, individual cardiomyocytes appear to drift away from the main populations (Trinh and Stainier, 2004). In particular, examination of molecular markers indicates that *nat* mutant cardiomyocytes do not exhibit normal apicobasal polarity or create proper junctional complexes; these epithelialization defects are likely to account for the migration defects in *nat* mutants.

The coupling of migration and epithelialization defects in *nat* mutants clearly raises interest in the identity of the *nat* gene. Through candidate gene analysis, Trinh and Stainier demonstrate that the *nat* locus encodes Fibronectin (Fn), a major component of the extracellular matrix. Fn deposition is detectable in a variety of tissues during embryogenesis; two particular locations appear most germane to cardiomyocyte migration. During the relevant stages, Fn is found laterally at the basal surface of the cardiomyocytes and also medially adjacent to the endocardium, an endothelial population located near the destination of the migrating cardiomyocytes. Does cardiomyocyte migration require Fn deposition in both of these areas? It appears that the medial deposition of Fn is generated by the endocardium: when the endocardium fails to form, as in *cloche* (*clo*) mutants (Stainier et al., 1995), medial Fn deposition is absent, but lateral deposition appears normal. Interestingly, cardiomyocyte migration does proceed in *clo* mutants, albeit at an altered pace, suggesting that lateral Fn deposition, adjacent to the cardiomyocytes, has the major impact on cardiomyocyte epithelialization and movement. Thus, cardiomyocyte-Fn interactions coordinate cardiac morphogenesis by maintaining cardiac epithelial integrity.

The importance of Fn during cardiac morphogenesis has also been noted in other vertebrates; for example, mice lacking Fn exhibit defects in cardiomyocyte migration (George et al., 1997), and an anti-Fn antibody can block heart formation in chick embryos (Linask and Lash, 1988). Building on these prior studies, Trinh and Stainier advance our understanding of the impact of Fn function on cell-cell interactions. During zebrafish cardiogenesis, Fn is not simply a passive substrate, paving the way for cardiomyocyte travel; rather, Fn plays an important role in promoting the interconnection of the overlying cardiac epithelium. This activity of Fn may be distinct from its roles in different developmental contexts, such as its function in regulating cellular intercalations during gastrulation (Marsden and DeSimone, 2001) or its function in cleft formation during branching morphogenesis (Sakai et al., 2003). Perhaps each of these contexts features different combinations of factors, such as specific sets of integrins, that interact with Fn,

thereby generating distinct impacts of Fn on cellular architecture. Along these lines, it will be particularly important to identify factors that collaborate with Fn during cardiomyocyte migration; the contributions of such factors may account for the phenotypic variability observed in *nat* mutants (Trinh and Stainier, 2004). Additional zebrafish mutants with cardiac migration defects will undoubtedly be key reagents for these future efforts. Analyses of mutations causing epithelial defects are likely to indicate more molecules required for cardiomyocyte coherence; furthermore, mutations that block migration while preserving epithelialization may lead to the identification of forces that drive integrated movement in a medial direction.

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## Formins Coming into Focus

**Two new crystal structures published in *Cell* and *Molecular Cell* provide the first clues about how fascinating proteins called formins interact with actin filaments.**

Multidomain proteins called “formins” initiate the actin filaments that form cytokinetic contractile rings as well as other actin filament bundles in both animal and fungal cells (reviewed by Wallar and Alberts, 2003). In a well-documented example, a formin-containing patch stimulates the growth of actin filament cables in budding yeast. These cables originate in the bud and grow into the mother cell to establish actin filament tracks for the transport of vesicles from mother to bud. These filaments grow by adding up to 100 subunits per second at their barbed ends in association with the formin patch (Yang and Pon, 2002). Two budding yeast formins participate in both cable formation and cytokinesis, while the three fission yeast formins function exclusively in one of three cellular processes, cytokinesis, interphase cable formation, or mating (Feierbach and Chang, 2001). Animal formins also participate in stress fiber formation and interact with the tips of microtubules (Wallar and Alberts, 2003).

The ability of formins to nucleate actin filaments *in vitro* requires only a conserved “formin homology-2 (FH2) domain” (Kovar et al., 2003; Li and Higgs, 2003; Moseley et al., 2003; Pruyne et al., 2002; Sagot et al., 2002), the subject of the two crystallographic studies. FH2 alone can influence monomer addition onto and capping protein interaction with the barbed end. An adjacent FH1 domain binds profilin and is required for function *in vivo*. Rho-family GTPases regulate formins by overcoming

### Selected Reading

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autoinhibition of the domains that interact with actin (Li and Higgs, 2003).

One of the exciting new structures is a homodimer of FH2 domains from budding yeast formin Bni1p, a construct with full actin nucleation activity (Xu et al., 2004; Figure 1). The other new structure is a monomer consisting of a proteolytically defined, mouse mDia1-FH2 domain that caps actin filament barbed ends but does not nucleate actin polymerization (Shimada et al., 2004). A monomer of Bni1pFH2 has similar properties (Moseley et al., 2003; Xu et al., 2004), so dimerization of FH2 domains appears to be crucial for nucleation activity.

The Bni1p FH2 construct is a donut-shaped homodimer held together by an extended N-terminal “lasso” that wraps around a “post” on the tail end of its partner. The face of the donut has dimensions of  $8 \times 10.5$  nm with a hole of  $3 \times 5.5$  nm, too small to accommodate either an actin monomer or the cross-section of an actin filament which is about  $5.5 \times 8.5$  nm. The shorter mDia1 FH2 construct of Shimada et al. lacks the N-terminal lasso, explaining why it is monomeric. Comparison of the two structures establishes that the FH2 structure has been highly conserved during evolution. The density of conserved surface residues is much higher on one side of the Bni1p FH2 donut, so Xu et al. postulate that this side interacts with actin. Accordingly, substitution of two residues on this side severely compromises function in actin polymerization assays.

The segment of 17 “linker” residues connecting the lasso to the body of the FH2 domain appears to be flexible given the following observations: the linker is mildly disordered in the Bni1p crystal; the linker is sensitive to proteolytic cleavage; linker segments vary in length and composition between formins; and Bni1p with 4 residues deleted from the linker crystallizes with the bodies of the two FH2 domains packed completely differently than the wild-type construct. This deletion construct is more active than the wild-type FH2 in spite