

Cdc42 and Tinman march to the same beat

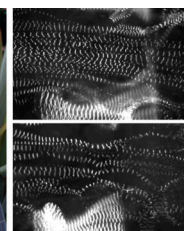
Study describes a conserved genetic network that regulates heart function in flies and mammals.

D*rosophila* may be small, but you can't accuse them of lacking heart. Unless they're missing the transcription factor Tinman, that is, in which case they fail to form a heart during embryogenesis (1, 2) or, if *tinman* is deleted later in development, they fail to maintain adult cardiac function (3). Qian et al. now reveal how Tinman works in conjunction with the small GTPase Cdc42 to regulate the heart in both flies and mice (4).

Tinman lies at the top of a complex hierarchical network that makes and maintains the heart. Much of this network—including Tinman itself—is conserved in mammals. "So we can use *Drosophila* to learn about the fundamental genetic mechanisms that are important for the development and function of the heart," says Rolf Bodmer, from the Sanford-Burnham Medical Research Institute in La Jolla, CA.

To discover new genes that work with *tinman* to maintain adult heart function, Bodmer and colleagues performed a genetic screen for mutations that cause heart failure in flies heterozygous for a deletion of *tinman* (4). Using this approach, Qian et al. found a genetic interaction between *tinman* and *Cdc42*. Whereas flies lacking one copy of either *tinman* or *Cdc42* were healthy, animals lacking one copy of both genes had multiple heart defects. "The flies' hearts didn't beat as regularly, and the heart myofibrils weren't arranged in a parallel, organized fashion," Bodmer recounts. Expressing a dominant-negative version of Cdc42 in cardiac tissue produced similar effects.

The heart arrhythmias in *tinman/Cdc42* double heterozygotes prompted Qian et al. to investigate the expression in these flies of several ion channels that regulate heart beat patterns. The expression of two potassium channels—dSUR and slowpoke—was down-regulated in double-heterozygous flies, and in vitro experiments revealed that Tinman and



FOCAL POINT

(Left to right) Li Qian, Joshua Wythe, Rolf Bodmer, and colleagues (not shown) describe a conserved genetic network that governs heart function in both flies and mice. The transcription factor Tinman (Nkx2-5 in mice) inhibits expression of the microRNA miR-1, which otherwise limits expression of the small GTPase Cdc42. Tinman and Cdc42 then interact to enhance expression of several genes required for cardiac myofibril organization and regular heart contraction, including the potassium channels dSUR and Slowpoke. Fly hearts lacking just one copy of *dSUR* (right image, top) show a regular myofibril arrangement, as indicated by α -actinin staining. But the additional loss of one copy of *Cdc42* disrupts this organization (bottom).

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Cdc42 act together genetically to promote *dSUR* expression by regulating its enhancer element. Furthermore, flies lacking one copy of *Cdc42* and one copy of either *dSUR* or *slowpoke* also had irregular heartbeats and disorganized myofibrils.

Complete loss of either *Cdc42* or *Nkx2-5* (the mammalian homologue of *tinman*) causes heart defects in mice (5, 6). "So we wanted to see if the combination of Tinman and Cdc42 also affected mouse heart function," Bodmer explains. Qian et al. found that the loss of one copy of each gene in the mouse, as in the fly, compromised cardiac contraction and rhythmicity, similar to double-heterozygous flies. "However, we didn't see any aberrations in myofibril organization in the mice," Bodmer says.

Therefore, although the precise phenotypes differ, Cdc42 and Tinman/Nkx2-5 genetically interact in both mouse and fly hearts. But what is the nature of this interaction? Qian et al. found that Nkx2-5 inhibited the

expression of a microRNA, miR-1, that targets Cdc42 mRNA both in vitro and in mice. miR-1 appears to serve as an intermediate between Tinman and Cdc42 in flies as well. *Drosophila* overexpressing miR-1 in their

hearts had decreased *Cdc42* and *slowpoke* levels as well as increased arrhythmias.

Mutations in the human homologue of Nkx2-5 are associated with a variety of cardiomyopathies (7), suggesting that Cdc42 may also influence human heart disease. "*Drosophila* genetics is a unique way of identifying polygenic interactions that might aggravate a disease," Bodmer says. Qian et al.'s collaborators performed a small-scale screen of human samples and found one congenital heart disease patient with a mutated version of Cdc42 not found in control samples. Bodmer now wants to examine families carrying mutations in Nkx2-5 to see whether they have different variants of Cdc42 that could explain differences in the severity of their cardiomyopathies. "We'd also like to look for more genes that interact with *tinman* or with other factors that we know are important for the heart," Bodmer says.

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