Gene regulatory networks consisting of subcircuits of transcription factors and intercellular signaling molecules are key to an understanding of the complex mechanisms of animal development and evolution [Davidson and Erwin 2006]. One of the most intensively studied genetic networks is that for the formation of the animal heart. Since the discovery of the “tinman” gene (Bodmer 1993) and its vertebrate homolog, Nkx2.5 [Lyons et al. 1995], many genes have been found to be involved in heart development in a wide range of animals, from insects to mammals. Actually, it is well established that a gene circuit consisting of GATA, Nkx, and Hand is evolutionarily highly conserved. This gene circuit constitutes a “kernel,” which is evolutionarily inflexible and performs essential regulatory functions in building a body part [Davidson and Erwin 2006]. Analyses of vertebrate heart development revealed that the differentiation of cardiac muscle cells and morphogenesis of the heart are governed by this heart kernel gene regulatory network [Cripps and Olson 2002; Harvey 2002, Buckingham et al. 2005]. An important question to be answered about the formation of the heart in vertebrates and other chordates is how this kernel is turned on at the earliest stages of the heart cell specification process to establish the heart field.

Another intriguing question in chordate heart formation is how the dual- or multichambered heart of vertebrates evolved. It is generally believed that the ancestral chordate resembled the present-day ascidian tadpole. The morphogenetic movement of heart precursor cells during ascidian larval development and metamorphosis is reminiscent of those in vertebrates [Davidson and Levine 2003]. However, the ascidian tube-like heart lacks chambers. The innovation of the chambered heart was a key event in vertebrate evolution, because the chambered heart generates one-way blood flow with high pressure, a critical requirement for the efficient blood supply of large-body vertebrates.

In this issue of Genes & Development, Davidson et al. (2006) addressed these questions by examining the function of an Ets-containing transcription factor in the tunicate, Ciona intestinalis. They found that Ci-Ets1/2 (because this is one of two Ciona orthologs for vertebrate Ets1 and Ets2 and Drosophila pointed, it was originally called Ci-ets/poindet2) [Yagi et al. 2003] establishes the heart field, probably through an FGF signal acting downstream from Ci-Mesp, a basic helix–loop–helix transcription factor gene required for the initial specification of heart precursor cells [Satou et al. 2004]. Targeted inhibition of Ci-Ets1/2 or FGF receptor function blocks heart formation. Moreover, targeted expression of a constitutively active Ci-Ets1/2 causes the expansion of the heart field by forced recruitment of larval tail muscle cells that express Ci-Mesp. Interestingly, this heart field expansion, evoked by the subtle alteration of the heart genetic program, caused a morphological change—that is, to a heart with two compartments.

Mesp, Ets, and FGF signaling cascade in Ciona heart development

Ascidians have an open blood–vascular system, and its blood flow, produced by peristaltic contractions of the heart, is regularly reversed [Ichikawa and Hoshino 1967]. The ascidian heart is formed after metamorphosis as a simple tube-like structure with a single-layered myoepithelium that is continuous with a single-layered pericardial wall. It lacks chambers and endocardium. Cell lineage tracing of heart-forming cells reveals that they descend from a pair of blastomeres named B7.5 of the 110-cell stage embryo [ Hirano and Nishida 1997]. B7.5 also contributes to the anterior tail muscle cells of the tadpole.

The heart regulatory kernel—GATA, Nkx, and Hand—is also conserved in the Ciona embryo. A previous study showed that this highly conserved kernel is under the control of Mesp [Satou et al. 2004]. Ciona Mesp, the sole ortholog of vertebrate Mesp1 and Mesp2, is expressed in B7.5 blastomeres where it regulates Nkx, HAND, and NoTrlc (a nonorthologous gene similar to vertebrate Hand, and also called Hand-like). Knockdown
of Mesp results in failure of proper specification and migration of the heart precursors [Satou et al. 2004]. In mice, Mesp is expressed in mesodermal cells including the region that forms the heart; no heart is formed in Mesp1 and Mesp2 double knockout mice [Kitajima et al. 2000]. There is no indication that Mesp is involved in heart formation in nonchordate animals. Thus, Mesp provides a critical link between the mesoderm and heart field in chordates [Fig. 1]. However, in both ascidian and mouse embryos, only some of the cells that express Mesp give rise to the heart. How is this achieved?

Davidson et al. (2006) have addressed this important question. By using elegant confocal imaging and transient transgenesis in Ciona embryos, they showed that Ci-Ets1/2, a transcriptional effector of receptor tyrosine kinase [RTK] signaling, acts downstream from Mesp to establish the heart field. Mesp expression begins at the 110-cell stage [immediately before gastrulation] in B7.5 and persists in B7.5-derived progenitors of the heart and larval tail muscles (Satou et al. 2004). Ci-Ets1/2 expression initiates during gastrulation in four cells derived from two Mesp-expressing founder cells. After gastrulation, these cells divide asymmetrically, and the smaller rostral daughter cells exhibit RTK activation and form the heart progenitors while the larger caudal daughters give rise to the larval tail muscle. Inhibition of RTK signaling and targeted inhibition of Ci-Ets1/2 function block heart specification. Conversely, targeted expression of a constitutively active form of Ci-Ets1/2 causes a fate change of the larger caudal daughters to form heart cells [Davidson et al. 2006].

The asymmetry of RTK activation in the smaller rostral daughter cells (heart) and larger caudal daughters (larval tail muscle) is probably induced by FGF signaling. The Ciona genome contains six Fgf genes: Fg3/7/10/22, Fg4/5/6, Fg8/17/18, Fg9/16/20, Fg11/12/13/14, and Fg1L (Satou et al. 2002). Although Davidson et al. (2006) did not show which FGF is responsible for heart induction, Fg9/16/20, which is the sole ortholog of vertebrate FGF9, FGF16, and FGF20, is the most probable candidate (they simply call this FGF9). This is because this Fgf gene is expressed at the right time and place [Imai et al. 2002, Tokuoka et al. 2004], and because knockdown of this gene by a morpholino oligonucleotide perturbs the expression of at least some of the heart precursor-specific genes [Imai et al. 2006]. Indeed, targeted inhibition of FGF receptor function blocks heart specification.

The expression of Mesp1 is the earliest sign of heart formation in mouse embryos, but the detailed molecular mechanisms that connects it to the GATA/Nkx/Hand regulatory kernel in vertebrate systems has not yet been uncovered. A series of studies of ascidian heart development provides a foundation for exploring the more complicated mechanisms of heart formation in vertebrates. Although there are several reports for FGF signaling in vertebrate heart development [e.g., Marguerie et al. 2006], the underlying mechanism is not known [Fig. 1]. Because the chordate heart kernel is probably highly conserved, FGF signaling may work similarly in vertebrates and ascidians.

Insight into an evolutionary change in morphology evoked by changes in the genetic program

Invertebrate chordates have nonchambered hearts while all extant vertebrates have hearts with two or more chambers [Moorman and Christoffels 2003]. As mentioned above, the ascidian heart is a simple tube-like organ, while the vertebrate heart has chambers partitioned by septa, and the chambers sequentially contract under the control of the conduction system. It is highly likely that vertebrates evolved the dual-chambered heart through modification of the gene regulatory networks responsible for heart formation in ancestral chordates. Davidson et al. (2006) have also presented a suggestive result on the evolution of the vertebrate multichambered heart.

All Mesp-expressing cells of Ciona embryos [half normally form larval tail muscle cells] were converted into heart fate by targeted expression of constitutively activated Ci-Ets1/2. This treatment resulted in a doubling of the heart field. Some of the resulting juveniles have a heart with two compartments that beat sometimes independently and sometimes in a coordinated fashion [see Fig. 7 in Davidson et al. 2006]. This mutant heart clearly needs to be further characterized morphologically, anatomically, developmentally and electrophysiologically. This two-compartment heart should lack a conduction system and other accessory structures characteristic of vertebrate hearts, like valves and septa. The mutant heart is expected to beat by peristalsis, rather than the sequential contraction of two compartments. It also probably lacks the dynamic suction pumping seen in the heart tube appearing at the beginning of vertebrate heart development [Forouhar et al. 2006]. Nevertheless, the hypothesis proposed by Davidson et al. (2006) presents a new possibility for our understanding of evolution.

The hypothesis includes two major points. First, increasing the number of primordial heart cells can occur by recruitment of competent cells that normally follow an alternative fate, rather than by excess cell divisions. Excess cell divisions do not expand the net volume of cells following a given fate, whereas cell recruit-
dependent compartments, how was synchrony achieved through subtle changes in a genetic program, such as altered expression of FGF9/16/20. It is therefore possible that this strategy is a general pathway of evolutionary change.

One important suggestion here is that anterior tail muscle cells, which were recruited to the mutant heart, may be dispensable because they do not contribute to any adult tissues in normal development. That is, these tail muscle cells are not set-aside cells, as proposed by Eric Davidson’s group [e.g., Davidson and Erwin 2006]. The life history of most animals is biphasic, consisting of larval and adult stages. An important question is how tissues and organs of the adult are formed through this biphasic mode of development. The concept of set-aside cells stipulates that the progenitors of adult organs are kept developmentally quiescent in larvae, separated from embryonic cells engaged in the formation of larval organs and tissues. Results of Davidson et al. [2005, 2006] clearly indicate that when the fate of larval organ-forming cells is changed to form the adult organ, they can be engaged in the formation of adult organs, suggesting the developmental flexibility of non-set-aside cells.

Second, the model for heart evolution proposed by Davidson et al. [2006] also requires that ascidians possess an intrinsic mechanism for making multiple compartments. What does this mean? One possibility is that the ancestral chordate had a multicompartment heart that worked by peristalsis. If so, this ancestral multicompartment heart evolved into the multichambered vertebrate heart with sequential contractions. On the other hand, compartmentalization was lost in the tunicate lineage, possibly by reduction in the number of heart precursor cells, but the ascidian has retained an intrinsic gene circuit for making compartments. A simpler possibility is that expansion of cardiac field produced two interconnected hearts in one place. Although either of these possibilities [and maybe other possibilities] cannot be rejected, the latter looks more likely. In this case, there is no need to invoke the evolution of new gene regulatory circuits. Nor do we need to change our view that the ancient chordate contained a simple tube-like heart (an ancient nerve cord, ventrally by endodermal strand, and laterally by muscle (Corbo et al. 2001; Satoh 2003; Satoh et al. 2003). This means that the basic body plan common to the chordates can be studied much more easily in the ascidian system than in vertebrates. Ciona heart speci-
fication is a good example. Single-cell resolution was obtained based on the sole ortholog of three Mesp genes in vertebrates.

Third, it is worth mentioning another characteristic feature of ascidian embryogenesis, namely, its mosaic mode of development. “Mosaic” contrasts with the “regulatory” mode of development. The former depends on the autonomous specification of individual embryonic cells, while the latter depends on cell–cell communication. In ascidians, the mosaic mode of development extends to the formation of entire organs and tissues. Ciona tadpole larvae with knockdown of Mesp function metamorphose into juveniles that look normal but lack hearts. The heartless, “tinman”-like juveniles can be alive for a few weeks before they die (Satou et al. 2004). Ciona larvae with knockdown of Twist-like1 function metamorphose into juveniles with normal morphology but lacking most blood cells. These bloodless juveniles also live for several days before death (Tokuoka et al. 2004). Thus, the development of heart and blood are independent of other organ-forming processes in juveniles. This high degree of mosaicism permits the formation of juveniles with mutant two-compartment hearts upon ectopic activation of Ets1/2. This feature of ascidian organogenesis is particularly advantageous for future studies of the gene regulatory networks underlying the adult body plans.

Toward a comprehensive understanding of gene regulatory networks for heart development in chordates

One of the final goals of molecular developmental biology is describing the developmental processes in the context of gene regulatory networks, beginning with maternal factors and ending with the expression of heart-specific genes. The expression of Mesp depends on localized maternal factors, including β-catenin and the Zic-like transcription factor, Macho1 (Satou et al. 2004). Macho1 activates Tbx6b and/or Tbx6c, which in turn, activates Mesp (Davidson et al. 2005). β-Catenin might also contribute directly toward the regulation of Mesp. This should be resolved at the single cell level in the near future. In addition to these early studies, the study of Davidson et al. (2006) has revealed that Ets1/2, which is probably activated by Fgf9/16/20 or another Fgf, fill the gap between Mesp and the GATA/Nkx/Hand regulatory kernel. Thus, the ascidian heart system may be closest to the goal of a comprehensive understanding of molecular mechanisms from the egg to organogenesis.

As we described above, C. intestinalis provides a valuable experimental system both for developmental biology and for evolutionary biology. The simplicity of its development and compactness of its genome makes this animal ideally suited for studying the molecular mechanisms of development. As it belongs to the closest sister group of the vertebrates (Delsuc et al. 2006), understanding the Ciona system provides a key for understanding the origin and evolution of chordates and/or vertebrates. To date, only limited conservation has been found in the gene regulatory networks for early fate specification of this animal and vertebrates (e.g., Imai et al. 2006). The heart differentiation mechanism represents such an example. Developmental mechanisms of Ciona, even if divergent, will illuminate developmental and evolutionary mechanisms of the chordate embryos.

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Gene regulatory networks for the development and evolution of the chordate heart

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