genesis 51:383–409 (2013)

REVIEW

Encoding Anatomy: Developmental Gene Regulatory Networks and Morphogenesis

Charles A. Ettensohn*

Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, Pennsylvania

Received 11 December 2012; Revised 6 February 2013; Accepted 7 February 2013

Summary: A central challenge of developmental and evolutionary biology is to explain how anatomy is encoded in the genome. Anatomy emerges progressively during embryonic development, as a consequence of morphogenetic processes. The specialized properties of embryonic cells and tissues that drive morphogenesis, like other specialized properties of cells, arise as a consequence of differential gene expression. Recently, gene regulatory networks (GRNs) have proven to be powerful conceptual and experimental tools for analyzing the genetic control and evolution of developmental processes. A major current goal is to link these transcriptional networks directly to morphogenetic processes. This review highlights three experimental models (sea urchin skeletogenesis, ascidian notochord morphogenesis, and the formation of somatic muscles in *Drosophila*) that are currently being used to analyze the genetic control of anatomy by integrating information of several important kinds: (1) morphogenetic mechanisms at the molecular, cellular and tissue levels that are responsible for shaping a specific anatomical feature, (2) the underlying GRN circuitry deployed in the relevant cells, and (3) modifications to gene regulatory circuitry that have accompanied evolutionary changes in the anatomical feature. genesis 51:383–409. © 2013 Wiley Periodicals, Inc.

Key words: ascidian; notochord morphogenesis; drosophila; muscle development; sea urchin; skeletogenesis; transcriptional networks

INTRODUCTION: MORPHOGENESIS AND GENE REGULATORY NETWORKS

The anatomy of multicellular organisms is encoded in the genome. It is the process of development that deciphers this genetic information, creating three-dimensional (3D) morphology from a linear string of nucleotides. The genomic control of anatomy is not only the central problem of development, it is also of critical importance to evolutionary biology, as changes in morphology arise through modifications to the developmental programs that transform DNA sequence into anatomy. By analyzing the development of diverse species, it is possible to uncover evolutionary changes in gene regulatory programs that are associated with morphological evolution.

There is a rich tradition of experimental work that has addressed the cellular and molecular mechanisms of morphogenesis (the shaping of tissues and organs during development). It is widely accepted that an understanding of morphogenesis will require the integration of information at multiple levels of biological organization, from genes and the biochemical functions of the proteins they encode, to the properties of individual cells (e.g., cell shape, motility, adhesion, proliferation, etc.), to the behaviors and mechanical characteristics of tissues (Fig. 1). Morphogenesis is even more complicated than this simple, linear flow of information might suggest. For example, there is an interplay between gene expression and cellular mechanics whereby differential gene expression drives the morphogenetic behaviors of cells and, in reciprocal fashion, the mechanical forces that are exerted on cells and tissues during morphogenetic processes act as signals that regulate gene expression in the cells that experience these forces (Dupont *et al*., 2011; Poh *et al*., 2012).

*Correspondence to: Charles A. Ettensohn, Department of Biological Sciences, Carnegie Mellon University, 4400 Fifth Avenue, Pittsburgh, PA 15213. E-mail: ettensohn@andrew.cmu.edu

Contract grant sponsor: National Science Foundation
Published online 21 February 2013 in
Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/dvg.22380

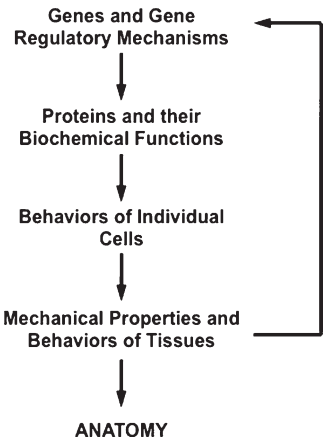

FIG. 1. A hierarchical view of morphogenesis. The development of form is encoded by the genome. Gene regulatory mechanisms direct the formation of gene products with biochemical functions that determine the properties of cells at an individual level (e.g., cell shape, motility, adhesion, and proliferation). These cellular properties generate mechanical forces that are integrated within larger cell collectives (e.g., cell sheets), thereby controlling the form of tissues and, ultimately, higher-order anatomy. Feedback occurs when mechanical forces acting on cells or tissues alter programs of gene expression.

The specialized properties of cells and tissues that drive morphogenetic processes, like other specialized properties of cells, arise as a consequence of differential gene expression. Until recently, the genetic control of development (and morphogenesis) had been studied primarily at the level of single genes, that is, by identifying genes with developmental functions through the use of genetic screens, targeted gene knockouts, and other approaches. Using these methods, hundreds of genes have been shown to play essential roles in various morphogenetic processes. These genes encode proteins of every known functional class, including transcription factors (TFs), signaling molecules, metabolic regulators, and various classes of proteins that likely play a more direct role in morphogenesis, such as cytoskeletal proteins and their regulators, cell adhesion proteins, and many others. Studies concerning the genetic basis of morphogenesis are often limited in two significant ways, however, (1) the genetic control of the morphogenetic process is limited to the identification of a single gene, or perhaps one gene product and a small number of interacting proteins, and (2) the relationship between the gene(s) and the morphogenetic process is murky, usually because the cellular and molecular mechanisms of the morphogenetic process are poorly understood (i.e., the morphogenetic machine is a "black box"). In an extreme case, the function of a specific TF or signaling molecule might be perturbed experimentally, leading to a disruption of the morphology of a particular organ or tissue. By itself, this kind of observation provides little insight concerning the mechanisms of the morphogenetic process, either at the genetic level or at the level of cell and tissue behavior.

Gene regulatory networks (GRNs) have proven to be powerful conceptual and experimental tools for analyzing the genetic control and evolution of developmental processes (Stathopoulos and Levine, 2005; Davidson, 2010; Peter and Davidson, 2011; Van Nostrand and Kim, 2011; Wunderlich and DePace, 2011). GRN analysis has been driven by many important technical advances, including next-generation DNA sequencing, new approaches for interfering with gene function, and genome-wide approaches for quantifying gene expression, mapping active promoters and enhancers, and identifying TF binding sites. GRNs can be viewed as elaborate networks of regulatory genes (that is, genes that encode TFs, and the cis-regulatory elements (CREs) to which these TFs bind) within a single cell type or embryonic lineage (Fig. 2). It is convenient to depict GRNs as wiring diagrams that represent interactions among genes in the network, although such diagrams are clearly abstractions, especially in the case of developmental networks, which are highly dynamic. Depending upon the resolution of the particular experimental analysis, regulatory inputs may be understood only at the level of functional (epistatic) gene interactions, or they may be understood much more completely, that is, at the level of direct interactions betweens specific TFs and their binding sites within CREs. Some of the same methods that are used in GRN analysis can be applied to various post-transcriptional regulatory networks that operate in embryonic cells, for example, splicing networks and miRNA networks (Hobert, 2006; Taliaferro *et al.*, 2011; Gagan *et al.*, 2012), and in the future it should be possible to integrate such networks with transcriptional GRNs.

Developmental GRNs are elaborated in a progressive fashion within specific embryonic lineages or territories. Their deployment can be viewed as layered; for example, cell specification is driven by interactions among early regulatory genes, which leads to the engagement of additional layers of regulatory genes, and finally to the activation of various nonregulatory genes (sometimes referred to as "differentiation" genes) that represent the output of the network (Fig. 2). The functions of these terminal genes are often poorly understood, but some encode proteins that play a direct role in driving key morphogenetic processes. The identification of such "morphoeffector genes" and the elucidation of their regulatory control are critically important, because it is precisely these regulatory connections that provide a glimpse of how anatomy is encoded in the genome.

This review highlights three experimental models that are currently being used to analyze the genetic control of anatomy by integrating information of several

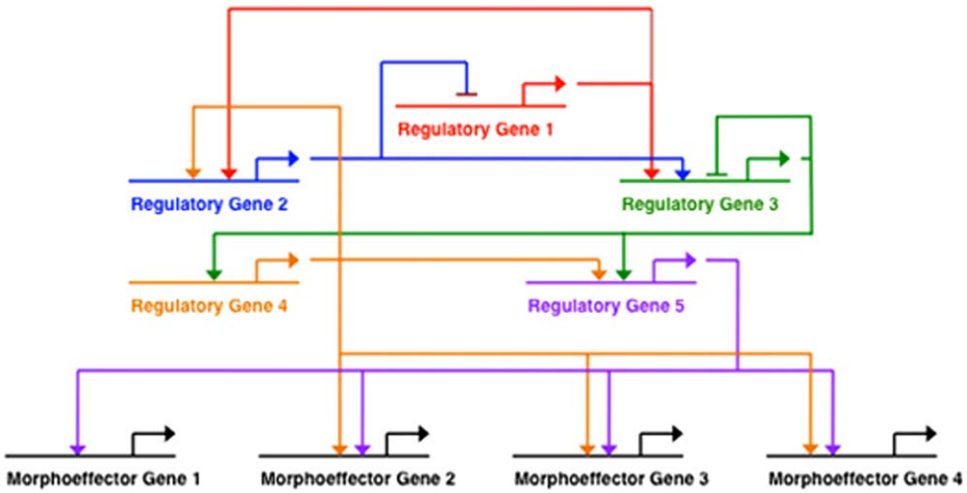

FIG. 2. A simple, hypothetical developmental GRN. The upper layers of the network consist of "regulatory" genes (i.e., genes that encode TFs), while the final output of the network is the activation of a suite of "morphoeffector" genes (i.e., genes that encode proteins that have a direct role in morphogenesis, including adhesion proteins, cytoskeletal regulators, ECM proteins, etc). Examples of positive (arrowheads) and negative (bars) interactions are included, representing transcriptional activation and repression, respectively. Ideally, interactions represent the direct binding of TFs to CREs of target genes (note that CREs are not shown directly), but often they represent epistatic interactions. Included are several examples of feed-forward interactions (e.g., Reg. Gene 1 > Reg. Gene 2, Reg. Gene 2 > Reg. Gene 3, Reg. Gene 1 > Reg. Gene 3). A circuit diagram like this might represent a snapshot of a GRN at a single developmental stage or a time-averaged view of several developmental stages.

important kinds: (1) morphogenetic mechanisms at the molecular, cellular and tissue levels that are responsible for shaping a specific anatomical feature, (2) the underlying GRN circuitry deployed in the relevant cells, and (3) modifications to this gene regulatory circuitry that have accompanied evolutionary changes in the anatomical feature. Few experimental models are amenable to the various methodologies that are required to address all of these issues. Each of the three model systems considered in this review has been analyzed by a combination of gene perturbation and gene expression studies, *in vivo* light optical imaging, cell lineage analysis, and comparative embryology, and all three have significant resources for genomics and systems biology.

CASE STUDIES

Skeletogenesis in Sea Urchins

All adult echinoderms possess an endoskeleton composed of calcite, a crystalline form of calcium carbonate. The skeleton supports the body and probably provides some defense against predation. With respect to members of the phylum that produce a skeleton early in development, including sea urchins (see below), the skeleton is the primary determinant of the distinctive, angular shape of the larva and influences its orientation, swimming, and feeding (Strathmann, 1971; Pennington and Strathmann, 1990; Hart and Strathmann, 1994; Strathman and Grunbaum, 2006). The growth of skeletal rods is required for the extension of the larval arms, which are decorated with a sinuous band of ciliated cells (the ciliary band) that moves food toward the mouth. Echinoderm larvae that have relatively long arms, and therefore a long ciliary band, clear algae from the seawater more rapidly than do larvae with short arms (Strathman, 1971). Moreover, sea urchin larvae are capable of regulating the rate of skeletal growth in response to the availability of food; they form relatively short arms when food is abundant and longer arms when food is scarce (Boidron-Metairon, 1988; Hart and Strathman, 1994; Miner, 2007). When food is abundant, dopamine-based signaling slows the growth of the skeletal rods that support the larval arms (Adams *et al.*, 2011).

Morphogenetic mechanisms. The skeleton of the sea urchin embryo is produced by primary mesenchyme cells (PMCs), a specialized population of migratory mesoderm cells. PMCs are descendants of the micromeres, four small blastomeres that arise at the vegetal pole at the 16-cell stage as a result of unequal cleavage. Each micromere undergoes an additional unequal division at the fifth cleavage, producing one small daughter cell (small micromere) and one large daughter cell (large micromere, or LM). The four LMs are the founder cells of the PMC lineage. Each LM divides three to four additional times (the number is characteristic of a species) and all LM descendants give rise exclusively to PMCs.

The descendants of the LMs become incorporated into the monolayered, epithelial wall of the blastula in a torus-shaped territory that surrounds the vegetal pole. At the onset of gastrulation, these cells initiate a

sequence of striking morphogenetic behaviors that includes: (1) ingression (an epithelial-mesenchymal transition, or EMT), (2) directional cell migration, (3) cell–cell fusion, and (4) biomineral deposition. PMC EMT is accompanied by elongation along the apical-basal axis of the cells, vigorous pulsatory activity at the basal surface, and changes in adhesive properties, including a loss of adhesion to neighboring cells and an increase in adhesion to the basal lamina that lines the blastocoel (Gustafson and Wolpert, 1967; Amemiya, 1989; Fink and McClay, 1985). These changes in the adhesive properties of PMCs may be a consequence of a burst of endocytosis and exocytosis that remodels the PMC surface (Wu *et al*., 2007). Among the proteins cleared from the plasma membrane is G-cadherin (Miller and McClay, 1997).

After a brief period of quiescence, PMCs migrate along the blastocoel wall. During the early phase of migration, PMCs remain in the vegetal hemisphere and mostly within the quadrant of the embryo from which they originally ingressed (Gustafson and Wolpert, 1967; Peterson and McClay, 2003). They move exclusively by means of filopodia, the dynamic behavior of which has been analyzed quantitatively in vivo (Malinda *et al*., 1995; Miller *et al*., 1995). PMC filopodia establish contacts primarily with the basal lamina, but they can also penetrate this thin layer. PMC filopodia interact selectively with basal lamina fibers that contain ECM3, the sea urchin ortholog of the vertebrate Frem2 protein (Hodor *et al*., 2000). By the mid-gastrula stage, the PMCs accumulate in a characteristic, ring-like pattern (the subequatorial PMC ring). Within the subequatorial ring, at two specific positions along its the ventrolateral aspects, clusters of PMCs form. The formation of the subequatorial ring and two ventrolateral PMC clusters is directed by ectoderm-derived guidance cues that arise in a progressive fashion during gastrulation. One critically important guidance signal is VEGF, which is expressed selectively by PMC target sites in the ectoderm and interacts with a PMC-specific receptor, VEGFR-Ig10 (Duloquin *et al*., 2007; Adomako-Ankomah and Ettensohn, in press).

During their initial phase of migration, PMCs undergo homotypic cell–cell fusion via their filopodia, forming cable-like structures that link the cells. By the time that the subequatorial ring is formed, all PMCs are joined in a single, common syncytial network (Hodor and Ettensohn, 1998, 2008). PMCs express many of the same proteins that have been shown to play a role in myoblast fusion in *Drosophila* and vertebrates, including Mbc/Dock1, Rac1, WASp/N-WASP, Arf6, and several others (Abmayr and Pavlath, 2012; Rafiq *et al*., 2012), although the role of these proteins in PMC fusion has not been tested directly. At the late gastrula stage, after cell–cell fusion is complete, PMCs in the two ventrolateral clusters extend numerous filopodia toward the animal pole, and a syncytial strand of PMCs migrates from each cluster toward the pole.

The formation of the embryonic skeleton begins at the late gastrula stage with the deposition of one triradiate skeletal rudiment in each of the two ventrolateral PMC clusters. The arms of the two skeletal rudiments subsequently elongate and branch in a stereotypical pattern to produce the embryonic skeleton, each rudiment giving rise to a half-skeleton that is the mirror image of its partner. The skeletal rods are deposited along the filopodial cables of the PMC syncytium, within a "privileged" extracellular compartment that is almost completely enshrouded by PMC membranes (Wilt and Ettensohn, 2007). At the end of embryonic development, when the larva begins to feed, the skeleton consists of an elaborate, bilaterally symmetrical network of 14 interconnected rods (Fig. 3).

The skeletal rods are composed primarily of mineral (calcite containing ~5% magnesium carbonate), within which small amounts of secreted proteins are occluded. Although these secreted proteins make up less than 0.1% of the mass of the biomineral, they play an important role in controlling its mechanical properties and growth (Wilt and Ettensohn, 2007; Kim *et al*., 2011). In the sea urchin, the most abundant of these secreted proteins are the spicule matrix (SM) proteins, a family of 17 closely related proteins, each of which contains a single C-type lectin domain and a variable number of proline/glycine-rich repeats (Livingston *et al*., 2006; Mann *et al*., 2010; Rafiq *et al*., 2012). One important function of SM proteins is probably to regulate the conversion of amorphous calcium carbonate (ACC), a precursor of calcite, to the crystalline state (Gong *et al*., 2012). SM proteins slow this transformation, thereby ensuring that it does not occur prematurely inside the cell, but only after the ACC has been secreted and added to the biomineral.

Several other gene products produced by PMCs play important roles in biomineralization. A PMC-specific, GPI-anchored carbonic anhydrase is likely involved in biomineral remodeling (Livingston *et al*., 2006). Nonfibrillar collagens produced by PMCs serve as an essential substrate for the cells, although they do not appear to be a structural component of the biomineral (Mitsunaga *et al*., 1986; Wessel *et al*., 1991; Livingston *et al*., 2006). Several PMC-specific, Type I transmembrane proteins, including the P16 family of genes (*p16*, *p16rel1*, and *p16rel2*) and *p58a/p58b*, play essential and nonredundant roles in biomineral deposition (Cheers and Ettensohn, 2005; Adomako-Ankomah and Ettensohn, 2011). Morpholino (MO)-based knockdown of any of these proteins inhibits skeletal growth without perturbing PMC specification, migration, or fusion. The genes that

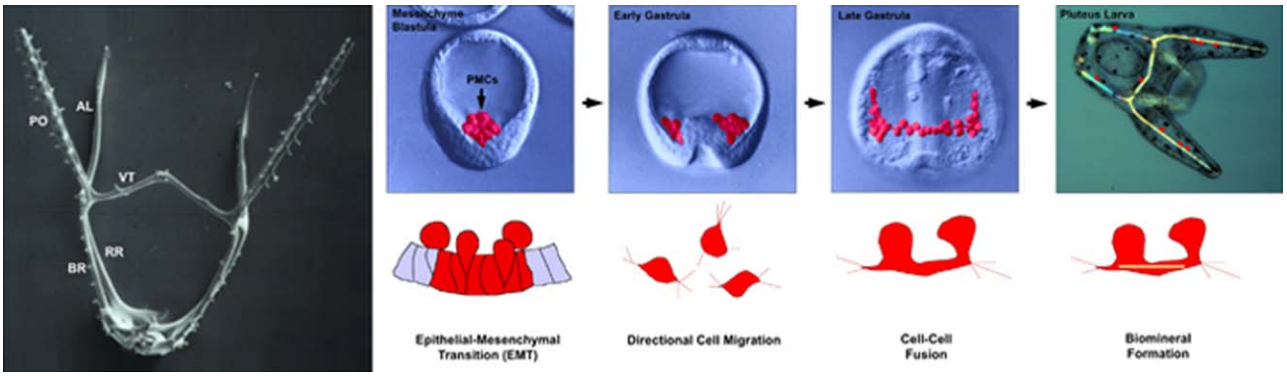

FIG. 3. Morphogenesis of the sea urchin embryonic skeleton. Left—Scanning electron micrograph of the late embryonic/early larval skeleton. All cellular material has been removed, revealing the bilaterally symmetrical network of calcareous rods that serves as an endoskeleton. AL = anterolateral rod, BR = body rod, PO = postoral rod, RR = recurrent rod, VT = ventral transverse rod (two additional rods, the dorso-ventral connecting rod and anonymous rod, are not visible in this view). Right—Summary of the major morphogenetic behaviors of PMCs during embryogenesis. Upper panels show images of living embryos viewed with differential interference contrast optics, with PMCs pseudocolored in red. Bottom diagrams illustrate cell behaviors that are most prominent at different stages.

encode these biomineralization-related proteins, like most of the SM genes, are organized in clusters in the genome, indicating that they have expanded relatively recently by duplication (Livingston *et al*., 2006; Rafiq *et al*., 2012).

Gene network analysis. The micromere-PMC GRN is currently one of the most complete developmental GRNs in any experimental model (Oliveri *et al*., 2008; Ettensohn, 2009; Rafiq *et al*., 2012) (Fig. 4). This network is initially deployed through the activity of vegetally localized, maternal proteins, which include β-catenin and probably other, as yet unidentified, maternal factors. These maternal inputs activate a small set of early regulatory genes (*pmar1/micro1*, *ets1*, *alx1*, and *tbr*) selectively in the micromere-PMC lineage (Kurokawa *et al*., 1999; Fuchikami *et al*., 2002; Kitamura *et al*., 2002; Oliveri *et al*., 2002; Ettensohn *et al*., 2003). *pmar1* is activated transiently and specifically in the micromeres at the 16-cell stage, but it activates the PMC GRN only in the LM descendants, perhaps because the small micromeres produce the germ-line, which is transcriptionally repressed in many organisms (Nakamura *et al*., 2010; Yajima and Wessel, 2011, 2012). *pmar1* is regulated directly by β-catenin and is likely to be its only essential target with respect to the activation of the PMC GRN (Oliveri *et al*., 2003; Nishimura *et al*., 2004). Because Pmar1 is a transcriptional repressor, it presumably functions by repressing other repressors. One important downstream target is *hesC*, although the repression of this gene occurs too late in development to account for the LM-specific activation of the network during cleavage (Revilla-i-Domingo *et al*., 2007; Yamazaki *et al*., 2009; Sharma and Ettensohn, 2010). Misexpression of *pmar1* results in a striking transformation of most of the cells of the embryo to a PMC-like fate (Oliveri *et al*., 2002; Nishimura *et al*., 2004; Yamazaki *et al*., 2009). Two critically important, indirect regulatory targets of *pmar1* are *alx1* and *ets1*. *alx1*, which encodes a homeodomain protein, is the earliest regulatory gene activated specifically in the LM-PMC lineage and its expression is restricted to this lineage throughout development. *ets1* is more broadly expressed; *ets1* mRNA and protein are present maternally and this gene is expressed zygotically not only in PMCs, but also in secondary mesenchyme cells (SMCs), a population of migratory mesoderm cells that ingresses later in gastrulation (Kurokawa *et al*., 1999; Yajima *et al*., 2010; Flynn *et al*., 2011). *alx1* and *ets1* are each required for PMC ingression and all subsequent aspects of PMC morphogenesis (Kurokawa *et al*., 1999; Ettensohn *et al*., 2003). Mis-expression of *alx1* or *ets1* has more limited effects than that of *pmar1*; mis-expression of *alx1* results in the conversion of macromere descendants to a PMC fate (Ettensohn *et al*., 2007) while mis-expression of *ets1* transforms most of the cells of the embryo into mesenchymal cells, but does not activate *alx1* or the full skeletogenic GRN (Kurokawa *et al*., 1999; Rottinger *et al*., 2004; Sharma and Ettensohn, 2010).

The early TFs in the GRN engage additional layers of regulatory genes, which include *hex*, *tgif*, *erg*, *tel*, *snail*, *foxN2/3*, *foxO*, *foxB*, *fos*, *smad1/5/8*, and *jun* (Wu and McClay, 2007; Oliveri *et al*., 2008; Rho and McClay, 2011; Rafiq *et al*., 2012). Multiple feedback and feedforward interactions subsequently stabilize the transcriptional network and drive it forward (Oliveri *et al*., 2008) (Fig. 4). Recently, many effector genes in the PMC GRN were identified through an in situ hybridization screen, and the inputs of three early regulatory genes (*ets1*, *alx1*, and *tbr*) into all known effector genes in the network were determined (Rafiq *et al*., 2012). This work showed that *ets1* and *alx1* have extensive and parallel connectivity within the network; both genes provide essential (direct or indirect) inputs into

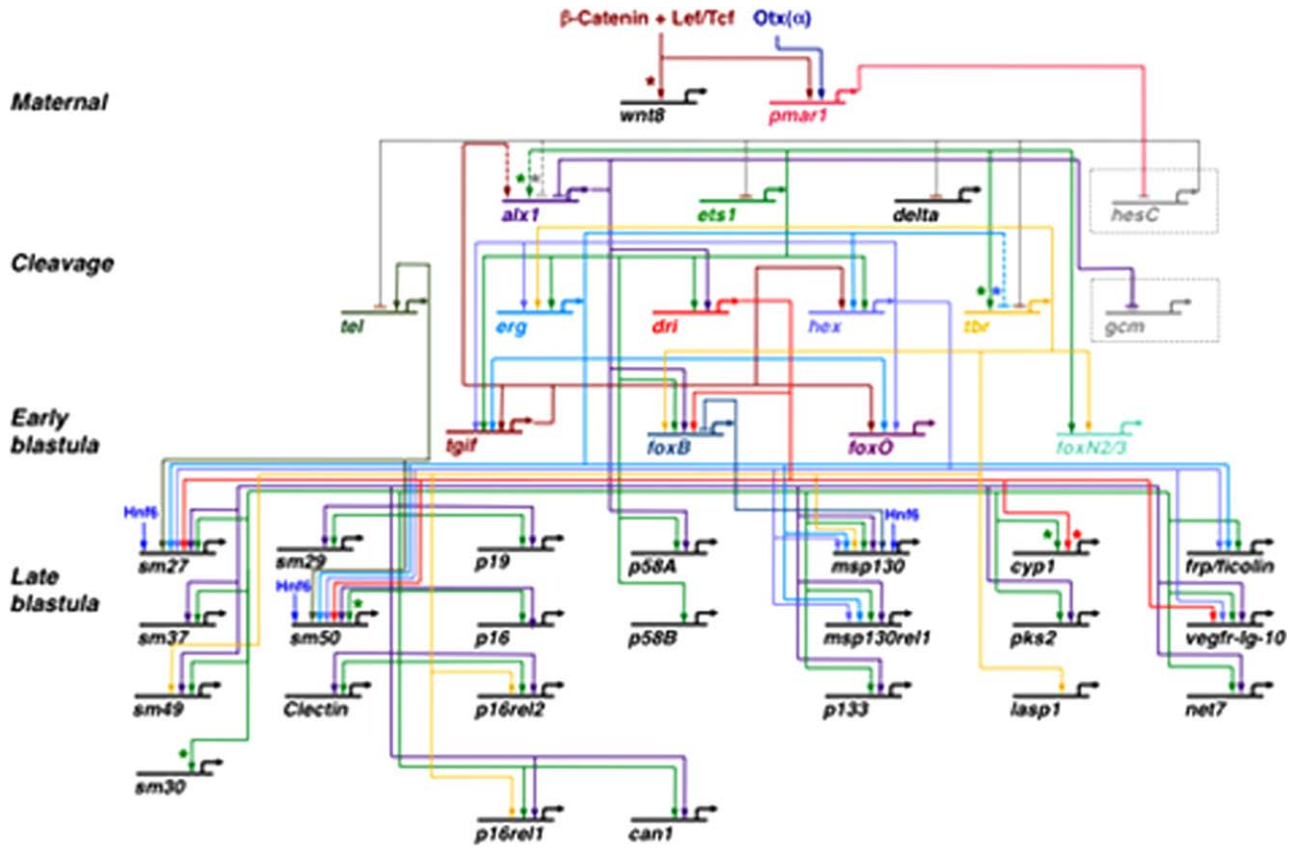

FIG. 4. GRN deployed in the skeletogenic, large micromere-PMC lineage of the sea urchin. A time-averaged view is shown, with approximate developmental stages indicated at left. Genes shown in gray and surrounded by dashed boxes (*hesC*, *gcm*) are expressed only in non-PMC territories, where they function to repress skeletogenic genes. Dashed arrows show late inputs into *alx1* that do not account for the initial activation of this gene during cleavage. Asterisks indicate interactions that have been shown to be direct. Modified from Rafiq and Ettensohn, 2012. Note that genome-wide RNAseq studies have identified many additional targets of Ets1 and Alx1 in PMCs (including ~60 co-regulated targets), although some of these regulatory interactions may be indirect (Rafiq and Ettensohn, unpublished observations).

the great majority of effector genes, although the molecular basis of the parallel regulation by *ets1* and *alx1* is unknown. In contrast, *tbr* provides regulatory inputs into very few effector genes, a finding consistent with a variety of evidence which suggests that *tbr* was recruited into the network relatively recently (see Rafiq *et al.*, 2012 and references therein). Recently, a genome-wide analysis of functional targets of *ets1* and *alx1* at the early gastrula stage has identified ~90 co-regulated targets and has provided a comprehensive picture of the set of PMC effector genes that receive inputs from one or both of these two important regulatory genes (Rafiq *et al.*, unpublished observations).

Of the >100 genes in the current model of the PMC GRN, 10 have been subjected to cis-regulatory analysis, predominantly through the mutational analysis of reporter gene contructs (Raman *et al.*, 1993; Frudakis and Wilt, 1995; Makabe *et al.*, 1995; Yamasu and Wilt, 1999; Revilla-i-Domingo *et al.*, 2004; Minokawa *et al.*, 2005; Amore and Davidson, 2006; Ochiai *et al.*, 2008; Smith and Davidson, 2008; Wahl *et al.*, 2009; Yajima *et al.*, 2010; Damle and Davidson, 2011). These include several genes that are expressed exclusively by cells of the LM-PMC lineage and other genes that have broader domains of expression. As yet, however, no general patterns have emerged that can fully account for the coordinated expression of these PMC-specific mRNAs.

The deployment of the PMC GRN, including the activation of all known morphoeffector genes, is complete prior to PMC ingression (Harkey and Whiteley, 1983; Rafiq *et al.*, 2012). In rapidly developing species, this occurs during a 6–7 h period between micromere formation and the onset of PMC ingression. The initial deployment of the network is driven by maternal inputs and occurs entirely autonomously within the LM descendants; no signals from other embryonic cell populations are required (Wilt and Ettensohn, 2007). During gastrulation, however, the PMC GRN becomes subject to the influence of signals from neighboring ectoderm cells. These ectoderm-derived cues determine the specific sites of skeletal rudiment formation and control the pattern of skeletal morphogenesis such that it is coordinated with the morphogenesis of the

ectoderm. One ectoderm-derived cue is VEGF, which has a role in PMC differentiation that can be separated experimentally from its effects on cell guidance (Duloquin *et al*., 2007; Adomako-Ankomah and Ettensohn, in press). To understand skeletal patterning, it will be essential to elucidate the regulatory mechanisms by which ectoderm-derived cues impinge on the PMC GRN.

Evolutionary modifications. Present-day echinoderms are grouped into five classes: crinoids (sea lilies and feather stars), asteroids (sea stars), ophiuroids (brittle stars), holothuroids (sea cucumbers), and echinoids (sea urchins and sand dollars) (Fig. 5). The late larval and adult forms of all echinoderms contain calcified endoskeletal tissue (sometimes referred to as stereom), which first appeared more than 500 Mya and is considered a major synapomorphy of the phylum (Bottjer *et al*., 2006). The recent demonstration of widespread, calcified endoskeletal elements in adult hemichordates (Cameron and Bishop, 2012) raises the possibility that a calcified endoskeleton was also present in the last common ancestor of the echinoderms and hemichordates, although the difference in crystal polymorph (aragonite in hemichordates and calcite in echinoderms) and apparent lack of SM proteins in hemichordates are more consistent with an independent onset of biomineralization in the two groups (Porter, 2010). It is universally accepted that the hydroxyapatite skeleton of vertebrates and the calcite endoskeleton of echinoderms appeared independently (Livingston *et al*., 2006; Kawasaki *et al*., 2009; Murdock and Donoghue, 2011). The extent to which biominerals appeared in these deuterostomes via the co-option of a shared, ancestral developmental program, however (e.g., mesoderm cells that produce a collagenous matrix), is an intriguing and open question.

Most echinoderms exhibit maximal indirect development, a mode distinguished by the formation of a larva that bears little anatomical resemblance to the adult. Characteristic features of the tests of fossil echinoids, and well-documented examples of the recent appearance of direct development in various clades of sea urchins, demonstrate that indirect development was the ancestral mode at least within this group (Emlet, 1985; Smith, 1997). Strikingly, although all adult echinoderms possess a biomineralized endoskeleton, only euechinoid sea urchins form micromeres, PMCs, and an elaborate, embryonic skeleton (brittle stars also produce an extensive embryonic skeleton, but do so without forming micromeres). Moreover, the closest

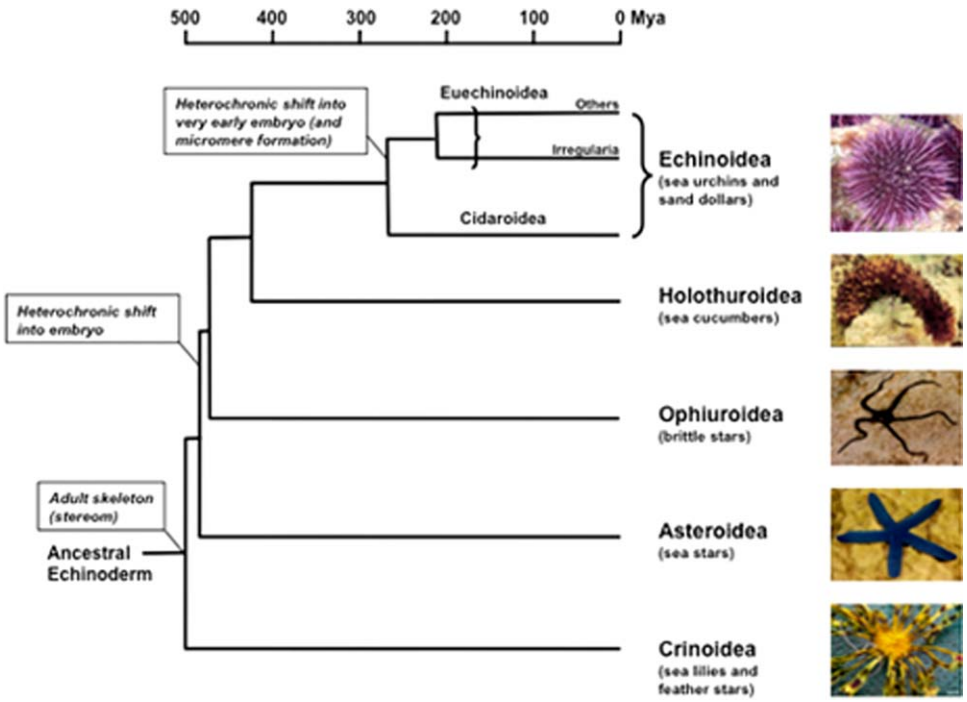

FIG. 5. Echinoderm phylogeny. The adult forms of all five classes of modern echinoderms have extensive, calcite-based endoskeletons. Deployment of the skeletogenic network is also observed in late blastula/gastrula-stage embryos of ophiuroids, holothuroids, and some echinoids, but not in more basal echinoderms (asteroids and crinoids), suggesting that a heterochronic importation of the adult program into the embryo occurred in the common ancestor of these three groups. Modern cidaroid sea urchins, which closely resemble the ancestral stock that gave rise to all modern echinoids, do not reliably form micromeres and do not activate the skeletogenic network during early cleavage. These developmental features are exhibited by all euechinoids, however, pointing to a second, more recent, heterochronic shift in the deployment of the GRN. Class relationships and divergence times shown here are based on Smith *et al*. (2006) and Pisani *et al*. (2012).

relatives of the euechinoid sea urchins, the cidaroid urchins, exhibit an intermediate pattern of development characterized by variable numbers of micromeres (0–4) and a skeletogenic mesenchyme that ingresses after the onset of gastrulation (Schroeder, 1981; Emlet, 1988; Wray and McClay, 1988; Yamazaki *et al*., 2012). These considerations strongly support the view that, in the euechinoid lineage, the adult skeletogenic program was imported into the early (cleavage stage) embryo, via the evolution of new regulatory linkages and the coupling of these regulatory inputs to the unequal cell division that produces micromeres (Gao and Davidson, 2008; Ettensohn, 2009; Sharma and Ettensohn, 2010) (Fig. 5). This heterochronic shift created a new embryonic cell population, the LM-PMC lineage, the morphogenetic activities of which modified the shape, swimming behavior, and feeding of the larva. This evolutionary change likely occurred in two stages; a shift from the adult into the late blastula/early gastrula (to produce patterns of development seen in modern cidaroids and sea cucumbers) and a subsequent shift into the micromeres of the cleavage stage embryo (to produce the developmental pattern seen in present-day euechinoids). The latter shift can be dated relatively precisely. Present day sea urchins and sand dollars arose from the radiation of a small number of cidaroid-like species that survived the Permian extinction ~252 Mya. The creation of micromeres, PMCs, and an early embryonic skeleton must have occurred after this time but before the extensive adaptive radiation of the euechinoidea, which is evident in the fossil record by ~200 Mya (Smith *et al*., 2006) (Fig. 5).

The heterochronic shift in the deployment of the GRN accounts for the striking similarities between the skeletogenic GRN as it is deployed in the embryo and in mineralized tissues of adult sea urchins, which include the test, teeth, and spines. Many regulatory genes that are expressed selectively in PMCs are also expressed selectively in skeletogenic centers of the adult, with the exception of *tbr*, which has relatively few targets in the embryonic network (Gao and Davidson, 2008; Rafiq *et al*., 2012). Recent proteomic studies have confirmed that many biomineralization genes are expressed in the mineralized tissues of both the adult and embryo (Mann *et al*., 2010). There are subtle differences, however, in the expression of biomineralization genes in the embryo and the adult that point to a limited divergence of the adult and embryonic networks after their temporal separation. For example, some members of biomineralization gene families are expressed selectively in the embryo or in one or more of the mineralized tissues of the adult (Livingston *et al*., 2006; Killian *et al*., 2010; Mann *et al*., 2010).

Heterochrony in the deployment of the skeletogenic GRN is also reflected in a program of late larval skeletogenesis in euechinoids. After the larva begins to feed, several additional skeletal elements arise that are separate from the early, embryonic skeleton (Okazaki, 1975; Smith *et al*., 2008). These skeletal elements are secreted, at least in part, by SMCs that ingress late in gastrulation (Yajima, 2007). SMCs are a heterogeneous population of cells and the subpopulation that participates in the formation of the postfeeding larval skeleton has not been identified. In euechinoids, SMCs are derived from *veg2* blastomeres, which are the immediate neighbors of the LMs (Ruffins and Ettensohn, 1996). Therefore, the heterochronic shift into the micromeres was not associated with a transfer of the GRN into a radically new spatial domain of the embryo; instead, it probably coincided with relatively subtle shifts in the positions of the fourth and fifth cleavage planes in vegetal blastomeres and the coupling of this cleavage pattern to the precocious activation of the network (Sharma and Ettensohn, 2010).

The regulatory states of SMCs and PMCs are similar in many respects, and it has been proposed that this reflects an ancestral program that directed the specification of migratory mesoderm cells (Ettensohn *et al*., 2007; Ettensohn, 2009; Rafiq *et al*., 2012). One significant difference between the two cell types, however, is that the embryonic expression of a key skeletogenic regulatory gene, *alx1*, is entirely restricted to PMCs (Ettensohn *et al*., 2003). Strikingly, misexpression of *alx1* alone is sufficient to convert SMCs to a skeletogenic fate early in development (Ettensohn *et al*., 2007). These findings suggest that a heterochronic shift in *alx1* expression played a key role in the precocious deployment of the skeletogenic GRN in the LM-PMC lineage.

Recent comparative studies have described the expression patterns of regulatory genes of the PMC GRN in several echinoderm clades. The expression patterns of many of these genes are highly conserved among euechinoid species that are separated by >200 My of evolution (Smith *et al*., 2006; Yamazaki *et al*., 2010). An exception is *tbr*, the expression of which became restricted to the LM-PMC lineage relatively recently, after the divergence of irregular echinoids from other euechinoids (Minemura *et al*., 2009) (Fig. 5). Studies of more distantly related echinoderms, sea stars and sea cucumbers, also reveal many conserved features and point to an ancestral program of mesoderm formation that included the expression of *ets1*, *erg*, *dri*, *hex*, *tgif*, *tbr*, and *foxN2/3* in the vegetal plate (Shoguchi *et al*., 2000; Koga *et al*., 2010; McCauley *et al*., 2010). One conspicuous difference, however, again concerns the expression of *alx1*, which is active in the skeletogenic centers of adult starfish (Gao and Davidson, 2008) but is not expressed (or is expressed only at extremely low levels) in the embryo. This finding again points to changes in *alx1* expression as pivotal in driving evolutionary changes in skeletogenesis.

The *alx1* gene arose via a relatively recent duplication of an ancestral *alx4*-like gene in the echinoderm lineage (Rafiq *et al*., 2012) and this may have allowed a shift in its pattern of expression.

Recent work indicates that the appearance of the embryonic skeleton was also accompanied by a heterochronic shift in the expression of VEGF in the ectoderm (Morino *et al*., 2012). The expression of VEGFR in adult skeletogenic centers of sea stars and in the embryos of brittle stars indicates that VEGF/VEGFR signaling was an ancient feature of echinoderm skeletogenesis (Gao and Davidson, 2008; Morino *et al*., 2012), and an evolutionary change in VEGF expression was probably a prerequisite for the formation of an embryonic skeleton. Although the order of evolutionary changes is unknown, one hypothesis is that a change in VEGF expression set the stage for a subsequent shift in the activation of the skeletogenic GRN (including *alx1* and one of its key targets, *vegfr*), which would have led quickly to the precocious appearance of skeletal structures in the embryo.

Notochord Formation in Ascidians

The notochord is a defining feature of the Chordata and may have originally served as a stiff but flexible structure that supported the muscular movements of a small, free-swimming chordate ancestor. The cellular and genetic mechanisms of notochord formation are particularly well understood in ascidians (Subphylum Urochordata), partly because the notochord is constructed from only a few cells. Notochord morphogenesis takes place after gastrulation is complete, and the shaping of this structure contributes to the elongation of the posterior part of the embryo (Reverberi *et al*., 1960), as it does in vertebrates (Keller, 2006). In ascidians, the notochord is a transient structure that is lost at metamorphosis.

Morphogenetic mechanisms. The notochord cells of the larva arise from two distinct embryonic lineages: a primary lineage (A-line) that derives from four anterior founder blastomeres, and a secondary lineage (B-line) that arises from two posterior founder blastomeres. At the onset of gastrulation, 10 presumptive notochord cells, eight from the primary lineage and two from the secondary lineage, form an arc at the anterior edge of the blastopore (Fig. 6). Two additional rounds of cell division result in the generation of 40 definitive notochord cells. These cells are transiently organized as a monolayered, polarized epithelium, in a territory shaped like a disk.

A sequence of four post-mitotic cell behaviors- invagination, convergent extension (a kind of polarized cell rearrangement), cell elongation, and tubulation- transforms the disk-shaped population of notochord cells into a long rod (Fig. 6). Two morphogenetic movements, invagination and convergent extension, transform the sheet of notochord cells into a single column of flattened, disk-shaped cells that resembles a stack of coins (Munro *et al*., 2006; Jiang and Smith, 2007). These processes together account for about 50% of the overall elongation of the notochord. Subsequently, the column of notochord cells elongates further through changes in the shape of individual cells (the elongation of each notochord cell along the anterior–axis of the embryo) and through tubulation, a complex process that creates a continuous, extracellular matrix (ECM)-filled lumen that extends the length of the notochord.

The cell behaviors that accompany notochord morphogenesis have been analyzed in vivo (Miyamoto and Crowther, 1985; Munro and Odell, 2002a; Rhee *et al*., 2005; Dong *et al*., 2009). These studies have shown that polarized protrusive activity and oriented cell rearrangements accompany the directional intercalation of the cells during convergent extension. Such cellular behaviors are similar in many respects to those that underlie convergent extension in other organisms (Keller *et al*., 2000). The polarization of cell protrusive activity associated with convergent extension is dependent upon components of the planar cell polarity (PCP) pathway, including Disheveled, Prickle, and Wnt5 (Keys *et al*., 2002; Jiang *et al*., 2005; Niwano *et al*., 2009). These molecules act downstream of notochord specification and their function is required in the notochord cells. In other organisms, PCP components also coordinate the polarized protrusive activity and rearrangement of cells that undergo convergent extension, although the underlying mechanisms are not fully understood and may not be conserved (Goto *et al*., 2005).

Contact with lateral tissues plays a role in orienting the protrusive activity and resultant rearrangements of notochord cells (Munro and Odell, 2002b). FGF3, which is produced by the ventral midline of the nerve cord, organizes the convergent extension movements of the notochord cells (Shi *et al*., 2009). This localized signal may act as an extracellular positional cue that directionally activates the intracellular PCP pathway in the adjacent notochord cells and polarizes their movements. Another potential player in this signaling process is the α-laminin protein, chongmague, which is concentrated at the outer boundary of the notochord and is required for later steps in convergent extension (Veeman *et al*., 2008).

Recent studies have revealed cellular and cytoskeletal events that underlie the second phase of notochord extension, which occurs by cell elongation and tubulation (Dong *et al*., 2009, 2011; Denker and Jiang, 2012). Cell elongation appears to be driven by an actomyosin ring, which is located near the equator of the cell and functions like a pseudocleavage furrow. During tubulation, apical/luminal domains form at the anterior and

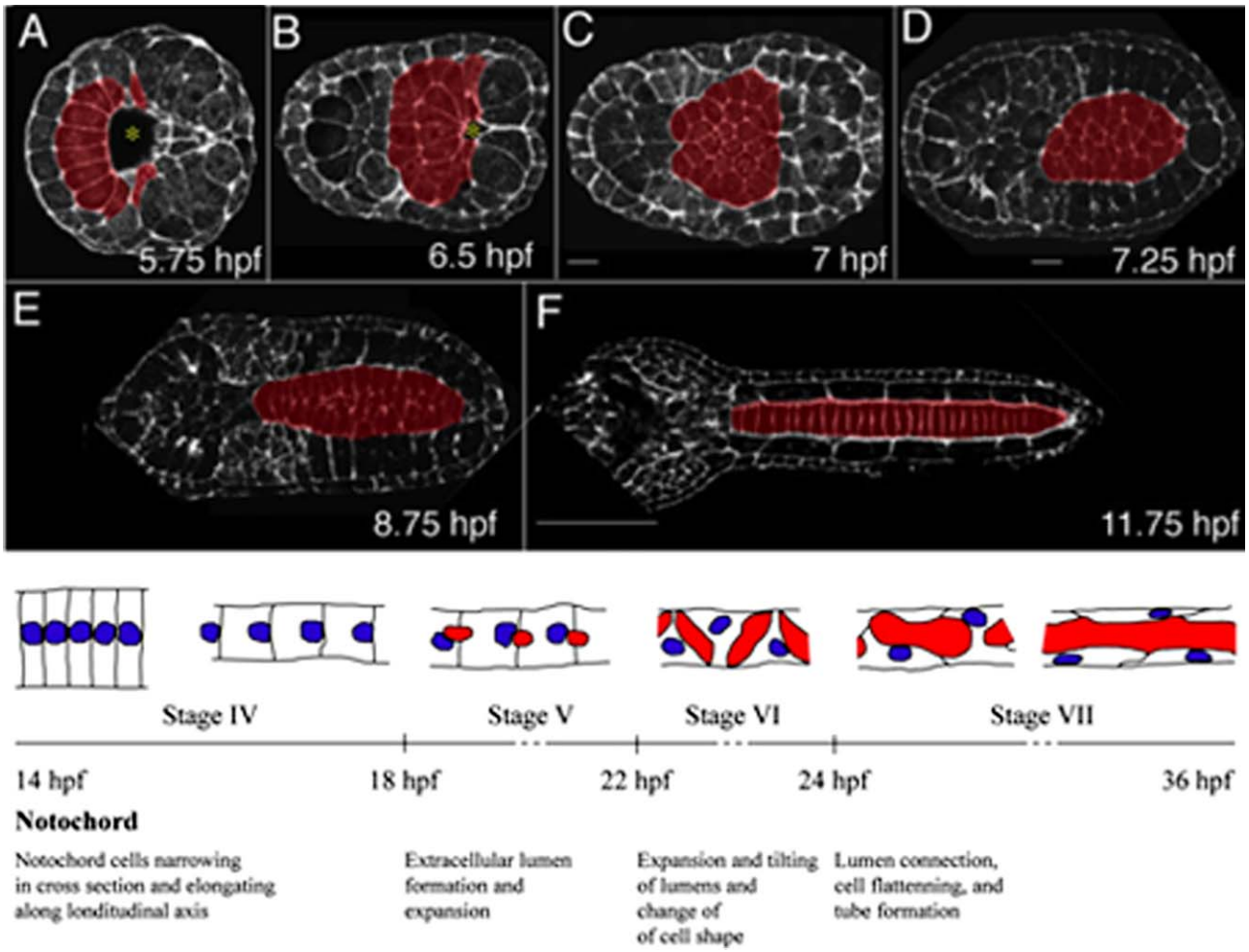

FIG. 6. Ascidian notochord morphogenesis. Upper panels (**A–F**): Notochord morphogenesis from the onset of gastrulation to the completion of convergent extension. (**A**) Ten presumptive notochord cells (pseudocolored red) form a semicircular arc anterior to the blastopore (*). (**B** and **C**) Two rounds of cell division generate 20 and finally 40 notochord cells that form a monolayered epithelium. (**D–F**) Invagination (not shown) and convergent extension transform the notochord precursor into a column of 40 stacked cells. These images are of *Ciona savignyi* embryos stained with bodipy–phalloidin and imaged by confocal microscopy (reprinted with permission from Jiang and Smith, 2007). Lower diagrams: Later elongation of the notochord, after the completion of convergent extension, illustrating cell elongation and lumen formation. Nuclei and vacuoles are colored blue and red, respectively (reprinted with permission from Dong *et al*., 2009). All images and diagrams show dorsal views with anterior to the left. Hpf = hours postfertilization at 18°C (upper panels) or 16°C (lower diagrams).

posterior ends of each notochord cell, and extracellular lumens appear between the cells. These lumens enlarge and eventually join with one another to create a continuous, central cavity (Fig. 6), while the notochord cells engage in active, migratory rearrangements and adopt an endothelial-like arrangement surrounding the cavity. This sequence of events resembles a general mechanism of tube formation that has been termed "cord hollowing" or "cell hollowing" (Andrew and Ewald, 2010; Dong *et al*., 2009). Tubulation in ascidians is dependent on ERM (ezri/radixin/moesin), which is expressed selectively in notochord cells at the tailbud stage (Dong *et al*., 2011).

Gene network analysis. Studies of the GRN deployed in ascidian notochord cells have focused on the T-box gene *brachyury* (*bra*), which is expressed specifically by these cells. Expression of *bra* can first be detected in the primary and secondary founder cells in the first cell cycle after the asymmetric cell divisions that produce these cells, that is, in the four presumptive A-line notochord founder cells at the 64-cell stage and, one cleavage division later, in the two B-line founder cells (Nakatani *et al*., 1996). *bra* is required for the normal specification and morphogenesis of the notochord (Chiba *et al*., 2009), and mis-expression of *bra* is sufficient to cause the ectopic expression of notochord-specific features in other cell lineages (Yasuo and Satoh, 1998; Takahashi *et al*., 1999).

The expression of *bra* in prospective notochord cells requires a combination of several regulatory inputs. Interestingly, these inputs are somewhat different in the

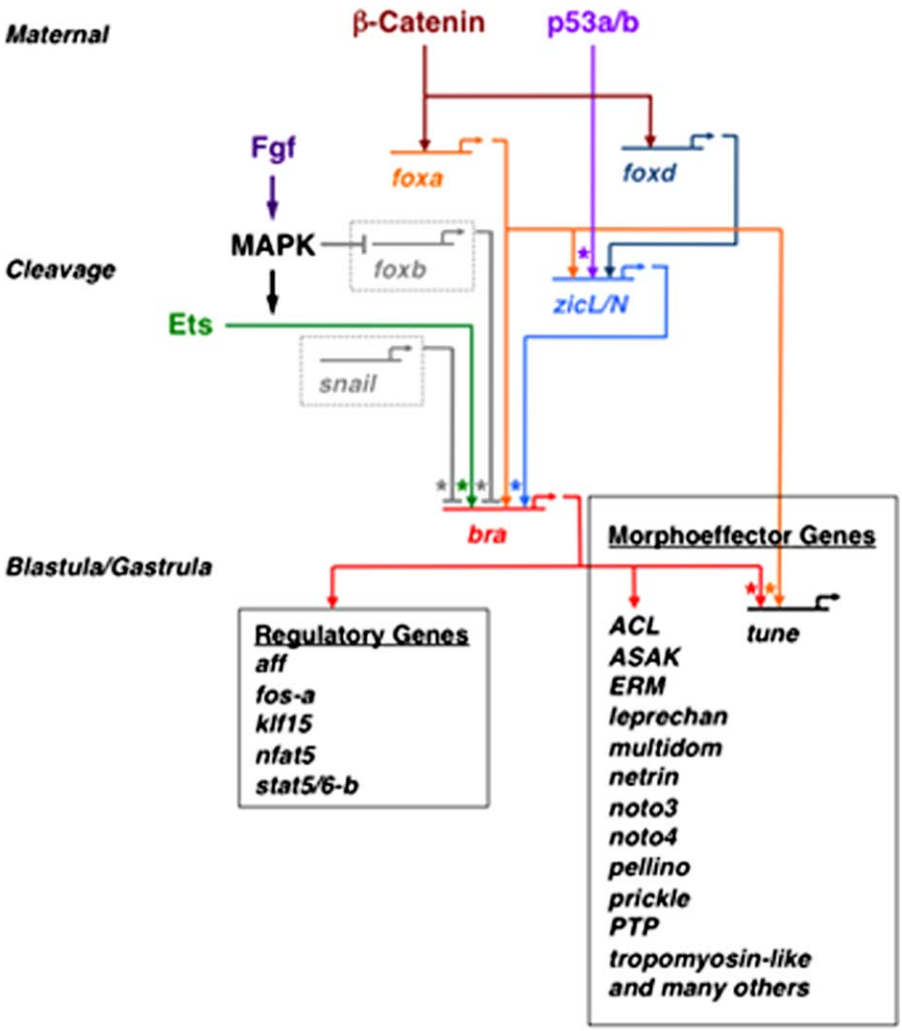

FIG. 7. GRN deployed in presumptive notochord cells of the A-lineage in the ascidian embryo (Ciona and Halocynthia sp.). A time-averaged view is shown, with approximate developmental stages indicated at left. Genes shown in gray and surrounded by dashed boxes (*snail*, *foxb*) are expressed only in non-notochord territories, where they function to repress *brachyury*. Asterisks indicate interactions that have been shown to be direct.

A and B lineages. Because all terminally differentiated notochord cells appear to be equivalent, this indicates that the same GRN can be activated in prospective notochord cells by more than one regulatory pathway. In the A-line, maternal β-catenin is required for the early zygotic activation of *foxA* and *foxD* in blastomeres that will give rise to mesoderm and endoderm (Imai *et al*., 2002a; Kumano *et al*., 2006) (Fig. 7). FoxA and FoxD, acting in concert with maternal p53 proteins, are required for the activation of the zinc-finger gene, *zicL/N* (Imai *et al*., 2002b; Wada and Saiga, 2002; Noda *et al*., 2011). ZicL/N binds directly to the *bra* promoter and provides an essential input (Yagi *et al*., 2004; Matsumoto *et al*., 2007). *zicL/N* is expressed more broadly than the prospective notochord lineage, however, and additional factors restrict *bra* expression. One critical input is provided by FGF signaling. FGF9/16/20, which is expressed in the vegetal region at the 32-cell stage, activates the MAPK pathway and promotes the phosphorylation of Ets, which functions as a direct activator of *bra*, in concert with ZicL/N (Nakatani *et al*., 1996; Shimauchi *et al*., 2001; Imai *et al*., 2002c; Miya and Nishida, 2003; Yagi *et al*., 2004; Matsumoto *et al*., 2007; Yasuo and Hudson, 2007). Ephrin-Eph signaling also acts to restrict the notochord field. Each progenitor of a notochord founder cell divides asymmetrically to produce one presumptive neural cell and one presumptive notochord cell. Signals from animal blastomeres, acting through ephrin-Eph, restrict MAPK signaling to the notochord founder cell, thereby preventing the expression of a direct repressor of *bra*, *foxb*, which is activated in the prospective neural cell (Picco *et al*., 2007; Hashimoto *et al*., 2011). These various findings demonstrate that the specification of notochord cells in

the A-line, as reflected by the restricted expression of *bra*, results from the integration of multiple, spatially restricted inputs (Kumano *et al*., 2006).

In the B-line, the activation of *bra* requires some, but not all, of the inputs that are utilized in the A lineage. FGF9/16/20 and Ets are required in both lineages, but inputs from P53 and ZicL/N are not required in the B-line (Imai *et al*., 2002b; but see also Kumano *et al*., 2006; Noda *et al*., 2011). Strikingly, in the B-line only, *bra* expression is dependent upon Nodal, which acts in part via a relay mechanism that also involves Delta signaling (Hudson and Yasuo, 2006). A Delta/Notch-mediated mechanism of *bra* expression is supported by the identification of functional Su(H) sites within a *bra* enhancer (Corbo *et al*., 1998; Hudson and Yasuo, 2006).

Early studies identified approximately 40 functional targets of *bra* expressed exclusively in the notochord lineage (Takahashi *et al*., 1999; Hotta *et al*., 2000; Hotta *et al*., 2008). These genes encode a broad spectrum of proteins, including Prickle, ERM, a tropomyosin-like protein, Leprecan (a prolyl hydroxylase), and Noto4 (a protein with a C-terminal phoshotyrosine-interaction domain). MO knockdowns of several of these genes (including Prickle, ERM, Tropomyosin-like, and Noto4) have effects on the rearrangements or elongation of notochord cells (Hotta *et al*., 2007; Yamada *et al*., 2011), pointing to these genes as key regulators of morphogenesis. In most cases, it is not known whether the regulation by *bra* is direct or indirect, although there is evidence for direct inputs in the case of *tropomyosin-like* (Di Gregorio and Levine, 1999) and *leprecan* (Dunn and Di Gregorio, 2009). Minimal enhancers that drive notochord-specific expression of several other *bra*-target genes have been isolated although their regulatory sequences have not yet been dissected in detail (Takahashi *et al*., 2010). *bra* provides inputs into a number of other regulatory genes in the prospective notochord cells, suggesting that some of the effects of *bra* on morphoregulatory genes are indirect (Jose-Edwards *et al*., 2011). At present, the intermediate wiring of the notochord GRN has not been analyzed in detail and further work will be needed to clarify and expand the network in this regard. There is some evidence of feed-forward regulatory loops; for example, *foxA* is required for *bra* expression and both FoxA and Bra bind directly to cis-regulatory sequences of *Ci-tune*, a gene that encodes a notochord-specific protein of unknown function (Passamaneck *et al*., 2009).

Evolutionary modifications. Comparative studies have revealed diverse patterns of notochord development among urochordates, although the underlying network changes have generally not been explored in detail. Some ascidians form a notochord that lacks any obvious central lumen, and it has been proposed that the transition between forms with or without a lumen may have been as simple as controlling whether ECM-filled vesicles are transported to the surface for secretion (Jiang and Smith, 2007). Molgulid ascidians have evolved tailless larvae several times independently, and these species have reduced numbers of notochord cells that fail to converge and extend. Notochord cells in molgulids express *bra*, but only transiently, and these cells undergo programmed cell death (Jeffery, 2002; Takada *et al*., 2002).

Several recent studies have examined notochord development in *Oikopleura dioica*, a member of the appendicularians (larvaceans), a sister group to the ascidians. Unlike ascidians, larvaceans exist as free-swimming organisms throughout their life cycle and do not under metamorphosis to produce a sessile adult. In *Oikopleura*, the larval notochord contains only 20 cells, but these cells proliferate later in development and the structure persists into the adult (Soviknes and Glover, 2008). In *Oikopleura*, as in vertebrates, *bra* is expressed not only in the notochord, but also in the developing endoderm (Bassham and Postlethwait, 2000). This finding suggests that the notochord-specific expression and function of *bra* observed in ascidian embryos is derived. Surprisingly, *Oikopleura* appears to lack orthologs of a several genes that are known to be targets of *bra* in ascidians, and the orthologs of other *bra* targets are not expressed in the notochord of *Oikopleura* (Kugler *et al*., 2011). These studies point to considerable evolutionary plasticity downstream of *bra* in the molecular program of notochord development in urochordates. Consistent with this view, several *hox* genes are differentially expressed in the *Oikopleura* notochord, while there is no detectable expression of *hox* genes in the notochord of at least one ascidian (Ikura *et al*., 2004; Seo *et al*., 2004). The biochemical properties of the Bra protein have remained well-conserved, at least within the invertebrate deuterostomes, as misexpression in ascidian embryos of Bra protein from hemichordates, sea urchins, or amphioxus in nerve cord and endoderm cells under the control of the *fkh* promoter mimics the misexpression of endogenous, ascidian Bra and activates notochord-specific target genes ectopically (Satoh *et al*., 2000). It appears more likely, therefore, that evolutionary changes in the *bra* promoter (Takahashi *et al*., 1999) and CREs of *bra* target genes are responsible for recent evolutionary changes in the developmental expression and function of *bra*.

Somatic Muscle Development in Drosophila

Striated, contractile muscle is often considered to be an evolutionary invention of the bilaterians, although its evolutionary relationship to the striated muscle of diploblastic metazoans is debated (Seipel and Schmid, 2005; Burton, 2008). There are many striking

similarities in the cellular and molecular mechanisms of myogenesis among distantly related bilaterians which point to an ancient developmental program within the group (Maqbool and Jagla, 2007; Ciglar and Furlong, 2009).

The muscles of *Drosophila* larvae, including the somatic (body wall) muscles, are derived from the mesoderm and develop over a period of 10–12 h (Fig. 8). A stereotypical arrangement of 30 body wall muscles arises in mirror-image duplication in each of the abdominal hemisegments A2 to A7, with minor variations in more anterior and posterior hemisegments (Maqbool and Jagla, 2007; Tixier *et al*., 2010; Abmayr and Pavlath, 2012). Each somatic muscle in this hemisegmental set is unique and exhibits a distinctive program of gene expression, position, size, shape, insertion sites on the epidermis and innervation. Together, the body wall muscles provide the larva with mobility and the ability to search for food.

Some mesodermal cells are set aside during embryonic myogenesis and give rise to adult-specific muscles, which arise at the time of metamorphosis. Unlike embryonic myoblasts, these adult muscle precursors (AMPs) postpone their differentiation and proliferate actively during larval stages. AMPs maintain the expression of an early regulatory gene, *twist*, which is expressed only transiently by embryonic myoblasts (Bate *et al*., 1991). Most larval muscles degenerate during metamorphosis, but some persist and serve as scaffolds for the assembly of adult muscles.

Morphogenetic mechanisms. Early mesoderm morphogenesis can be divided into several discrete phases: invagination, epithelial–mesenchymal transition (EMT), and dorsal–lateral migration/monolayer formation (reviewed by Leptin, 1999; Winklbauer and Muller, 2011; Davidson, 2012). The invagination of the prospective mesoderm, a rapid (~20 min) event that initiates gastrulation, internalizes the cells by an epithelial

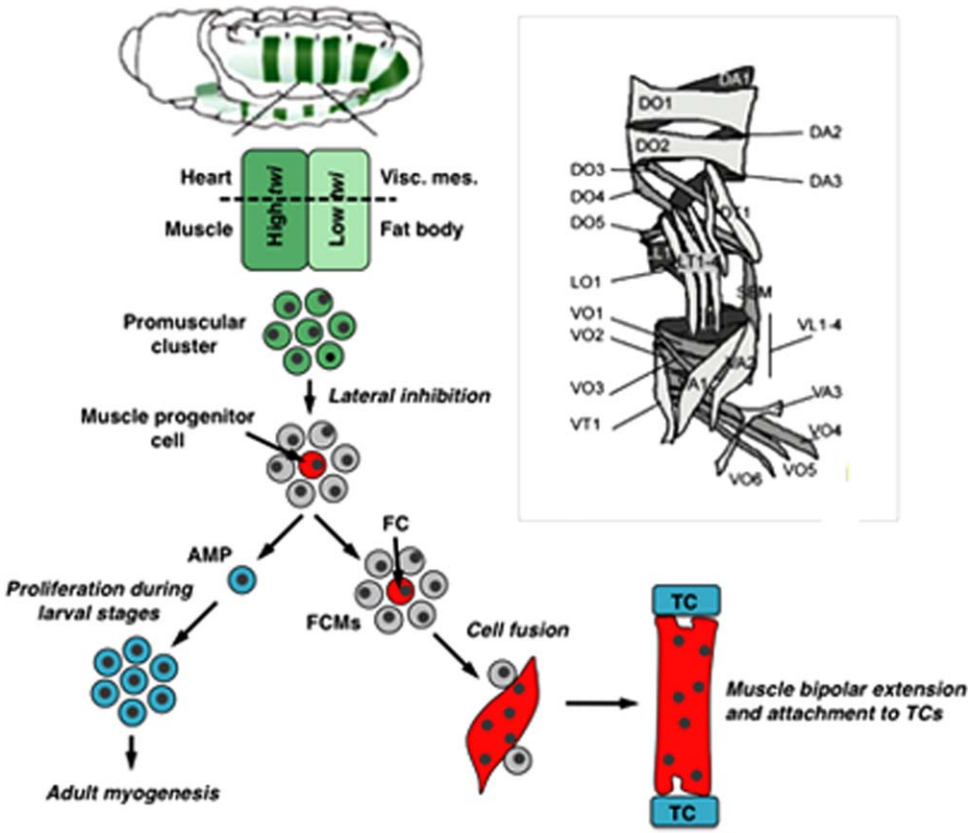

FIG. 8. Overview of the development of somatic muscles in *Drosophila* (adapted from Maqbool and Jagla, 2007; de Joussineau *et al*., 2012). During early embryogenesis, the mesoderm becomes regionalized and promuscular clusters emerge. Lateral inhibition results in the specification of a single muscle precursor, which divides asymmetrically to produce a founder cell (FC) and an AMP (or two FCs). Surrounding cells become FCMs. FCMs fuse with the FC to produce a developing myotube; the number of fusion events is characteristic of each muscle. After fusion, FCMs adopt the gene expression program of the FC. The developing muscle extends at each end and establishes specific contacts with TCs. AMPs proliferate and give rise to adult muscles arise during pupal stages (left side of diagram). Some larval muscles serve as templates for adult muscles, but most adult muscles arise *de novo* and most larval muscles degenerate during metamorphosis. Inset: Diagram illustrating the arrangement of the 30 larval somatic muscles in a single abdominal body segment (only one side of the embryo is shown).

folding process which is probably driven by apical constriction. During EMT, the mesoderm loses its epithelial character and attaches as a multilayered aggregate of mesenchymal cells to the basal surface of the ectoderm along the ventral midline. Subsequently, the outward movement of the mesoderm in the dorsal–lateral direction is associated with a reorganization of the cells and the formation of cellular protrusions at their free dorsal edges. As the mesoderm spreads along the surface of the ectoderm, the inner cells of the mesodermal mass undergo radial cell intercalation and a monolayer forms. In addition to this dorso-lateral movement, the mesoderm also expands along the anterior–posterior axis during germ band extension, in close register with the ectoderm. Some of the spreading mesoderm cells produce the body wall muscles (somatic muscles), the muscles of the gut (visceral muscles), and the heart.

Recent in vivo imaging studies have revealed new features of the internal movements of mesoderm cells and clarified the essential role of FGF signaling in these movements (Schumacher *et al*., 2004; Wilson *et al*., 2005; McMahon *et al*., 2008, 2010, Murray and Saint, 2007; Klingseisen *et al*., 2009; Clark *et al*., 2011). The FGF receptor Heartless (Htl) is expressed in the mesoderm and two FGF8-like ligands, Pyramus (Pyr) and Thisbe (Ths), are expressed in dynamic patterns in the ectoderm (Beiman *et al*., 1996, Gryzik and Muller 2004; Shishido *et al*., 1993, Stathopoulos *et al*., 2004). Analysis of mutant phenotypes indicates that FGF signaling regulates the timing of EMT and is required for the formation of cell protrusions and for both radial and dorsolateral migration. Of the two known FGF ligands, Pyr plays a particularly important role and may function as a dorso-lateral chemoattractant (Winklbauer and Muller, 2011). Guidance of mesodermal cells by growth factors and RTKs is a common feature of gastrulation in animal embryos; it also plays an important role in Xenopus and sea urchins, although different growth factors and receptors appear to be used (viz., PDGF and VEGF, and their cognate receptors, respectively).

In contrast to vertebrate skeletal muscle, each somatic muscle in the *Drosophila* larva is a single, syncytial myofiber. The unique properties of each muscle depend on the prior specification of a single myoblast known as a founder cell (FC), the identity of which is determined by a distinctive, combinatorial pattern of expression of 10–15 regulatory genes ("muscle identity genes") (Bate, 1990; Carmena *et al*., 1995; Rushton *et al*., 1995; Ruiz-Gomez *et al*., 1997; Jagla *et al*., 1998). Each FC fuses with neighboring, fusion-competent myoblasts (FCMs), and the syncytium takes on the identity and properties of the original FC. The number of cell–cell fusion events that occur is a characteristic of each muscle and determines the final size of the fiber. The fusion process itself is usually divided into the following sequence of events; cell migration, cell recognition/adhesion, and membrane fusion (Richardson *et al*., 2008; Haralalka and Abamyr, 2010; Rochlin *et al*., 2010; Abmayr and Pavlath, 2012). Like the myoblasts of vertebrates, FCMs extend filopodia and lamellipodia and are migratory. Most FCMs develop in close proximity to FCs/myotubes, however, and therefore do not engage in long distance migrations. Forward genetic screens and other approaches have identified ~20 proteins that participate in various aspects of the fusion process (Maqbool and Jagla, 2007; Abmayr and Pavlath, 2012). Among these are three transmembrane proteins of the immunoglobulin superfamily (IgS), Kin-of-IrreC (Kirre), Roughest (Rst), and Sticks-and-Stones (SnS) (Bour *et al*., 2000; Strünkelnberg *et al*., 2001), which are believed to mediate adhesion between FCs and FCMs, and a number of actin remodeling proteins, including several that function selectively in either the FCs/myotubes or the FCMs and others that function in both fusion partners. The interaction of the IgS adhesion molecules in "trans" triggers distinct signal transduction cascades within the FC and FCM, leading to the recruitment of actin regulators to the two membranes and the formation of an asymmetric, fusogenic cell–cell contact (Jin *et al*., 2011; Abmayr and Pavlath, 2012). There are likely to be additional plasma membrane proteins that promote the final step in the process of myoblast fusion—the formation of fusion pores and the merging of the two lipid bilayers—but these have not yet been identified in *Drosophila* or other organisms.

The body wall muscles make attachments to specific, epidermal tendon cells (TCs) that arise within the ectoderm in an intricate pattern, in parallel with the appearance of FCs (Schweitzer *et al*., 2010). The initial positioning of TCs at the parasegment boundaries is achieved through the expression of Stripe, an early growth response (Egr)-like TF (Vorbrüggen *et al*., 1997). The initial specification of TCs is independent of muscle-derived signals, but the later differentiation of these cells is triggered by Vein, a neuregulin-like ligand of the Egfr pathway, which is secreted by muscle cells and activates the Egfr pathway specifically in muscle-bound tendon progenitors, driving them to differentiate into mature TCs (Yarnitzky *et al*., 1997). As cell fusion occurs, the developing myotubes adopt a bipolar shape and filopodia are extended at each end. The two, opposite leading edges of this multinucleate cell seek out and contact TCs, while the central region remains relatively stationary (Schnorrer and Dickson, 2004). Correct guidance of myotube filopodia toward the TCs requires some of the same signaling pathways that mediate axon pathfinding, including the Slit-Robo and the Wnt5-Derailed pathways (Schnorrer and Dickson, 2004; Schnorrer *et al*., 2010; Schweitzer *et al*., 2010; Lahaye *et al*., 2012). Muscle cells express the Robo receptor, while its ligand, Slit, is secreted by tendon cells and by the ventral cord midline. Derailed and

Doughnut, which are related, atypical receptor tyrosine kinases, are required autonomously in certain muscles (a subset of the lateral transverse muscles) where they respond to local Wnt5 to direct myotubes to the correct TCs (Lahaye *et al*., 2012). In addition, a novel protein complex expressed by ventral longitudinal muscles is required for the migration of these cells towards TCs. This complex includes the transmembrane protein Kontiki and its cytoplasmic partner, the PDZ domain protein, Grip (Schnorrer *et al*., 2007). A variety of guidance cues have therefore been identified and these appear to act in a muscle-specific fashion to produce the stereotypical patterns of TC attachment that are observed in vivo. Contact between the ends of myotubes and the TCs is followed by a cessation of filopodial activity and the progressive assembly of specialized myotendinous junctions (Schnorrer and Dickson, 2004, Schweitzer *et al*., 2010). These junctions consist of hemidesmosomes on the muscle cells and TCs, and an intervening ECM, which is secreted by both cells. There are many proteins that contribute to the formation of the myotendinous junction; in the myotube, an essential function is provided by the integrin heterodimer, αPS2βPS, which is required for proper muscle–tendon attachment (Leptin *et al*., 1989).

Gene network analysis. In each segment, the mesoderm is patterned through the activities of multiple signals, including Wingless, Hedgehog, and Decapentaplegic (reviewed by Tixier *et al*., 2010; de Joussineau *et al*., 2012). Somatic myoblasts arise primarily from the ventral, anterior portion of each segment (Fig. 8). Within this region, by a process akin to proneural cluster formation and neuroblast specification, a territory of competent cells, marked by the expression of *lethal of scute (l'sc*), is refined through FGF and EFG signaling into several smaller promuscular clusters, within which muscle-specific regulatory genes are expressed. Within each cluster, lateral inhibition establishes a single muscle progenitor cell, which maintains both elevated MAPK activity and *l'sc* expression. This cell divides asymmetrically to produce one FC and a second cell, which is usually a distinct FC or an AMP. Nonselected cells in promuscular clusters activate the FCM-specific *lame duck* (*lmd*) gene, through elevated Notch signaling.

In vertebrates, a well-described set of basic helix-loop-helix bHLH myogenic regulatory factors (MRFs), Myf5, MyoD, Mrf4, and myogenin, controls myogenesis (reviewed by Berkes and Tapscott, 2005) (Fig. 9). Nautilus (Nau), the closest *Drosophila* relative of vertebrate MRFs, is expressed specifically by most, if not all, myogenic FCs and is required for the proper development of many somatic muscles (Michelson *et al*., 1990; Paterson *et al*., 1991; Keller *et al*., 1997, 1998; Misquitta and Paterson, 1999; Wei *et al*., 2007). Nau appears to have both a general role in muscle development and also a more selective function in muscle-specific properties such as attachment and shape (Enriquez *et al*., 2012), but the upstream regulators and downstream targets of Nau are poorly understood. Much more is known of the biology of the bHLH TF, *twist* (*twi)*, which functions as a key regulator of myogenesis and several other aspects of early mesoderm development (Baylies and Bate, 1996). Twi homodimers are responsible for activating target genes that direct cells to the somatic myogenic lineage, while heterodimers between Twi and a different bHLH protein, Daughterless, repress this specification pathway (Castanon *et al*., 2001). Recent studies have shown that the expression of a subset of Twi-regulated genes involves the interaction of Twi with a cofactor, Akirin, that colocalizes and genetically interacts with subunits of the Brahma SWI/SNF-class chromatin remodeling complex (Nowak *et al*., 2012).

Genome-wide ChIP-chip and ChIPseq have been used to identify Twi-binding sites at stages that correspond to gastrulation and early mesoderm specification, prior to the appearance of promuscular clusters. These studies have identified 500–3,000 Twi-bound CREs that are associated with several hundred genes (Sandmann *et al*., 2007; Zeitlinger *et al*., 2007; Ozdemir *et al*., 2011). Many Twi-binding sites are conserved in several *Drosophila* species (He *et al*., 2011). Regulatory genes are common targets, but other classes of putative target genes include genes that mediate FGF signaling during gastrulation (for example, *htl*) and cell division. Comparisons of Twi-binding sites with those of other early mesodermal regulators (Dorsal, Tinman, and Mef2, see below) provide evidence of combinatorial binding within CREs and examples of feed-forward loops (e.g., dorsal > twist > dorsal + twist). Genome-wide mapping of Twi-binding sites has also been carried out in DmD8 cells, which exhibit several characteristics of adult muscle progenitor cells. In these cells, binding of Twi to CREs may facilitate the utilization of nearby Suppressor of Hairless-binding sites, and it has been proposed that functional cooperation of these two TFs may underlie the roles of *twi* and Notch signaling in maintaining the undifferentiated state of AMPs (Bernard *et al*., 2010). Although many hundreds of Twi-binding sites have been identified in genome-wide studies, the number of functional sites (i.e., those that actually modulate rates of target gene transcription in response to changing levels of Twi) is probably considerably smaller (Ozdemir *et al*., 2011).

Several regulatory genes are controlled directly by Twi and are activated in the somatic muscle lineage later in development, where they participate in a transcriptional network that supports muscle development (reviewed by Ciglar and Furlong, 2009; Fig 9). Tinman is an Nkx homeodomain protein required for the specification of dorsal mesoderm, including heart muscle (a

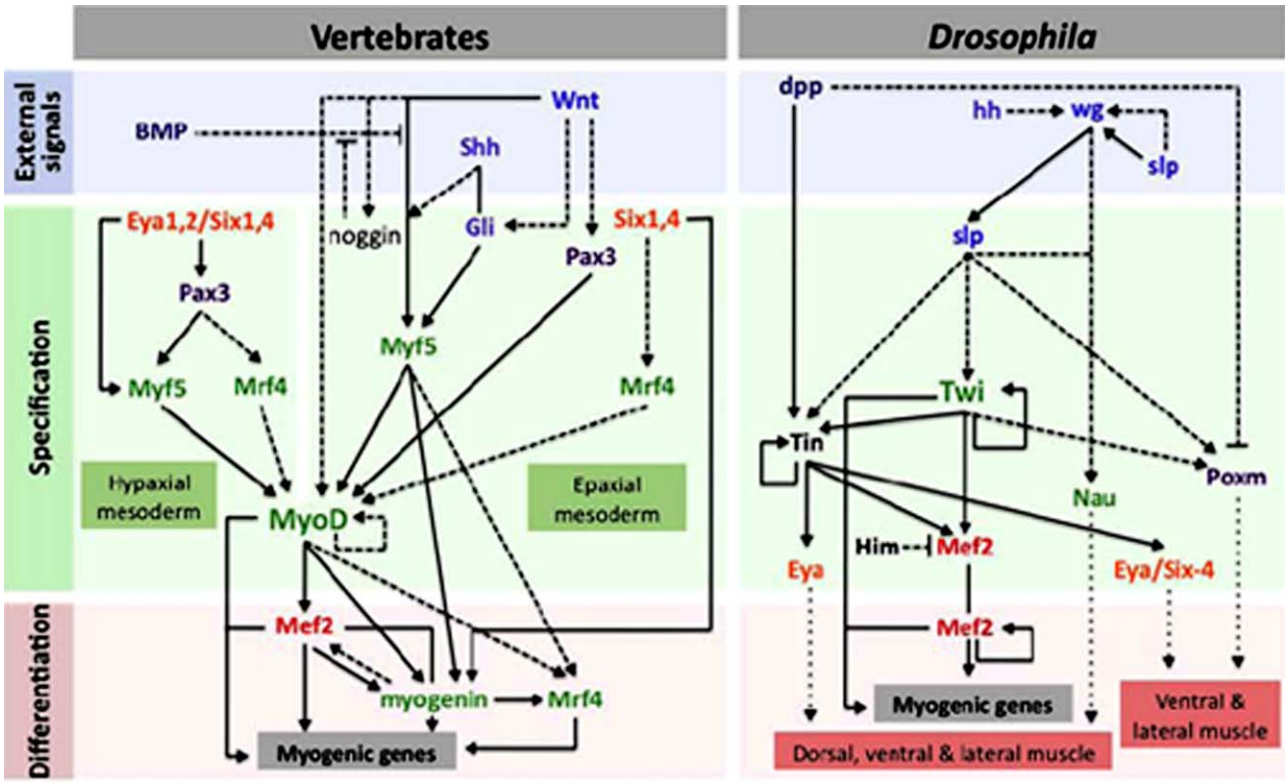

FIG. 9. Comparison of the core myogenic network in vertebrates and *Drosophila* (reprinted with permission from Ciglar and Furlong, 2009). Solid lines show direct transcriptional regulation, dashed lines represent genetic interactions that may be direct or indirect, and dotted lines indicate mutant phenotypes (*Drosophila*). Blue—Components of signalling pathways initiated in nonmesodermal tissues (Wnt/BMP/Shh). Green—bHLH MRFs. Orange—Eya-Six proteins. Purple—Pax proteins. Red—MADS/SRF proteins. Black- regulators from other protein families.

function of Tinman that is conserved in vertebrates), dorsal body wall muscles, and visceral muscle (Azpiazu and Frasch, 1993; Bodmer, 1993). ChIP-chip analysis at three developmental stages from gastrulation to the formation of muscle primordia has identified ~500 Tinman-bound sites in the genome, collectively associated with 260 genes (Liu *et al.*, 2009). Among these putative target genes are several regulatory genes that mediate somatic muscle development (e.g, *eya*, *six4*, and *him*) as well as morphoregulatory genes that function in myoblast fusion and guidance (e.g., *sns*, *mbc*, *robo*).

Mef-2, a MADS-box protein, is expressed in all muscle types in a Twi-dependent fashion and plays an essential role in the development of all larval muscles as well as many adult muscles (Nguyen *et al.*, 1994; Bour *et al.*, 1995; Lilly *et al.*, 1995; Ranganayakulu *et al.*, 1995; Bryantsev *et al.*, 2012a; Soler *et al.*, 2012). The activity of Mef-2 is modulated both positively and negatively by a number of co-factors, including Vestigial and Scalloped (Deng *et al.*, 2009), Chorion Factor 2 (Tanaka *et al.*, 2008), Him (Liotta *et al.*, 2007; Elgar *et al.*, 2008; Soler and Taylor, 2009), and Lmd (Cunha *et al.*, 2010). Genome-wide analysis of Mef-2 binding sites at several developmental stages has identified >600 bound CREs and revealed striking temporal changes in CRE occupancy during embryogenesis (Sandmann *et al.*, 2006). Analysis of gene expression changes in Mef-2 mutant embryos suggests that ~50% of Mef-2 binding sites are functional. Comparison of TF occupancy and expression-profiling data for Twi and Mef-2 indicates that both factors co-regulate a large battery of direct targets by a feed-forward mechanism (*twi* > *mef-2* > *twi* + *mef-2*) (Sandmann *et al.*, 2007). More recently, a comparison of Mef-2 and Lmd binding sites at multiple stages of muscle development, coupled with genome-wide transcriptome profiling of Mef-2 and Lmd mutant embryos, has revealed many examples of combinatorial inputs of these proteins into CREs of muscle-expressed genes, with diverse regulatory consequences that presumably reflect additional, as yet unidentified, inputs into these same CREs (Cunha *et al.*, 2010). Zinzen *et al.*, (2009) developed an atlas of genome-wide binding sites of Twi, Mef-2, Tinman, and other TFs, and found that combinatorial binding of multiple proteins was a reliable predictor of CREs. A number of binding "signatures" were identified that were associated with transcription specifically in somatic muscle (or in somatic muscle and other tissues) but, surprisingly, combinatorial binding

signatures were not highly constrained for any given, tissue-specific pattern of gene expression.

A recent, large-scale analysis of a genome-wide RNAi library expressed in muscle cells via a Mef2-Gal4 driver identified 66 genes that affect the overall morphology of the larval body wall muscles (Schnorrer *et al*., 2010). These morphoeffector genes encode proteins of the myotendinous junction (βPS and αPS2 integrins, integrin linked kinase, talin, α-actinin, parvin, and others), signaling proteins (e.g., the FGF-receptor, Htl, described above) and other proteins with uncharacterized functions in muscle morphogenesis. Although the upstream regulation of most of these genes has not been explored in detail, several were identified independently in genome-wide analyses of Twi and/or Mef-2 targets (see above).

Most of the genes described above function in most or all muscle cells and mediate the various morphogenetic behaviors that are common to all somatic myoblasts. A striking feature of the embryonic musculature of *Drosophila*, however, is that each body wall muscle displays specific properties, including shape, size (largely a function of the number of the cell–cell fusion events that generate a particular syncytial fiber), position, innervation, and attachment points (reviewed by de Joussineau *et al*., 2012). These muscle-specific properties are directed, at least in part, by a unique combinatorial code of 10–15 regulatory genes that specifies each FC (Ruiz-Gomez *et al*., 1997; Knirr *et al*., 1999; Bataille *et al*., 2010; Enriquez *et al*., 2012). The specific morphological features of individual body wall muscles present an intriguing opportunity to link genomic regulatory programs to morphogenesis. Bataille *et al*. (2010) provided evidence that the number of fusion events characteristic of several somatic muscles (DA1, SBM, DT1, VA2, and VT1), is regulated by the identity genes *even-skipped* (*eve*), *ladybird* (*lb*), and *slouch* (*slou*), through the effect of these regulatory genes on the expression levels of three morphoeffector genes: *muscle protein 20* (*mp20*), *paxillin* (*pax*), and *m-spondin* (*mspo*). Based on work in other systems, Mp20 and Pax are likely to regulate the actin cytoskeleton, while Mspo is an ECM protein that may regulate cell motility or adhesion. The precise step(s) in the complex cell fusion process influenced by these proteins, and the regulatory mechanisms that determine their levels of expression in specific muscle types, will be important to explore. There is also evidence that muscle-specific patterns of attachment and innervation are mediated by the expression of guidance/adhesion proteins, such as Kon-tiki, Derailed, and Toll, in specific subsets of muscle cells (Schnorrer and Dickson, 2004; Schnorrer *et al*., 2007; Inaki *et al*., 2010; Schweitzer *et al*., 2010; Lahaye *et al*., 2012). Little is known concerning the regulatory control of these genes, and establishing links between their expression and the muscle-specific, combinatorial identity gene code will also be of considerable interest.

Evolutionary modifications. Studies of the genetic control of myogenesis in *Drosophila* have generally emphasized features that are conserved across wide evolutionary distances (e.g., features that are shared with vertebrates) rather than the evolutionary plasticity of the myogenic program. Nevertheless, the myogenic networks in three widely separated taxa-flies, vertebrates, and nematodes, are different in many respects (Ciglar and Furlong, 2009) (Fig. 9). For example, there have been evolutionary changes in the identity, upstream regulation, and downstream targets of MRFs, although some general features of network topology (e.g., the widespread use of feed-forward circuitry) and regulatory gene usage (e.g., related bHLH proteins functioning as MRFs) are shared by all three taxa.

As noted above, some *twi*-expressing cells, siblings of muscle founder cells, are set aside during embryonic myogenesis and contribute to adult-specific muscles. Unlike embryonic myoblasts, these AMPs postpone their differentiation, persistently express *twi*, and proliferate continuously during larval life. Some adult muscles form via the fusion of AMPs with existing larva muscles that serve as scaffolds, while others arise *de novo*. As in the case of skeletogenesis in sea urchins, the maximal indirect mode of development exhibited by *Drosophila* provides an opportunity to explore the deployment of a cell-type specific GRN at distinct developmental phases widely separated in time. There are a number of differences in the myogenic program during these two phases of development. Myogenesis in adult muscles is regulated by neural and endocrine signals that play no role in embryonic myogenesis, pointing to very different linkages to cell signaling pathways (reviewed by Roy and VijayRaghavan, 1999). Furthermore, certain regulatory genes appear to function in only one of the two pathways. For example, the TF Spalt major (Salm) has no known function in embryonic myogenesis, but in the adult, plays a conserved, central role in controlling the morphology and contractile properties of stretch-activated, indirect flight muscles in *Drosophila* and other insects, through its effects on the expression and splicing of several components of the sarcomere (Schönbauer *et al*., 2011). Salm acts in concert with the homeodomain proteins Homothorax (Hth) and Extradenticle (Exd), which also promote the differentiation of muscle precursors into flight muscle, rather than jump muscle (Bryantsev *et al*., 2012b). Additional differences between the embryonic and adult myogenic programs may emerge as both networks are dissected further.

CONCLUSIONS

Elucidating the genomic encoding of anatomy is central to our understanding of both development and evolution. The experimental systems reviewed here illustrate

current progress in this area and provide a tantalizing glimpse of opportunities that lie ahead for dissecting the regulatory control of morphogenetic processes and the evolution of anatomy. Other experimental models, not highlighted in this review, are being used to address similar questions (e.g., Reim and Frasch, 2010; Vincent and Buckingham, 2010; Ghabrial *et al*., 2011; Bronner and LeDouarin, 2012; Felix *et al*., 2012). What have we learned thus far? It should be noted that this review has focused on organisms that undergo indirect development, and the early morphogenesis of such organisms may be distinctive in certain ways. For example, heterochronic shifts in GRN deployment may be especially prominent, while the regulation of cell proliferation and tissue growth is likely to be less significant during prefeeding embryogenesis, which occurs in the absence of growth. Nevertheless, several general themes emerge that are likely to be shared by most developing systems: (1) Territory- or lineage-specific transcriptional networks drive distinctive programs of cell behaviors which, in turn, shape embryonic tissues. (2) The transcriptional networks that regulate morphogenesis are modulated in critically important ways by inputs from extracellular signals. (3) The same GRN (or, at least, networks that are remarkably similar) can be activated by very different inputs in distinct cell lineages and/or at different developmental stages. Examples include the activation of notochord-specific genes in the A and B lineages in ascidians, skeletogenic genes in the PMCs, SMCs, and adult skeletogenic cells of sea urchins, and muscle-specific genes in embryonic myoblasts and AMPs in *Drosophila*. (4) A given kind of cellular behavior (e.g., EMT) can be produced by different transcriptional programs, even in a single organism (Lim and Thiery, 2012). (5) Evolutionary changes in developmental anatomy are associated with modifications to the transcriptional programs that drive morphogenesis; these can be changes in the timing of GRN deployment (heterochronic shifts) or other modifications to the structure of these networks.

To complete the connection between genes and anatomy, it will be necessary to elucidate in greater detail the architecture of selected, model transcriptional gene networks that underlie specific morphogenetic processes. Developing systems present special challenges for network studies because of the dynamic nature of developmental networks and the cellular heterogeneity of embryonic tissues. There is a need to work with pure populations of embryonic cells, rather than whole embryos or mixed tissues, to ensure that (1) the cells under study exhibit uniform morphogenetic behaviors and (2) any genetic or biochemical interactions that are inferred from GRN analysis are, in fact, occurring in a single cell type. Because developmental networks are inherently dynamic, temporal control over molecular manipulations (e.g., gene knockdowns) and gene expression profiling with high temporal resolution are also important. Transcriptional networks are, of course, only a starting point and one may envision integrating such networks with the complex suite of epigenetic and post-transcriptional mechanisms that regulate gene expression (Qian *et al*., 2011; Gagan *et al*., 2012). Finally, there is a overarching need to better understand the "morphogenetic machine" itself, not just with respect to the identification of specific morphoeffector molecules that play essential roles in particular morphogenetic processes, but with respect to the cellular behaviors that drive such processes and the mechanical forces they generate within tissues (Davidson, 2011). Although these are significant challenges, a conceptual and technical framework exists to address all these issues. In the near future, therefore, we can anticipate exciting new insights concerning the organization and evolution of the genomic circuitry that controls anatomy.

LITERATURE CITED

Abmayr SM, Pavlath GK. 2012. Myoblast fusion: Lessons from flies and mice. Development 139:641–656.

Adams DK, Sewell MA, Angerer RC, Angerer LM. 2011. Rapid adaptation to food availability by a dopamine-mediated morphogenetic response. Nat Commun 2:592.

Adomako-Ankomah A, Ettensohn CA. 2011. P58-A and P58-B: Novel proteins that mediate skeletogenesis in the sea urchin embryo. Dev Biol 353:81–93.

Adomako-Ankomah A, Ettensohn CA. Dissecting the roles of VEGF and FGF signaling in sea urchin gastrulation and skeletogenesis. Development, in press.

Amemiya S. 1989. Electron microscopic studies on primary mesenchyme cell ingression and gastrulation in relation to vegetal pole cell behavior in sea urchin embryos. Exp Cell Res 183:453–462.

Amore G, Davidson EH. 2006. cis-Regulatory control of cyclophilin, a member of the ETS-DRI skeletogenic gene battery in the sea urchin embryo. Dev Biol 293:555–564.

Andrew DJ, Ewald AJ. 2010. Morphogenesis of epithelial tubes: Insights into tube formation, elongation, and elaboration. Dev Biol 341:34–55.

Azpiazu N, Frasch M. 1993. *tinman* and *bagpipe*: Two homeo box genes that determine cell fates in the dorsal mesoderm of Drosophila. Genes Dev 7:1325–1340.

Bassham S, Postlethwait J. 2000. Brachyury (T) expression in embryos of a larvacean urochordate, *Oikopleura dioica*, and the ancestral role of T. Dev Biol 220:322–332.

Bataillé L, Delon I, Da Ponte JP, Brown NH, Jagla K. 2010. Downstream of identity genes: Muscle-type-

specific regulation of the fusion process. Dev Cell 19:317–328.

Bate M, Rushton E, Currie DA. 1991. Cells with persistent twist expression are the embryonic precursors of adult muscles in Drosophila. Development 113:79–89.

Bate M. 1990. The embryonic development of larval muscles in Drosophila. Development 110:791–804.

Baylies MK, Bate M. 1996. *twist*: A myogenic switch in Drosophila. Science 272:1481–1484.

Beiman M, Shilo BZ, Volk T. 1996. Heartless, a Drosophila FGF receptor homolog, is essential for cell migration and establishment of several mesodermal lineages. Genes Dev 10:2993–3002.

Berkes CA, Tapscott SJ. 2005. MyoD and the transcriptional control of myogenesis. Semin Cell Dev Biol 16:585–595.

Bernard F, Krejci A, Housden B, Adryan B, Bray SJ. 2010. Specificity of Notch pathway activation: *twist* controls the transcriptional output in adult muscle progenitors. Development 137:2633–2642.

Bodmer R. 1993. The gene *tinman* is required for specification of the heart and visceral muscles in Drosophila. Development 118:719–729.

Boidron-Metairon IF. 1988. Morphological plasticity in laboratory-reared echinoplutei of *Dendraster excentricus* (Eschscholtz) and *Lytechinus variegatus* (Lamarck) in response to food conditions. J Exp Mar Biol Ecol 119:31–41.

Bottjer DJ, Davidson EH, Peterson KJ, Cameron RA. 2006. Paleogenomics of echinoderms. Science 314:956–960.

Bour BA, Chakravarti M, West JM, Abmayr SM. 2000. Drosophila SNS, a member of the immunoglobulin superfamily that is essential for myoblast fusion. Genes Dev 14:1498–1511.

Bour BA, O'Brien MA, Lockwood WL, Goldstein ES, Bodmer R, Taghert PH, Abmayr SM, Nguyen HT. 1995. Drosophila Mef2, a transcription factor that is essential for myogenesis. Genes Dev 9:730–741.

Bronner ME, LeDouarin NM. 2012. Development and evolution of the neural crest: An overview. Dev Biol 366:2–9.

Bryantsev AL, Baker PW, Lovato TL, Jaramillo MS, Cripps RM. 2012a. Differential requirements for Myocyte Enhancer Factor-2 during adult myogenesis in Drosophila. Dev Biol 361:191–207.

Bryantsev AL, Duong S, Brunetti TM, Chechenova MB, Lovato TL, Nelson C, Shaw E, Uhl JD, Gebelein B, Cripps RM. 2012b. *Extradenticle* and *homothorax* control adult muscle fiber identity in Drosophila. Dev Cell 23:664–673.

Burton PM. 2008. Insights from diploblasts; the evolution of mesoderm and muscle. J Exp Zool B Mol Dev Evol 310:5–14.

Cameron CB, Bishop CD. 2012. Biomineral ultrastructure, elemental constitution and genomic analysis of biomineralization-related proteins in hemichordates. Proc Biol Sci Apr 279;3041–3048.

Carmena A, Bate M, Jiménez F. 1995. Lethal of scute, a proneural gene, participates in the specification of muscle progenitors during Drosophila embryogenesis. Genes Dev 9:2373–2383.

Castanon I, Von Stetina S, Kass J, Baylies MK. 2001. Dimerization partners determine the activity of the Twist bHLH protein during Drosophila mesoderm development. Development 128:3145–3159.

Cheers MS, Ettensohn CA. 2005. P16 is an essential regulator of skeletogenesis in the sea urchin embryo. Dev Biol 283:384–396.

Chiba S, Jiang D, Satoh N, Smith WC. 2009. Brachyury null mutant-induced defects in juvenile ascidian endodermal organs. Development 136:35–39.

Ciglar L, Furlong EE. 2009. Conservation and divergence in developmental networks: A view from Drosophila myogenesis. Curr Opin Cell Biol 21:754–760.

Clark IB, Muha V, Klingseisen A, Leptin M, Müller HA. 2011. Fibroblast growth factor signalling controls successive cell behaviours during mesoderm layer formation in Drosophila. Development 138:2705–2715.

Corbo JC, Fujiwara S, Levine M, Di Gregorio A. 1998. Suppressor of hairless activates *brachyury* expression in the *Ciona* embryo. Dev Biol 203:358–368.

Cunha PM, Sandmann T, Gustafson EH, Ciglar L, Eichenlaub MP, Furlong EE. 2010. Combinatorial binding leads to diverse regulatory responses: Lmd is a tissue-specific modulator of Mef2 activity. PLoS Genet 6:e1001014.

Damle S, Davidson EH. 2011. Precise cis-regulatory control of spatial and temporal expression of the *alx1* gene in the skeletogenic lineage of *S. purpuratus*. Dev Biol 357:505–517.

Davidson EH. 2010. Emerging properties of animal gene regulatory networks. Nature 468:911–920.

Davidson LA. 2011. Embryo mechanics: Balancing force production with elastic resistance during morphogenesis. Curr Top Dev Biol 95:215–241.

Davidson LA. 2012. Epithelial machines that shape the embryo. Trends Cell Biol 22:82–87.

de Joussineau C, Bataillé L, Jagla T, Jagla K. 2012. Diversification of muscle types in Drosophila: Upstream and downstream of identity genes. Curr Top Dev Biol 98:277–301.

Deng H, Hughes SC, Bell JB, Simmonds AJ. 2009. Alternative requirements for Vestigial, Scalloped, and Dmef2 during muscle differentiation in Drosophila melanogaster. Mol Biol Cell 20:256–269.

Denker E, Jiang D. 2012. *Ciona intestinalis* notochord as a new model to investigate the cellular and

molecular mechanisms of tubulogenesis. Semin Cell Dev Biol 23:308–319.

Di Gregorio A, Levine M. 1999. Regulation of Ci-tropomyosin-like, a Brachyury target gene in the ascidian, Ciona intestinalis. Development 126:5599–5609.

Dong B, Deng W, Jiang D. 2011. Distinct cytoskeleton populations and extensive crosstalk control *Ciona* notochord tubulogenesis. Development 138:1631–1641.

Dong B, Horie T, Denker E, Kusakabe T, Tsuda M, Smith WC, Jiang D. 2009. Tube formation by complex cellular processes in *Ciona intestinalis* notochord. Dev Biol 330:237–249.

Duloquin L, Lhomond G, Gache C. 2007. Localized VEGF signaling from ectoderm to mesenchyme cells controls morphogenesis of the sea urchin embryo skeleton. Development 134:2293–2302.

Dunn MP, Di Gregorio A. 2009. The evolutionarily conserved *leprecan* gene: Its regulation by Brachyury and its role in the developing *Ciona* notochord. Dev Biol 328:561–574.

Dupont S, Morsut L, Aragona M, Enzo E, Giulitti S, Cordenonsi M, Zanconato F, Le Digabel J, Forcato M, Bicciato S, Elvassore N, Piccolo S. 2011. Role of YAP/TAZ in mechanotransduction. Nature 474:179–183.

Elgar SJ, Han J, Taylor MV. 2008. mef2 activity levels differentially affects gene expression during Drosophila muscle development. Proc Natl Acad Sci USA 105:918–923.

Emlet RB. 1985. Crystal axes in recent and fossil adult echinoids indicate trophic mode in larval development. Science 230:937–940.

Emlet RB. 1988. Larval form and metamorphosis of a "primitive" sea urchin, *Eucidaris thouarsi* (Echinodermata: Echinoidea: Cidaroidea), with implications for developmental and phylogenetic studies. Biol Bull 174:4–19.

Enriquez J, de Taffin M, Crozatier M, Vincent A, Dubois L. 2012. Combinatorial coding of Drosophila muscle shape by Collier and Nautilus. Dev Biol 363:27–39.

Ettensohn CA, Illies MR, Oliveri P, De Jong DL. 2003. Alx1, a member of the Cart1/Alx3/Alx4 subfamily of Paired-class homeodomain proteins, is an essential component of the gene network controlling skeletogenic fate specification in the sea urchin embryo. Development 130:2917–2928.

Ettensohn CA, Kitazawa C, Cheers MS, Leonard JD, Sharma T. 2007. Gene regulatory networks and developmental plasticity in the early sea urchin embryo: Alternative deployment of the skeletogenic gene regulatory network. Development 134:3077–3087.

Ettensohn CA. 2009. Lessons from a gene regulatory network: Echinoderm skeletogenesis provides insights into evolution, plasticity, and morphogenesis. Development 136:11–21.

Félix MA, Barkoulas M. 2012. Robustness and flexibility in nematode vulva development. Trends Genet 28:185–195.

Fink RD, McClay DR. 1985. Three cell recognition changes accompany the ingression of sea urchin primary mesenchyme cells. Dev Biol 107:66–74.

Flynn CJ, Sharma T, Ruffins SW, Guerra SL, Crowley JC, Ettensohn CA. 2011. High-resolution, three-dimensional mapping of gene expression using GeneExpressMap (GEM). Dev Biol 357:532–540.

Frudakis TN, Wilt F. 1995. Two cis elements collaborate to spatially repress transcription from a sea urchin promoter. Dev Biol 172:230–241.

Fuchikami T, Mitsunaga-Nakatsubo K, Amemiya S, Hosomi T, Watanabe T, Kurokawa D, Kataoka M, Harada Y, Satoh N, Kusunoki S, Takata K, Shimotori T, Yamamoto T, Sakamoto N, Shimada H, Akasaka K. 2002. T-brain homologue (HpTb) is involved in the archenteron induction signals of micromere descendant cells in the sea urchin embryo. Development 129:5205–5216.

Gagan J, Dey BK, Dutta A. 2012. MicroRNAs regulate and provide robustness to the myogenic transcriptional network. Curr Opin Pharmacol 12;383–388.

Gao F, Davidson EH. 2008. Transfer of a large gene regulatory apparatus to a new developmental address in echinoid evolution. Proc Natl Acad Sci USA 105:6091–6096.

Ghabrial AS, Levi BP, Krasnow MA. 2011. A systematic screen for tube morphogenesis and branching genes in the Drosophila tracheal system. PLoS Genet 7:e1002087.

Gong YU, Killian CE, Olson IC, Appathurai NP, Amasino AL, Martin MC, Holt LJ, Wilt FH, Gilbert PU. 2012. Phase transitions in biogenic amorphous calcium carbonate. Proc Natl Acad Sci USA 109:6088–6093.

Goto T, Davidson L, Asashima M, Keller R. 2005. Planar cell polarity genes regulate polarized extracellular matrix deposition during frog gastrulation. Curr Biol 15:787–793.

Gryzik T, Müller HA. 2004. FGF8-like1 and FGF8-like2 encode putative ligands of the FGF receptor Htl and are required for mesoderm migration in the Drosophila gastrula. Curr Biol 14:659–667.

Gustafson T, Wolpert L. 1967. Cellular movement and contact in sea urchin morphogenesis. Biol Rev 42:442–498.

Haralalka S, Abmayr SM. 2010. Myoblast fusion in Drosophila. Exp Cell Res 316:3007–3013.

Harkey MA, Whiteley AH. 1983. The program of protein synthesis during the development of the micromere-primary mesenchyme cell line in the sea urchin embryo. Dev Biol 100:12–28.

Hart MW, Strathmann RR. 1994. Functional consequences of phenotypic plasticity in echinoid larvae. Biol Bull 186:291–299.

Hashimoto H, Enomoto A, Kumano G, Nishida H. 2011. The transcription factor FoxB mediates temporal loss of cellular competence for notochord induction in ascidian embryos. Development 138:2591–2600.

He Q, Bardet AF, Patton B, Purvis J, Johnston J, Paulson A, Gogol M, Stark A, Zeitlinger J. 2011. High conservation of transcription factor binding and evidence for combinatorial regulation across six Drosophila species. Nat Genet 43:414–420.

Hobert O. 2006. Architecture of a microRNA-controlled gene regulatory network that diversifies neuronal cell fates. Cold Spring Harb Symp Quant Biol 71:181–188.

Hodor PG, Ettensohn CA. 1998. The dynamics and regulation of mesenchymal cell fusion in the sea urchin embryo. Dev Biol 199:111–124.

Hodor PG, Ettensohn CA. 2008. Mesenchymal cell fusion in the sea urchin embryo. Methods Mol Biol 475:315–334.

Hodor PG, Illies MR, Broadley S, Ettensohn CA. 2000. Cell-substrate interactions during sea urchin gastrulation: Migrating primary mesenchyme cells interact with and align extracellular matrix fibers that contain ECM3, a molecule with NG2-like and multiple calcium-binding domains. Dev Biol 222:181–194.

Hotta K, Takahashi H, Asakura T, Saitoh B, Takatori N, Satou Y, Satoh N. 2000. Characterization of Brachyury-downstream notochord genes in the *Ciona intestinalis* embryo. Dev Biol 224:69–80.

Hotta K, Takahashi H, Satoh N, Gojobori T. 2008. Brachyury-downstream gene sets in a chordate, *Ciona intestinalis*: Integrating notochord specification, morphogenesis and chordate evolution. Evol Dev 10:37–51.

Hotta K., Yamada S., Ueno N., Satoh N., Takahashi H. 2007. Brachyury-downstream notochord genes and convergent extension in *Ciona intestinalis* embryos. Dev Growth Differ 49:373–382.

Hudson C, Yasuo H. 2006. A signalling relay involving Nodal and Delta ligands acts during secondary notochord induction in *Ciona* embryos. Development 133:2855–2864.

Ikuta T, Yoshida N, Satoh N, Saiga H. 2004. *Ciona intestinalis* Hox gene cluster: Its dispersed structure and residual colinear expression in development. Proc Natl Acad Sci USA 101:15118–15123.

Imai KS, Satoh N, Satou Y. 2002a. An essential role of a FoxD gene in notochord induction in Ciona embryos. Development 129:3441–3453.

Imai KS, Satoh N, Satou Y. 2002c. Early embryonic expression of FGF4/6/9 gene and its role in the induction of mesenchyme and notochord in *Ciona savignyi* embryos. Development 129:1729–1738.

Imai KS, Satou Y, Satoh N. 2002b. Multiple functions of a Zic-like gene in the differentiation of notochord, central nervous system and muscle in *Ciona savignyi* embryos. Development 129:2723–2732.

Inaki M, Shinza-Kameda M, Ismat A, Frasch M, Nose A. 2010. Drosophila Tey represses transcription of the repulsive cue Toll and generates neuromuscular target specificity. Development 137:2139–2146.

Jagla T, Bellard F, Lutz Y, Dretzen G, Bellard M, Jagla K. 1998. *ladybird* determines cell fate decisions during diversification of Drosophila somatic muscles. Development 125:3699–3708.

Jeffery WR. 2002. Programmed cell death in the ascidian embryo: Modulation by FoxA5 and Manx and roles in the evolution of larval development. Mech Dev 118:111–124.

Jiang D, Munro EM, Smith WC. 2005. Ascidian prickle regulates both mediolateral and anterior-posterior cell polarity of notochord cells. Curr Biol 15:79–85.

Jiang D, Smith WC. 2007. Ascidian notochord morphogenesis. Dev Dyn 236:1748–1757.

Jin P, Duan R, Luo F, Zhang G, Hong SN, Chen EH. 2011. Competition between Blown fuse and WASP for WIP binding regulates the dynamics of WASP-dependent actin polymerization *in vivo*. Dev Cell 20:623–638.

José-Edwards DS, Kerner P, Kugler JE, Deng W, Jiang D, Di Gregorio A. 2011. The identification of transcription factors expressed in the notochord of *Ciona intestinalis* adds new potential players to the *brachyury* gene regulatory network. Dev Dyn 240:1793–1805.

Kawasaki K, Buchanan AV, Weiss KM. 2009. Biomineralization in humans: Making the hard choices in life. Annu Rev Genet 43:119–142.

Keller CA, Erickson MS, Abmayr SM. 1997. Misexpression of nautilus induces myogenesis in cardioblasts and alters the pattern of somatic muscle fibers. Dev Biol 181:197–212.

Keller CA, Grill MA, Abmayr SM. 1998. A role for nautilus in the differentiation of muscle precursors. Dev Biol 202:157–171.

Keller R, Davidson L, Edlund A, Elul T, Ezin M, Shook D, Skoglund P. 2000. Mechanisms of convergence and extension by cell intercalation. Philos Trans R Soc Lond B Biol Sci 355:897–922.

Keller R. 2006. Mechanisms of elongation in embryogenesis. Development 133:2291–2302.

Keys DN, Levine M, Harland RM, Wallingford JB. 2002. Control of intercalation is cell-autonomous in the notochord of *Ciona intestinalis*. Dev Biol 246:329–340.

Killian CE, Croker L, Wilt FH. 2010. SpSM30 gene family expression patterns in embryonic and adult biomineralized tissues of the sea urchin, *Strongylocentrotus purpuratus*. Gene Expr Patterns 10:135–139.

Kim YY, Ganesan K, Yang P, Kulak AN, Borukhin S, Pechook S, Ribeiro L, Kröger R, Eichhorn SJ, Armes SP, Pokroy B, Meldrum FC. 2011. An artificial biomineral formed by incorporation of copolymer micelles in calcite crystals. Nat Mater 10:890–896.

Kitamura K, Nishimura Y, Kubotera N, Higuchi Y, Yamaguchi M. 2002. Transient activation of the *micro1* homeobox gene family in the sea urchin (*Hemicentrotus pulcherrimus*) micromere. Dev Genes Evol 212:1–10.

Klingseisen A, Clark IB, Gryzik T, Müller HA. 2009. Differential and overlapping functions of two closely related Drosophila FGF8-like growth factors in mesoderm development. Development 136:2393–2402.

Knirr S, Azpiazu N, Frasch M. 1999. The role of the NK-homeobox gene slouch (S59) in somatic muscle patterning. Development 126:4525–4535.

Koga H, Matsubara M, Fujitani H, Miyamoto N, Komatsu M, Kiyomoto M, Akasaka K, Wada H. 2010. Functional evolution of Ets in echinoderms with focus on the evolution of echinoderm larval skeletons. Dev Genes Evol 220:107–115.

Kugler JE, Kerner P, Bouquet JM, Jiang D, Di Gregorio A. 2011. Evolutionary changes in the notochord genetic toolkit: a comparative analysis of notochord genes in the ascidian Ciona and the larvacean Oikopleura. BMC Evol Biol 20:11–21.

Kumano G, Yamaguchi S, Nishida H. 2006. Overlapping expression of FoxA and Zic confers responsiveness to FGF signaling to specify notochord in ascidian embryos. Dev Biol 300:770–784.

Kurokawa D, Kitajima T, Mitsunaga-Nakatsubo K, Amemiya S, Shimada H, Akasaka K. 1999. HpEts, an ets-related transcription factor implicated in primary mesenchyme cell differentiation in the sea urchin embryo. Mech Dev 80:41–52.

Lahaye LL, Wouda RR, de Jong AW, Fradkin LG, Noordermeer JN. 2012. WNT5 interacts with the Ryk receptors Doughnut and Derailed to mediate muscle attachment site selection in Drosophila melanogaster. PLoS One 7:e32297.

Leptin M, Bogaert T, Lehmann R, Wilcox M. 1989. The function of PS integrins during Drosophila embryogenesis. Cell 56:401–408.

Leptin M. 1999. Gastrulation in Drosophila: The logic and the cellular mechanisms. EMBO J 18:3187–3192.

Lilly B, Zhao B, Ranganayakulu G, Paterson BM, Schulz RA, Olson EN. 1995. Requirement of Mads domain transcription factor D-Mef2 for muscle formation in Drosophila. Science 267:688–693.

Lim J, Thiery JP. 2012. Epithelial-mesenchymal transitions: Insights from development. Development 139:3471–3486.

Liotta D, Han J, Elgar S, Garvey C, Han Z, Taylor MV. 2007. The Him gene reveals a balance of inputs controlling muscle differentiation in Drosophila. Curr Biol 17:1409–1413.

Liu YH, Jakobsen JS, Valentin G, Amarantos I, Gilmour DT, Furlong EE. 2009. A systematic analysis of Tinman function reveals Eya and JAK-STAT signaling as essential regulators of muscle development. Dev Cell 16:280–291.

Livingston BT, Killian CE, Wilt F, Cameron A, Landrum MJ, Ermolaeva O, Sapojnikov V, Maglott DR, Buchanan AM, Ettensohn CA. 2006. A genome-wide analysis of biomineralization-related proteins in the sea urchin *Strongylocentrotus purpuratus*. Dev Biol 300:335–348.

Makabe KW, Kirchhamer CV, Britten RJ, Davidson EH. 1995. Cis-regulatory control of the SM50 gene, an early marker of skeletogenic lineage specification in the sea urchin embryo. Development 121:1957–1970.

Malinda KM, Fisher GW, Ettensohn CA. 1995. Four-dimensional microscopic analysis of the filopodial behavior of primary mesenchyme cells during gastrulation in the sea urchin embryo. Dev Biol 172:552–566.

Mann K, Wilt FH, Poustka AJ. 2010. Proteomic analysis of sea urchin (*Strongylocentrotus purpuratus*) spicule matrix. Proteome Sci 8:33–44.

Maqbool T, Jagla K. 2007. Genetic control of muscle development: Learning from Drosophila. J Muscle Res Cell Motil 28:397–407.

Matsumoto J, Kumano G, Nishida H. 2007. Direct activation by Ets and Zic is required for initial expression of the Brachyury gene in the ascidian notochord. Dev Biol 306:870–882.

McCauley BS, Weideman EP, Hinman VF. 2010. A conserved gene regulatory network subcircuit drives different developmental fates in the vegetal pole of highly divergent echinoderm embryos. Dev Biol 340:200–208.

McMahon A, Reeves GT, Supatto W, Stathopoulos A. 2010. Mesoderm migration in Drosophila is a multi-step process requiring FGF signaling and integrin activity. Development 137:2167–2175.

McMahon A, Supatto W, Fraser SE, Stathopoulos A. 2008. Dynamic analyses of Drosophila gastrulation provide insights into collective cell migration. Science 322:1546–1550.

Michelson AM, Abmayr SM, Bate M, Arias AM, Maniatis T. 1990. Expression of a MyoD family member prefigures muscle pattern in Drosophila embryos. Genes Dev 4:2086–2097.

Miller J, Fraser SE, McClay DR. 1995. Dynamics of thin filopodia during sea urchin gastrulation. Development 121:2501–2511.

Miller JR, McClay DR. 1997. Characterization of the role of cadherin in regulating cell adhesion during sea urchin development. Dev Biol 192:323–339.

Minemura K, Yamaguchi M, Minokawa T. 2009. Evolutionary modification of *T-brain* (*tbr*) expression patterns in sand dollar. Gene Expr Patterns 9:468–474.

Miner BG. 2007. Larval feeding structure plasticity during pre-feeding stages of echinoids: Not all species respond to the same cues. J Exp Mar Biol Ecol 343:158–165.

Minokawa T, Wikramanayake AH, Davidson EH. (2005) cis-Regulatory inputs of the *wnt8* gene in the sea urchin endomesoderm network. Dev Biol 288:545–558.

Misquitta L, Paterson BM. 1999. Targeted disruption of gene function in Drosophila by RNA interference (RNA-i): A role for nautilus in embryonic somatic muscle formation. Proc Natl Acad Sci USA 96:1451–1456.

Mitsunaga K, Akasaka K, Shimada H, Fujino Y, Yasumasu I, Numanoi H. 1986. Carbonic anhydrase activity in developing sea urchin embryos with special reference to calcification of spicules. Cell Differ 18:257–262.

Miya T, Nishida H. 2003. An Ets transcription factor, HrEts, is target of FGF signaling and involved in induction of notochord, mesenchyme, and brain in ascidian embryos. Dev Biol 261:25–38.

Miyamoto DM, Crowther RJ. 1985. Formation of the notochord in living ascidian embryos. J Embryol Exp Morphol 86:1–17.

Morino Y, Koga H, Tachibana K, Shoguchi E, Kiyomoto M, Wada H. 2012. Heterochronic activation of VEGF signaling and the evolution of the skeleton in echinoderm pluteus larvae. Evol Dev 14:428–436.

Munro E, Robin F, Lemaire P. 2006. Cellular morphogenesis in ascidians: How to shape a simple tadpole. Curr Opin Genet Dev 16:399–405.

Munro EM, Odell G. 2002b. Morphogenetic pattern formation during ascidian notochord formation is regulative and highly robust. Development 129:1–12.

Munro EM, Odell GM. 2002a. Polarized basolateral cell motility underlies invagination and convergent extension of the ascidian notochord. Development 129:13–24.

Murdock DJ, Donoghue PC. 2011. Evolutionary origins of animal skeletal biomineralization. Cells Tissues Organs 194:98–102.

Murray MJ, Saint R. 2007. Photoactivatable GFP resolves Drosophila mesoderm migration behaviour. Development 134:3975–3983.

Nakamura A, Shirae-Kurabayashi M, Hanyu-Nakamura K. 2010. Repression of early zygotic transcription in the germline. Curr Opin Cell Biol 22:709–714.

Nakatani Y, Yasuo H, Satoh N, Nishida H. 1996. Basic fibroblast growth factor induces notochord formation and the expression of As-T, a Brachyury homolog, during ascidian embryogenesis. Development 122:2023–2031.

Nguyen HT, Bodmer R, Abmayr SM, McDermott JC, Spoerel NA. 1994. D-mef2: A Drosophila mesoderm-specific MADS box-containing gene with a biphasic expression profile during embryogenesis. Proc Natl Acad Sci USA 91:7520–7524.

Nishimura Y, Sato T, Morita Y, Yamazaki A, Akasaka K, Yamaguchi M. 2004. Structure, regulation, and function of *micro1* in the sea urchin *Hemicentrotus pulcherrimus*. Dev Genes Evol 214:525–536.

Niwano T, Takatori N, Kumano G, Nishida H. 2009. Wnt5 is required for notochord cell intercalation in the ascidian *Halocynthia roretzi*. Biol Cell 101:645–659.

Noda T. 2011. The maternal genes Ci-p53/p73-a and Ci-p53/p73-b regulate zygotic ZicL expression and notochord differentiation in Ciona intestinalis embryos. Dev Biol 360:216–229.

Nowak SJ, Aihara H, Gonzalez K, Nibu Y, Baylies MK. 2012. Akirin links *twist*-regulated transcription with the Brahma chromatin remodeling complex during embryogenesis. PLoS Genet 8:e1002547. Epub Mar 1.

Ochiai H, Sakamoto N, Momiyama A, Akasaka K, Yamamoto T. 2008. Analysis of cis-regulatory elements controlling spatio-temporal expression of T-brain gene in sea urchin, *Hemicentrotus pulcherrimus*. Mech Dev 125:2–17.

Okazaki K. 1975. Normal development to metamorphosis. In: Czihak G, editor. The sea urchin embryo-biochemistry and morphogenesis. Berlin: Springer-Verlag. Ch. 7, pp 177–232.

Oliveri P, Carrick DM, Davidson EH. 2002. A regulatory gene network that directs micromere specification in the sea urchin embryo. Dev Biol 246:209–228.

Oliveri P, Davidson EH, McClay DR. 2003. Activation of pmar1 controls specification of micromeres in the sea urchin embryo. Dev Biol 258:32–43.

Oliveri P, Tu Q, Davidson EH. 2008. Global regulatory logic for specification of an embryonic cell lineage. Proc Natl Acad Sci USA 105:5955–5962.

Ozdemir A, Fisher-Aylor KI, Pepke S, Samanta M, Dunipace L, McCue K, Zeng L, Ogawa N, Wold BJ, Stathopoulos A. 2011. High resolution mapping of Twist to DNA in Drosophila embryos: Efficient functional analysis and evolutionary conservation. Genome Res 21:566–577.

Passamaneck YJ, Katikala L, Perrone L, Dunn MP, Oda-Ishii I, Di Gregorio A. 2009. Direct activation of a notochord cis-regulatory module by Brachyury and FoxA in the ascidian *Ciona intestinalis*. Development 136:3679–3689.

Paterson BM, Walldorf U, Eldridge J, Dubendorfer A, Frasch M, Gehring WJ. 1991. The Drosophila homologue of vertebrate myogenic-determination genes encodes a transiently expressed nuclear protein

marking primary myogenic cells. Proc Natl Acad Sci USA 88:3782–3786.

Pennington JT, Strathmann RR. 1990. Consequences of the calcite skeletons of planktonic echinoderm larvae for orientation, swimming, and shape. Biol Bull 179:121–133.

Peter IS, Davidson EH. 2011. Evolution of gene regulatory networks controlling body plan development. Cell 144:970–985.

Peterson RE, McClay DR. 2003. Primary mesenchyme cell patterning during the early stages following ingression. Dev Biol 254:68–78.

Picco V, Hudson C, Yasuo H. 2007. Ephrin-Eph signalling drives the asymmetric division of notochord/neural precursors in Ciona embryos. Development 134:1491–1497.

Pisani D, Feuda R, Peterson KJ, Smith AB. 2012. Resolving phylogenetic signal from noise when divergence is rapid: A new look at the old problem of echinoderm class relationships. Mol Phylogenet Evol 62:27–34.

Poh YC, Shevtsov SP, Chowdhury F, Wu DC, Na S, Dundr M, Wang N. 2012. Dynamic force-induced direct dissociation of protein complexes in a nuclear body in living cells. Nat Commun 3:866.

Porter SM. 2010. Calcite and aragonite seas and the de novo acquisition of carbonate skeletons. Geobiology 8:256–277.

Qian L, Wythe JD, Liu J, Cartry J, Vogler G, Mohapatra B, Otway RT, Huang Y, King IN, Maillet M, Zheng Y, Crawley T, Taghli-Lamallem O, Semsarian C, Dunwoodie S, Winlaw D, Harvey RP, Fatkin D, Towbin JA, Molkentin JD, Srivastava D, Ocorr K, Bruneau BG, Bodmer R. 2011. Tinman/Nkx2–5 acts via miR-1 and upstream of Cdc42 to regulate heart function across species. J Cell Biol 193:1181–1196.

Rafiq K, Cheers M, Ettensohn CA. 2012. The genomic regulatory control of skeletal morphogenesis in the sea urchin. Development 139:579–590.

Raman V, Andrews ME, Harkey MA, Raff RA. 1993. Protein-DNA interactions at putative regulatory regions of two coordinately expressed genes, *msp130* and *PM27*, during skeletogenesis in sea urchin embryos. Int J Dev Biol 37:499–507.

Ranganayakulu G, Zhao B, Dokidis A, Molkentin JD, Olson EN, Schulz RA. 1995. A series of mutations in the D-MEF2 transcription factor reveal multiple functions in larval and adult myogenesis in Drosophila. Dev Biol 171:169–181.

Reim I, Frasch M. 2010. Genetic and genomic dissection of cardiogenesis in the Drosophila model. Pediatr Cardiol 31:325–334.

Reverberi G. 1960. The causal formation of the brain in the ascidian larva. Acta Embryol Exp Morphol 3:296–336.

Revilla-i-Domingo R, Minokawa T, Davidson EH. 2004. R11: A cis-regulatory node of the sea urchin embryo gene network that controls early expression of SpDelta in micromeres. Dev Biol 274:438–451.

Revilla-i-Domingo R, Oliveri P, Davidson EH. 2007. A missing link in the sea urchin embryo gene regulatory network: hesC and the double-negative specification of micromeres. Proc Natl Acad Sci USA. 104:12383–12388.

Rhee JM, Oda-Ishii I, Passamaneck YJ, Hadjantonakis AK, Di Gregorio A. 2005. Live imaging and morphometric analysis of embryonic development in the ascidian *Ciona intestinalis*. Genesis 43:136–147.

Rho HK, McClay DR. 2011. The control of foxN2/3 expression in sea urchin embryos and its function in the skeletogenic gene regulatory network. Development 138:937–945.

Richardson B, Beckett K, Baylies M. 2008. Visualizing new dimensions in Drosophila myoblast fusion. Bioessays 30:423–431.

Rochlin K, Yu S, Roy S, Baylies MK. 2010. Myoblast fusion: When it takes more to make one. Dev Biol 341:66–83.

Röttinger E, Besnardeau L, Lepage T. 2004. A Raf/MEK/ERK signaling pathway is required for development of the sea urchin embryo micromere lineage through phosphorylation of the transcription factor Ets. Development 131:1075–1087.

Roy S, VijayRaghavan K. 1999. Muscle pattern diversification in Drosophila: The story of imaginal myogenesis. Bioessays 21:486–498.

Ruffins SW, Ettensohn CA. 1996. A fate map of the vegetal plate of the sea urchin (*Lytechinus variegatus*) mesenchyme blastula. Development 122:253–263.

Ruiz-Gómez M, Romani S, Hartmann C, Jäckle H, Bate M. 1997. Specific muscle identities are regulated by Krüppel during Drosophila embryogenesis. Development 124:3407–3414.

Rushton E, Drysdale R, Abmayr SM, Michelson AM, Bate M. 1995. Mutations in a novel gene, myoblast city, provide evidence in support of the founder cell hypothesis for Drosophila muscle development. Development 121:1979–1988.

Sandmann T, Girardot C, Brehme M, Tongprasit W, Stolc V, Furlong EE. 2007. A core transcriptional network for early mesoderm development in *Drosophila melanogaster*. Genes Dev 21:436–449.

Sandmann T, Jensen LJ, Jakobsen JS, Karzynski MM, Eichenlaub MP, Bork P, Furlong EE. 2006. A temporal map of transcription factor activity: mef2 directly regulates target genes at all stages of muscle development. Dev Cell 10:797–807.

Satoh G, Harada Y, Satoh N. 2000. The expression of nonchordate deuterostome Brachyury genes in the ascidian *Ciona* embryo can promote the

differentiation of extra notochord cells. Mech Dev 96:155–163.

Schnorrer F, Dickson BJ. 2004. Muscle building; mechanisms of myotube guidance and attachment site selection. Dev Cell 7:9–20.

Schnorrer F, Kalchhauser I, Dickson BJ. 2007. The transmembrane protein Kon-tiki couples to Dgrip to mediate myotube targeting in Drosophila. Dev Cell 12:751–766.

Schnorrer F, Schönbauer C, Langer CC, Dietzl G, Novatchkova M, Schernhuber K, Fellner M, Azaryan A, Radolf M, Stark A, Keleman K, Dickson BJ. 2010. Systematic genetic analysis of muscle morphogenesis and function in Drosophila. Nature 464:287–291.

Schönbauer C, Distler J, Jährling N, Radolf M, Dodt HU, Frasch M, Schnorrer F. 2011. Spalt mediates an evolutionarily conserved switch to fibrillar muscle fate in insects. Nature 479:406–409.

Schroeder TE. 1981. Development of a "primitive" sea urchin (*Eucidaris tribuloides*): Irregularities in the hyaline layer, micromeres, and primary mesenchyme. Biol Bull 161:141–151.

Schumacher S, Gryzik T, Tannebaum S, Müller HA. 2004. The RhoGEF Pebble is required for cell shape changes during cell migration triggered by the Drosophila FGF receptor Heartless. Development 131:2631–2640.

Schweitzer R, Zelzer E, Volk T. 2010. Connecting muscles to tendons: Tendons and musculoskeletal development in flies and vertebrates. Development 137:2807–2817.

Seipel K, Schmid V. 2005 Evolution of striated muscle: Jellyfish and the origin of triploblasty. Dev Biol 282:14–26.

Seo HC, Edvardsen RB, Maeland AD, Bjordal M, Jensen MF, Hansen A, Flaat M, Weissenbach J, Lehrach H, Wincker P, Reinhardt R, Chourrout D. 2004. Hox cluster disintegration with persistent anteroposterior order of expression in *Oikopleura dioica*. Nature 431:67–71.

Sharma T, Ettensohn CA. 2010. Activation of the skeletogenic gene regulatory network in the early sea urchin embryo. Development 137:1149–1157.

Shi W, Peyrot SM, Munro E, Levine M. 2009. FGF3 in the floor plate directs notochord convergent extension in the *Ciona* tadpole. Development 136:23–28.

Shimauchi Y, Murakami SD, Satoh N. 2001. FGF signals are involved in the differentiation of notochord cells and mesenchyme cells of the ascidian *Halocynthia roretzi*. Development 128:2711–2721.

Shishido E, Higashijima S, Emori Y, Saigo K. 1993. Two FGF-receptor homologues of Drosophila: One is expressed in mesodermal primordium in early embryos. Development 117:751–761.

Shoguchi E, Satoh N, Maruyama YK. 2000. A starfish homolog of mouse T-brain–1 is expressed in the archenteron of Asterina pectinifera embryos: Possible involvement of two T-box genes in starfish gastrulation. Dev Growth Differ 42:61–68.

Smith AB, Pisani D, Mackenzie-Dodds JA, Stockley B, Webster BL, Littlewood DT. 2006. Testing the molecular clock: Molecular and paleontological estimates of divergence times in the Echinoidea (Echinodermata). Mol Biol Evol 23:1832–1851.

Smith AB. 1997. Echinoderm larvae and phylogeny. Annu Rev Ecol Syst 28:219–241.

Smith J, Davidson EH. 2008. Gene regulatory network subcircuit controlling a dynamic spatial pattern of signaling in the sea urchin embryo. Proc Natl Acad Sci USA 105:20089–20094.

Smith MM, Cruz Smith L, Cameron RA, Urry LA. 2008. The larval stages of the sea urchin, *Strongylocentrotus purpuratus*. J Morphol 269:713–733.

Soler C, Han J, Taylor MV. 2012. The conserved transcription factor Mef2 has multiple roles in adult Drosophila musculature formation. Development 139:1270–1275.

Soler C, Taylor MV. 2009. The Him gene inhibits the development of Drosophila flight muscles during metamorphosis. Mech Dev 126:595–603.

Søviknes AM, Glover JC. 2008. Continued growth and cell proliferation into adulthood in the notochord of the appendicularian *Oikopleura dioica*. Biol Bull 214:17–28.

Stathopoulos A, Levine M. 2005. Genomic regulatory networks and animal development. Dev Cell 9:449–462.

Stathopoulos A, Tam B, Ronshaugen M, Frasch M, Levine M. 2004. *pyramus* and *thisbe*: FGF genes that pattern the mesoderm of Drosophila embryos. Genes Dev 18:687–699.

Strathmann RR, Grunbaum D. 2006. Good eaters, poor swimmers: Compromises in larval form. Integr Comp Biol 46:312–322.

Strathmann RR. 1971. The behavior of planktotrophic echinoderm larvae: Mechanisms, regulation, and rates of suspension feeding. J Exp Mar Biol Ecol 6:109–160.

Strünkelnberg M, Bonengel B, Moda LM, Hertenstein A, de Couet HG, Ramos RG, Fischbach KF. 2001. *rst* and its paralogue *kirre* act redundantly during embryonic muscle development in Drosophila. Development 128:4229–4239.

Takada N, York J, Davis JM, Schumpert B, Yasuo H, Satoh N, Swalla BJ. 2002. Brachyury expression in tailless Molgulid ascidian embryos. Evol Dev 4:205–211.

Takahashi H, Hotta K, Erives A, Di Gregorio A, Zeller RW, Levine M, Satoh N. 1999. Brachyury

downstream notochord differentiation in the ascidian embryo. Genes Dev 13:1519–1523.

Takahashi H, Hotta K, Takagi C, Ueno N, Satoh N, Shoguchi E. 2010. Regulation of notochord-specific expression of Ci-Bra downstream genes in *Ciona intestinalis* embryos. Zoolog Sci 27:110–118.

Takahashi H, Mitani Y, Satoh G, Satoh N. 1999. Evolutionary alterations of the minimal promoter for notochord-specific Brachyury expression in ascidian embryos. Development 126:3725–3734.

Taliaferro JM, Alvarez N, Green RE, Blanchette M, Rio DC. 2011. Evolution of a tissue-specific splicing network. Genes Dev 25:608–620.

Tanaka KK, Bryantsev AL, Cripps RM. 2008. Myocyte enhancer factor 2 and chorion factor 2 collaborate in activation of the myogenic program in Drosophila. Mol Cell Biol 28:1616–1629.

Tixier V, Bataillé L, Jagla K. 2010. Diversification of muscle types: Recent insights from Drosophila. Exp Cell Res 316:3019–3027.

Van Nostrand EL, Kim SK. 2011. Seeing elegance in gene regulatory networks of the worm. Curr Opin Genet Dev 21:776–786.

Veeman MT, Nakatani Y, Hendrickson C, Ericson V, Lin C, Smith WC. 2008. *Chongmague* reveals an essential role for laminin-mediated boundary formation in chordate convergence and extension movements. Development 135:33–41.

Vincent SD, Buckingham ME. 2010. How to make a heart: The origin and regulation of cardiac progenitor cells. Curr Top Dev Biol 90:1–41.

Vorbrüggen G, Jäckle H. 1997. Epidermal muscle attachment site-specific target gene expression and interference with myotube guidance in response to ectopic stripe expression in the developing Drosophila epidermis. Proc Natl Acad Sci USA 94:8606–8611.

Wada S, Saiga H. 2002. HrzicN, a new Zic family gene of ascidians, plays essential roles in the neural tube and notochord development. Development 129:5597–5608.

Wahl ME, Hahn J, Gora K, Davidson EH, Oliveri P. 2009. The cis-regulatory system of the *tbrain* gene: Alternative use of multiple modules to promote skeletogenic expression in the sea urchin embryo. Dev Biol 335:428–441.

Wei Q, Rong Y, Paterson BM. 2007. Stereotypic founder cell patterning and embryonic muscle formation in Drosophila require nautilus (MyoD) gene function. Proc Natl Acad Sci USA 104:5461–5466.

Wessel GM, Etkin M, Benson S. 1991. Primary mesenchyme cells of the sea urchin embryo require an autonomously produced, nonfibrillar collagen for spiculogenesis. Dev Biol 148:261–272.

Wilson R, Vogelsang E, Leptin M. 2005. FGF signalling and the mechanism of mesoderm spreading in Drosophila embryos. Development 132:491–501.

Wilt FH, Ettensohn CA. 2007. The morphogenesis and biomineralization of the sea urchin larval skeleton. In: Bauerlein E, editor. Handbook of biomineralization. Weinheim, Germany: Wiley-VCH Press. pp 183–210.

Winklbauer R, Müller HA. 2011. Mesoderm layer formation in Xenopus and Drosophila gastrulation. Phys Biol 8:045001.

Wray GA, McClay DR. 1988. The origin of spicule-forming cells in a 'primitive' sea urchin (*Eucidaris tribuloides*) which appears to lack primary mesenchyme cells. Development 103:305–15.

Wu SY, Ferkowicz M, McClay DR. 2007. Ingression of primary mesenchyme cells of the sea urchin embryo: A precisely timed epithelial mesenchymal transition. Birth Defects Res C Embryo Today 81:241–252.

Wu SY, McClay DR. 2007. The Snail repressor is required for PMC ingression in the sea urchin embryo. Development 2007 134:1061–1070.

Wunderlich Z, DePace AH. 2011. Modeling transcriptional networks in Drosophila development at multiple scales. Curr Opin Genet Dev 21:711–718.

Yagi K, Satou Y, Satoh N. 2004. A zinc finger transcription factor, ZicL, is a direct activator of Brachyury in the notochord specification of *Ciona intestinalis*. Development 131:1279–1288.

Yajima M, Umeda R, Fuchikami T, Kataoka M, Sakamoto N, Yamamoto T, Akasaka K. 2010. Implication of HpEts in gene regulatory networks responsible for specification of sea urchin skeletogenic primary mesenchyme cells. Zool Sci 27:638–646.

Yajima M, Wessel GM. 2011. Small micromeres contribute to the germline in the sea urchin. Development 138:237–243.

Yajima M, Wessel GM. 2012. Autonomy in specification of primordial germ cells and their passive translocation in the sea urchin. Development 139:3786–3794.

Yajima MA, 2007. A switch in the cellular basis of skeletogenesis in late-stage sea urchin larvae. Dev Biol 307:272–281.

Yamada S, Ueno N, Satoh N, Takahashi H. 2011. *Ciona intestinalis* Noto4 contains a phosphotyrosine interaction domain and is involved in the midline intercalation of notochord cells. Int J Dev Biol 55:11–18.

Yamasu K, Wilt FH. 1999. Functional organization of DNA elements regulating SM30alpha, a spicule matrix gene of sea urchin embryos. Dev Growth Differ 41:81–91.

Yamazaki A, Furuzawa Y, Yamaguchi M. 2010. Conserved early expression patterns of micromere specification genes in two echinoid species belonging to

the orders clypeasteroida and echinoida. Dev Dyn 239:3391–3403.

Yamazaki A, Ki S, Kokubo T, Yamaguchi M. 2009. Structure-function correlation of *micro1* for micromere specification in sea urchin embryos. Mech Dev 126:611–623.

Yamazaki A, Kidachi Y, Minokawa T. 2012. "Micromere" formation and expression of endomesoderm regulatory genes during embryogenesis of the primitive echinoid *Prionocidaris baculosa*. Dev Growth Differ 54:566–578.

Yarnitzky T, Min L, Volk T. 1997. The Drosophila neuregulin homolog Vein mediates inductive interactions between myotubes and their epidermal attachment cells. Genes Dev 11:2691–2700.

Yasuo H, Hudson C. 2007. FGF8/17/18 functions together with FGF9/16/20 during formation of the notochord in *Ciona* embryos. Dev Biol 302:92–103.

Yasuo H, Satoh N. 1998. Conservation of the developmental role of Brachyury in notochord formation in a urochordate, the ascidian *Balocynthia roretzi*. Dev Biol 200:158–170.

Zeitlinger J, Zinzen RP, Stark A, Kellis M, Zhang H, Young RA, Levine M. 2007. Whole-genome ChIP-chip analysis of Dorsal, Twist, and Snail suggests integration of diverse patterning processes in the Drosophila embryo. Genes Dev 21:385–390.

Zinzen RP, Girardot C, Gagneur J, Braun M, Furlong EE. 2009. Combinatorial binding predicts spatio-temporal cis-regulatory activity. Nature 462:65–70.