On a Possible Evolutionary Link of the Stomochord of Hemichordates to Pharyngeal Organs of Chordates

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Summary

As a group closely related to chordates, hemichordate acorn worms are in a key phylogenic position for addressing hypotheses of chordate origins. The stomochord of acorn worms is an anterior outgrowth of the pharynx endoderm into the proboscis. In 1886 Bateson proposed homology of this organ to the chordate notochord, crowning this animal group "hemichordates." Although this proposal has been debated for over a century, the question still remains unresolved. Here we review recent progress related to this question. First, the developmental mode of the stomochord completely differs from that of the notochord. Second, comparison of expression profiles of genes including *Brachyury*, a key regulator of notochord formation in chordates, does not support the stomochord/notochord homology. Third, *FoxE* that is expressed in the stomochord-forming region in acorn worm juveniles is expressed in the club-shaped gland and in the endostyle of amphioxus, in the endostyle of ascidians, and in the thyroid gland of vertebrates. Based on these findings, together with the anterior endodermal location of the stomochord, we propose that the stomochord has evolutionary relatedness to chordate organs deriving from the anterior pharynx rather than to the notochord.

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Recent molecular phylogenetic, genomic, and evolutionary developmental studies of deuterostomes have demonstrated that echinoderms and hemichordates form a clade called the Ambulacraria, whereas cephalochordates, urochordates (tunicates) and vertebrates are strongly supported as monophyletic Chordata, but the classical basal position of urochordates has been revised, placing them as the sister group to vertebrates (Bourlat *et al.*, 2006; Cameron *et al.*, 2000; Delsuc *et al.*, 2006; Halanych, 1995; Putnam *et al.*, 2008; Wada and Satoh, 1994). The Xenacoelomorpha is a newly recognized phylum some have assigned to the deuterostomes, but the phylogenetic position of this group is still uncertain (Philippe *et al.*, 2011; Telford, 2008). This revised deuterostome phylogeny has revitalized one of the largest questions in the evolution of metazoans, namely the origin and evolution of chordates (Brown *et al.*, 2008; Gerhart *et al.*, 2005; Lacalli, 2005; Ruppert, 2005; Satoh, 2008; Satoh *et al.*, 2012, 2014).

Although echinoderms and hemichordates are close relatives of chordates, hemichordates show several developmental and genomic features that are not seen in echinoderms and are quite chordate-like, such as distinctive gill-slits (Gillis *et al.*, 2012; Ogasawara *et al.*, 1999; Rychel and Swalla, 2007) and a tube-like nervous system that condenses dorsally in the collar region (Brown *et al.*, 2008; Kaul and Stach, 2010; Lowe *et al.*, 2003; Luttrell *et al.*, 2012; Miyamoto and Wada, 2013; Nomaksteinsky *et al.*, 2009). The Hox cluster of the echinoderm *Strongylocentrotus purpuratus* shows substantial reorganization including a major inversion and loss of *Hox4* (Cameron *et al.*, 2006). On the other hand, approximately 500-kb-length genomic regions of both hemichordates, *Ptychodera flava* and *Saccoglossus kowalevskii*, contain a well-organized cluster of *Hox1-Hox11/13c* (Freeman *et al.*, 2012), similar to the Hox cluster of *Branchiostoma floridae*, which consists of a basic set of *Hox* genes within approximately 200 kb of the genome (although the posterior *Hox* genes are further duplicated in the amphioxus) (Amemiya *et al.*, 2008; Holland *et al.*, 2008). In addition, *P. flava* contains an intact ParaHox cluster with a similar arrangement and a similar temporal expression order to that of chordate ParaHox genes (Ikuta *et al.*, 2013). Three ParaHox genes are not linked in *S. purpuratus* (Arnone *et al.*, 2006), although an intact ParaHox cluster was found in the starfish *Patiria miniata* (Annunziata *et al.*, 2013). All the data suggest that the hemichordate-chordate relationship requires further attention to bridge the gap between non-chordate and chordate deuterostomes (Brown *et al.*, 2008; Gerhart *et al.*, 2005; Ruppert, 2005; Tagawa *et al.*, 2001; Satoh, 2008).

During the development of acorn worm juveniles, a short diverticulum protrudes from the gut lumen anterodorsally into the protocoel (Kaul-Strehlow and Stach, 2013). This diverticulum possesses a central narrow lumen that is continuous with the lumen of the buccal cavity. As development proceeds, this structure becomes a rod-like protrusion from the dorsal buccal cavity, and is reinforced ventrally by the Y-shaped proboscis skeleton. This organ is called the "stomochord" or "buccal diverticulum." Bateson (1886) proposed homology of this organ to the chordate notochord, and together with other features

mentioned above he named this animal group "hemichordates" (Bateson, 1885). Following Bateson's proposal, the stomochord-notochord relationship has been debated for more than a century. The stomochord has been homologized to the vertebrate adenohypophysis (Komai, 1951), to a neurochord (Mookerjee *et al.*, 1955) or considered as an evolutionarily derived structure unique to hemichordates (Newell, 1952). Or others have restated the arguments in favor of the notochord homology (Balser and Ruppert, 1990).

The stomochord-notochord relationship has been revisited due to, for example, a recent recognition of a short tube-like nervous system that condenses dorsally in the collar region in acorn worms and the dorsoventral inversion hypothesis of chordate origin (Brown *et al.*, 2008; Gerhart *et al.*, 2005). However, it remains an open question whether the stomochord shares any evolutionary relationships with chordate characters. Recently, many developmental biology studies have either directly or indirectly elucidated molecular mechanisms involved in the formation of the chordate notochord as well as of the hemichordate stomochord. This article is aimed to review such works in an effort to answer this long-standing evo-devo question.

THE DEVELOPMENTAL MODE OF THE STOMOCHORD DIFFERS FROM THAT OF THE NOTOCHORD

First, comparative embryology shows that the developmental mode of the stomochord differs from that of the notochord (Table 1; Fig. 1). As shown in Figure 1a–d, during the development of acorn worm juveniles, a stomochord anlage first appears as a short diverticulum protruding from the gut lumen anterodorsally into the protocoel (Fig. 1a,b) and possesses a central narrow lumen that is continuous with the lumen of the buccal cavity (Fig. 1c). As development proceeds, this structure becomes a rod-like protrusion from the dorsal buccal cavity, reinforced ventrally by the Y-shaped proboscis skeleton (Fig. 1d). The primary structural role of the stomochord appears to be that of supporting the heart-glomerulus complex, which surrounds the anterior-dorsal aspect of this structure (Fig. 1d). Ultra-structually, the stomochord consists of a monolayered epithelium of highly columnar and vacuolated cells (Balser and Ruppert, 1990; Kaul-Strehlow and Stach, 2013).

Stomochord formation takes place in juveniles (during metamorphosis of indirect development species such as *Ptychodera*). In contrast, notochord formation takes place during early embryogenesis (Table 1; Fig. 1). In the cephalochordate amphioxus, during the time of neural tube formation, the notochord develops from the adjacent chordamesodermal plate that constitutes the roof of the archenteron (Fig. 1e–j). Namely, the amphioxus notochord is formed by an upward pouching off of midline cells along of the chordamesodermal plate (Conklin, 1932; Hatschek, 1893; Hirakow and Kajita, 1994). Later the notochord is filled with myofibrils and shows muscle-like properties (Ruppert, 1997).

The notochord of ascidians is composed of exactly 40 cells and runs through the dorsal midline of the larval tail (Satoh, 2014). The lineage of the cells is completely documented. The ascidian notochord is formed, from the onset of gastrulation to the completion of convergent extension, by the intercalation of primordial cells at the dorsal midline of the embryo (Table 1; Fig. 1k–n). Infolding and convergent extension transforms notochord

precursors into a column of stacked cells that form a monolayer epithelium (Jiang and Smith, 2007). Vacuolation within the cell increases the cell volume to lead to extension of the notochord as a midline organ (Fig. 1m,n).

The main reason why Batson (1886) proposed the evolutionary link of the stomochord and the notochord is the similarity between them since morphologically both organs consist of a monolayered epithelium of highly columnar and vacuolated cells. However, as mentioned above, their mode of development, namely timing and place of the organ formation is quite different. Ruppert (2005) discussed that the hemichordate stomochord is regionally restricted, lacks an association with the locomotory musculature and nerve cord, lies below rather than above the dorsal aorta, has a lumen that is confluent with the buccal cavity, and developmentally, the stomochord originates from dorsal endoderm but does not pass through a lumenless stack-of-coins stage.

GENES INVOLVED IN NOTOCHORD FORMATION

One approach to examine a possible evolutionary link of the stomochord to notochord is to identify toolkit genes and/or structural genes that are responsible for the formation of notochord and then ask whether these are expressed in the stomochord too. Genes involved in the notochord formation in the ascidian *Ciona intestinalis* have been characterized more fully than in other chordates. There are several studies on genes expressed in the notochord of amphioxus. In addition, recent studies attempted to elucidate genes that are expressed in the stomochord of acorn worms. Here we compare these data to gain some insight into stomochord/notochord relationship.

Gene Regulatory Cascade in *Ciona* Notochord Formation

Taking advantage of such a simple system of organ development (Fig. 1k–n) as well as of the well-characterized genome and a large quantity of transcriptome data, *Ciona intestinalis* stands out as a system in which a gene regulatory network underlying the notochord formation in chordates has been best characterized (reviewed by Satoh *et al.*, 2012).

A member of the T-box transcription factor family, *Brachyury*, plays a pivotal role in *Ciona* notochord formation. The gene (*Ci-Bra*) is expressed from the 64-cell stage onward exclusively in notochord precursor cells. Functional suppression of *Ci-Bra* results in the failure of notochord cell differentiation, and ectopic expression of *Ci-Bra* alters developmental fate of endoderm to notochord. Genes involved in the upstream cascade of *Ci-Bra* transcriptional activation are maternally expressed *β-catenin* and *P60*, and zygotically expressed *FoxD*, *FoxA*, *FGF9/16/20*, and *ZicL*.

In *Ciona* approximately 400 genes are characterized as *Brachyury* downstream cascade genes by subtraction of mRNAs in normal embryos from those in *Ci-Bra* ectopically expressed embryos (Takahashi *et al.*, 1999). Further analyses of these demonstrated that they are specifically or preferentially expressed in the notochord (Hotta *et al.*, 2000, 2008). The most prominent notochord-associated genes of *C. intestinalis* are *Brachyury*, *ATP-citrate-lyase* (*ACL*), *calumenin, collagenXI, ezrin-radixin-moesin (ERM), β-1,4-galactosyl transferase (β4GalT), leprecan, netrin, perlecan, prickle*, and others (Table 1; dataset-4).

Some of them including *leprecan* have counterparts expressed in vertebrate notochord (Capellini *et al.*, 2008), and some of them including *ACL, β4GalT* and *prickle* have been shown to be direct targets of *Ci-Bra* (Katikala *et al.*, 2013) (Table 1; dataset-4). However, no "organizer-like" genes including *chordin*, *admp*, *noggin*, and *shh* are expressed in the ascidian notochord.

Genes Involved in the Notochord Formation in Amphioxus

As described in the previous section, the amphioxus notochord is formed by an upward pouching off of midline cells along the chordamesodermal plate that constitutes the roof of the archenteron (Fig. 1e–j). The amphioxus *Bracnchiostoma belcheri* and *B. florida* each has two copies of *Brachyury*, of which spatiotemporal expression profiles are indistinguishable (Holland *et al.*, 1995; Putnam *et al.*, 2008; Terazawa and Satoh, 1995). Interestingly, *Amphi-Bra* is expressed not only in the notochord but also in the somite-forming region, as is also the case in vertebrates.

In relation to the muscle properties of the amphioxus notochord, structural genes that are expressed there were examined in the organ isolated from *B. belcheri* adults (Suzuki and Satoh, 2000). Analysis of a set of 257 ESTs showed that about 11% of the cDNAs are related to muscle genes, while 9% of them are genes for extracellular matrix proteins associated with formation of the notochordal sheath. The muscle related genes included *notochord-actin, troponin I (TropI), myosin regulatory light chain (nMRLC), myosin light chain kinase (nMLCK), myosin heavy chain 2 (MHC2), calponin (nCalponin)*, and others (Table 1, dataset-2). *In situ* analysis indicated that some of them are specific to the developing notochord (*nMRLC, nMLCK*, and *nCalponin*) while some others are expressed both in the developing notochord and muscle (*TropI* and *MHC2*) (Urano *et al.*, 2003). A recent comparative analysis of genes that are expressed in the notochord, muscle and neural tube demonstrated genes that are highly and predominantly expressed in the notochord, include *brachyury, globin, matrix metalloproteinase, myosin regulatory light chain* (*MRLC*), *myosin heavy chain, lysozyme, Pselectin*, and *ferritin* (Table 1, dataset-3). In addition, "organizer genes" such as *chordin, admp, foxA*, and others are expressed in the amphioxus notochord precursors (Yu *et al.*, 2007).

Since both amphioxus and ascidian notochords are enclosed by extracellular matrix, genes associated with this structure and function are recognized as those commonly expressed in the organ. However, in general, there are not always so many structural genes commonly expressed in both notochords due to difference of their structural components.

GENES INVOLVED IN STOMOCHORD FORMATION

Brachyury

Since *Brachyury* is a key regulator of notochord formation, its expression in the developing stomochord has been examined in both *Ptychodera flava* (Peterson *et al.*, 1999; Tagawa *et al.*, 1998) and *Saccoglossus kowalevskii* (Pani *et al.*, 2012). *Brachyury* of the acorn worms is not expressed in the developing stomochord but the gene is expressed in the archenteron invagination region and stomodeum invagination region of gastrulae and later in the tip of

adult anus. This is an important piece of evidence against a possible evolutionary link of the stomochord and notochord (Table 1).

Structural Genes

An argument on the evolutionary relationship of the two organs may be possible by identifying genes that are expressed in the stomochord and then comparing them with those expressed in the notochord of ascidians and amphioxus. Transcriptome analyses of *P. flava* have been recently reported (Chen *et al.*, 2014; Tagawa *et al.*, 2014). In a study (Tagawa *et al.*, 2014), the stomochord was isolated from *P. flava* adults for EST analysis, although the organ cannot completely separated from other organs (glomulus and heart) that surround the stomochord and firmly attach to it. Comparison of genes expressed in the stomochord with those expressed in other adult organs showed that *Pitx, Lhx9*, *disheveled-3, ferritin, myosin-10, NADH dehydrogenease, Type-III 5'-deiodinase*, and others are expressed highly and/or uniquely in the *P. flava* stomochord-containing tissues (Table 1, dataset-1; *Pitx* expression was shown to be predominant in the glomerulus). Although a few genes including *leprecan* (Fig. 2p) and *ferritin* are expressed in both hemichordate stomochord and chordate notochord (and those genes are expressed rather ubiquitously), we did not find evidence for expression similarities between the stomochord of hemichordates and notochord of ascidians or amphioxus in terms of these highly expressed genes (Table 1).

Toolkit Genes

Transcription factors and signaling molecules play pivotal roles in development of various organs of metazoans, and that evolutionary relatedness can be inferred between morphologically diversified organs that share the specific expression of these genes. Along this line, the stomochord of the direct-developing enteropneust *Saccoglossus kowalevskii* was examined by *in situ* hybridization for the expression of orthologs of genes of the vertebrate notochord, including those involved in early inductions (Ozair *et al.*, 2013), namely: *bra* (Fig. 2b; mentioned above), *chordin* (Fig. 2c), *noggin* (Fig. 2d), *admp* (Fig. 2e), *foxa* (Fig.2f), *hh* (Fig. 2g), and *ptch* (Fig. 2h). Of these, only *hh* is expressed prominently in the stomochord; however, it is also expressed throughout the endoderm. Thus, *in situ* evidence is weak for a stomochord/notochord homology.

We then surveyed orthologs of other chordate genes expressed anterior to the notochord, to see if any of these is stomochord-specific in acorn worms, since the stomochord is an anterior endodermal structure. Orthologs of vertebrate prechordal endomesoderm genes examined included *frzb* (*sfrp3/4*) (Fig. 2i), *gsc* (Fig. 2j), *hex* (Fig. 2k), *dmbx* (Fig. 2l), *otx* (Fig. 2m), *pitx* (Fig. 2n), and *dkk1/2/4* (Fig. 2o) (Lowe *et al.*, 2003; Lowe *et al.*, 2006; Pani *et al.*, 2012; unpublished data). Of these, *otx*, *hex*, and *dmbx* are expressed in the stomochord-forming region. However, *otx* is also widely expressed in endoderm at earlier stages and in neuroectoderm at later stages, *hex* is expressed along the entire dorsal midline of the endoderm, and *dmbx* expression is more restricted to the endoderm at the base of the stomochord. *Leprecan* is expressed in the pharynx near the stomochord, but not in it (Fig. 2p). Thus, their expression is not stomochord-specific.

Recently the Fox/Forkhead transcription factor family has been characterized in *S. kowalevskii* (Fritzenwanker *et al.*, 2014). Of them, it was found that *FoxE* is specifically expressed in buccal and pharyngeal region or stomochord-forming region (Fig. 3a,b) (Table 1). Since the specific *FoxE* expression in the stomochord is a cue to understanding what kind of organ the stomochord is, we now review the literature on the expression and function of *FoxE* in chordates.

In vertebrates, FoxE (TTF2) is a thyroid-specific transcription factor and plays key roles in thyroid development (Damante *et al.*, 2001). Amphioxus *FoxEa* is expressed in the club-shaped gland of the larvae (Fig. 3c,d) (Yu *et al.*, 2002), and later in the endostyle of the adult (Fig. 3c,d) (Hiruta *et al.*, 2005). Ascidian *Ci-FoxE* is expressed in the endostyle, prominently in zone 7 of the organ (Fig. 3e,f) (Hiruta *et al.*, 2005). Since the ascidian endostyle concentrates iodide, this organ is believed to be homologous to the vertebrate thyroid gland, which is derived by pouching of the pharynx. The common expression profile of *FoxE* in the hemichordate stomochord and chordate club-shaped gland/endostyle/thyroid gland strongly suggests that the stomochord is a related pharynx-derived organ.

CONCLUSION

Table 1 summarizes the developmental modes, morphological features, and regulatory genes of the stomochord of hemichordates and the notochord of chordates. First, the organs differ developmentally in at least four points of comparison, namely, developmental stage, mode of formation, structure formed, and possible function. In addition, *Brachyury* as well as a suite of other genes are expressed in the developing notochord but not in the stomochord-forming region, whereas *FoxE* is expressed in the stomochord outgrowth region but not in the developing notochord. Therefore, we conclude that evolutionarily the stomochord is not related to the notochord, but to pharynx-derived organs of chordates. According to the definition of a notochord, namely an organ supporting the larval tail of chordates (Satoh *et al.*, 2012), it is hard to accept that the axchord of annelids, recently reported by Larui *et al.* (2014) has a direct evolutionary link to the notochord. A question of how the notochord was originated during the evolution of chordates should be investigated by future studies, independently of the hemichordate stomochord (Satoh *et al.*, 2012).

If the stomochord of Bateson is not related to the notochord, the phylum Hemichordata loses part of its basis for naming. However, we prefer to retain this phylum name since acorn worms show several developmental and morphological features that are chordate-like, as Bateson pointed out, such as the distinctive gill-slits (Gillis *et al.*, 2012; Ogasawara *et al.*, 1999), and several that are not seen in echinoderms, such as the tube-like nervous system that condenses dorsally in the collar region (Brown *et al.*, 2008; Kaul and Stach, 2010; Luttrell *et al.*, 2012; Miyamoto and Wada, 2013; Nomaksteinsky *et al.*, 2009). At the same time, recent molecular phylogeny and genome studies support a sister grouping of hemichordates with echinoderms, named Ambulacraria. Both *Saccoglossus kowalevskii* and *Ptychodera flava* are now targets for genome sequencing and analysis. Genomic information of the acorn worms will provide us further tools to disclose molecular mechanisms underlying the evolution of deuterostomes and the origin of chordates.

Abbreviations

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ACL	ATP-citrate-lyase
ERM	ezrin-radixin-moesin
MRLC	myosin regulatory light chain
MLCK	myosin light chain kinase

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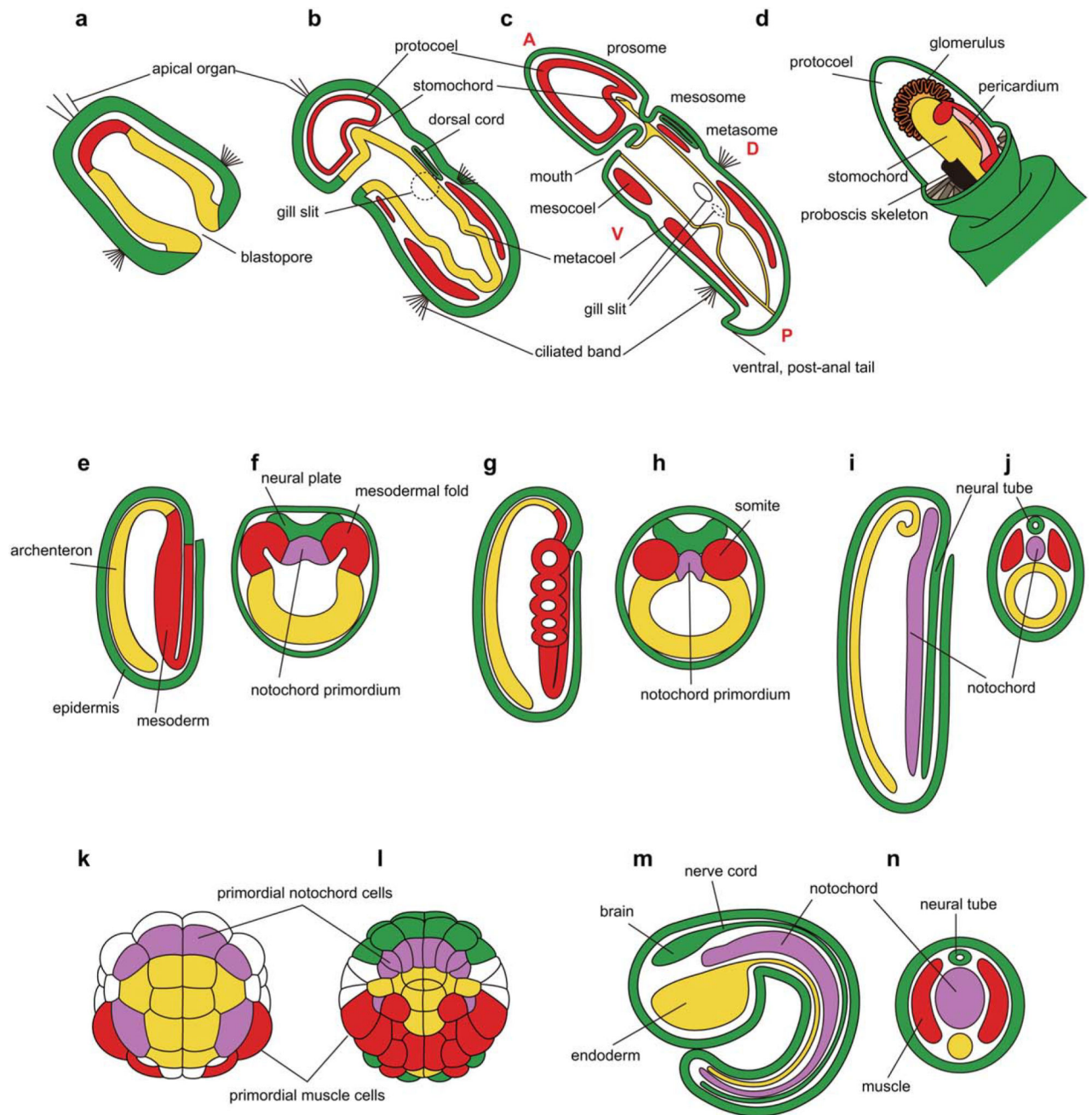
FIG. 1.

Development of the stomochord in hemichordates and the notochord in amphioxus and ascidians. (**a–c**) A schematic drawing of development of the stomochord in *Saccoglossus kowalevskii*. (**a**) A late gastrula (36 hrs postfertilization) shown in longitudinal section. Anterior is to the top left. Ectoderm shown in green, mesoderm in red, and endoderm in yellow. (**b**) A late neurula embryo (3 days post-fertilization) shown in sagittal section. Anterior is to the top left. Dorsal is to the top right. (**c**) The two gill slit embryo (14 days post-fertilization) in sagittal section. Note the extended stomochord protruding into the prosome, two paired gill-slits, and ventral post-anal tail (redrawn from Lowe et al., 2006).

(**d**) The stomochord in the proboscis of *Ptychodera* adult (redrawn from Peterson *et al.*, 1999). (**e–j**) A schematic drawing of development of the notochord in amphioxus embryos (based on Hatschek, 1893; Conklin, 1932; Hirakow and Kajita, 1994). (**e, f**) Mid neurula, (**g, h**) mid-to-late neurula, and (**I, j**) late neurula. (**e, g, i**) Mid-sagittal sectioned, (**f, h, j**) cross-sectioned. During the time of neural tube formation, the notochord develops from the adjacent chordamesodermal plate that constitutes the roof of the archenteron. Namely, the notochord is formed by an upward pouching off of midline cells along of the chordamesodermal plate. (**k–n**) Ascidian notochord development from the 64-cell stage embryo. (**k**) The 64-cell stage embryo, (**l**) 110-cell stage embryo, and (**m, n**) late tailbud embryo. Infolding and convergent extension transform notochord precursor into a column of 40 stacked cells.
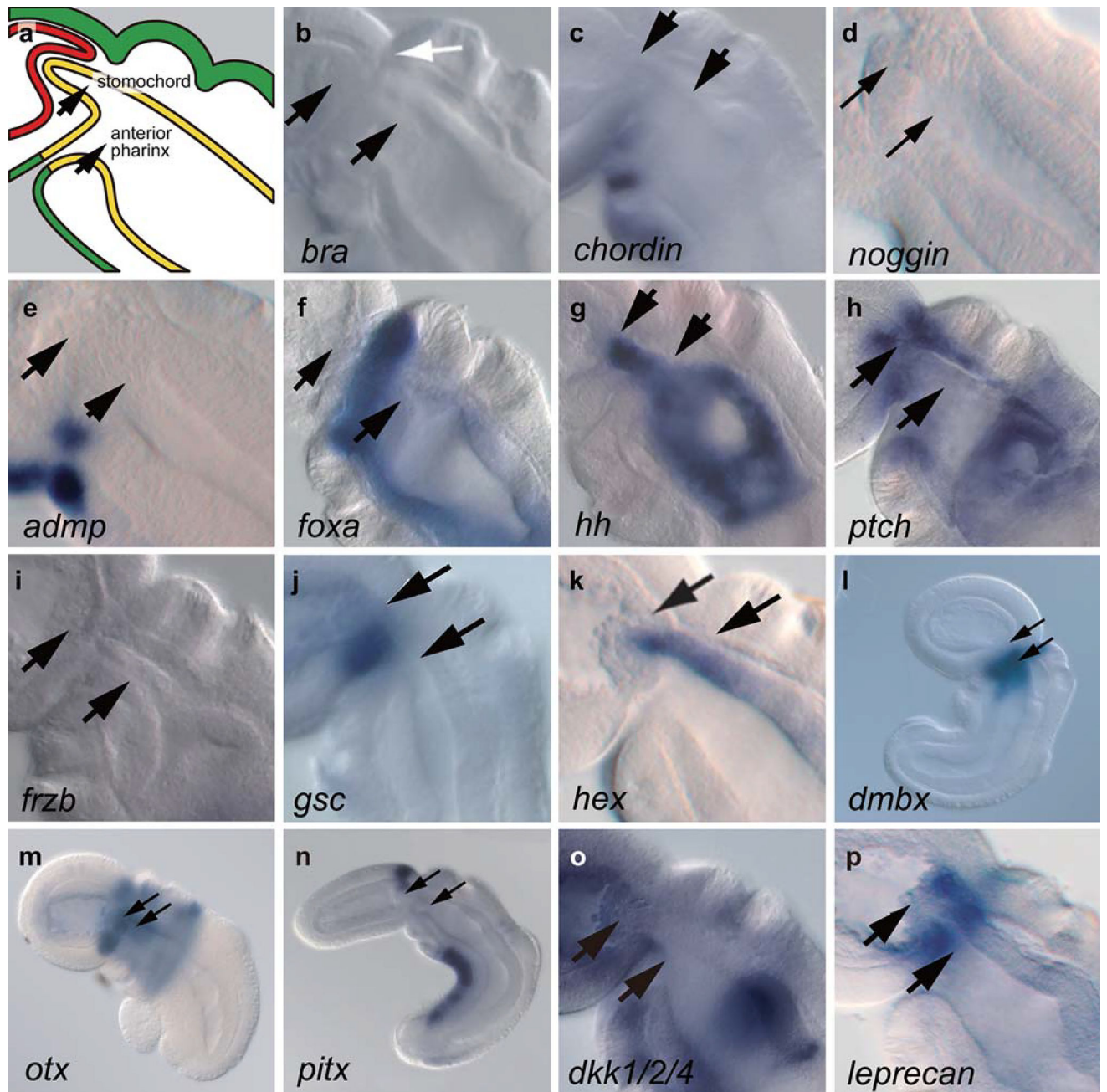
FIG. 2.
Expression of genes in the stomochord-forming region in *S. kowalevskii* juveniles. (**a**) A schematic drawing of the stomochord-forming region, (**b**) *bra*, (**c**) *chordin*, (**d**) *noggin*, (**e**) *admp*, (**f**) *foxA*, (**g**) *hh*, (**h**) *ptch*, (**i**) *frzb*, (**j**) *gsc*, (**k**) *hex*, (**l**) *dmbx*, (**m**) *otx*, (**n**) *pitx*, (**o**) *dkk1/2/4*, and (**p**) *leprecan* (Lowe *et al.*, 2003, 2006; Pani and Lowe, 2013) Black arrows indicate the stomochord-forming region and white arrow dorsal neural pit region.
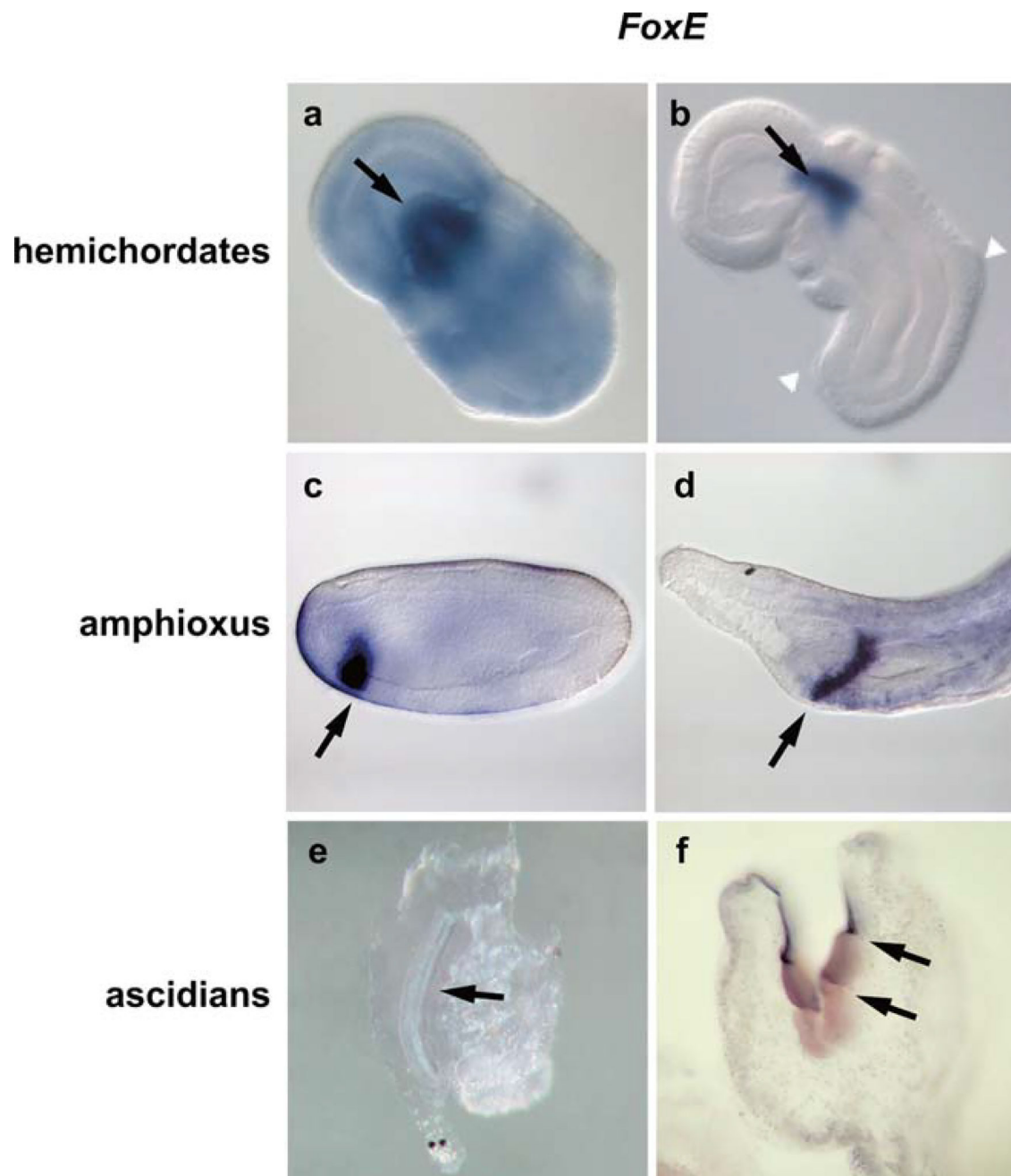
FoxE

FIG. 3.
Comparison of the expression of *foxE* among hemichordates, cephalochordates, and urochordates. (**a, b**) Expression of *foxE* in the stomochord-forming region of *Saccoglossus kowalevskii* juveniles. Arrows indicate the gene expression, and white arrowhead the ciliated band positions. (**c, d**) Expression of *foxE* in the forming region of the club-shaped gland and endostyle of amphioxus embryo (**c**) and juvenile (**d**), and (**e, f**) in the endostyle of ascidian juveniles, whole juvenile (**e**) and section of the endostyle (**f**).

Table 1

Comparison of Characteristic Features Between the Acorn Worm Stomochord and Chordate Notochord

		Notochord	
Organ	Stomochord	Cephalochordates	Urochordates
Developmental stage at which the organ is formed	Juveniles	Embryos	Embryos
The region in which the organ is formed	Proboscis of juveniles (anterior region of the body)	Dorsal midline of fish-like larvae (anterior to posterior region of the body)	Tail of tadpole-like larvae (posterior region of the body)
Developmental and morphological features	Formed by anterior outgrowth of pharynx (buccal diverticulum); layered cells, vacuolated; covered with extracellular matrix	Formed by pinching off of dorsal archenteron; coin-shaped cells with myofibrils; covered with the notochord sheath	Formed by convergence, intercalation, and extension of precursor cells; vacuolated; covered with the notochord sheath
Gene expression profile			
Brachyury	−	+	+
FoxE	+	−	−
Structural genes	**Data set-1** ([*1])	**Data set-2** ([*2])	**Data set-4** ([*4])
	pituitary homeobox 2 (Pitx2)	Notochord actin	Brachyury
	LIM/homeobox protein Lhx9	Tropomyosin	ATP citrate-lyase
	bHLH B7	Troponin I	aryl hydrocarbon receptor nuclear translocator
	bHLH protein Pod1	Calmodulin	ATP sulfurylase/APS kinase
	BMP3	Calponin	calumenin
	BMP4	Myosin light chain kinase	calcium/calmodulin-dependent protein kinase
	CREAP-1	Myosin regulatory light chain	cdc45
	DEAHbox Protein 34	Creatine kinase	collagen XVIII
	dishevelled-3	Muscle LIM protein	collagen XI
	krueppel-like factor 15		ezrin/radixin/moesin (ERM)
	growth/differentiation factor 16	**Data set-3** ([*3])	myomegalin
	ferritin	Brachyury	pellino
	Myoneurin	angiopoietin-1 isoform X3	fibrinogen-like
	myosin-10	connective tissue growth factor precursor	leprecan
	NADH dehydrogenase subunit-6	collagen alpha-1(IV) chain	netrin
	Nuclear Receptor Subfamily 4	complement component 1	proliferating cell nuclear antigen
	Nuclear Receptor Subfamily 5	cytoplasmic actin	perlecan
	peptidyl-prolyl cis-trans isomerase B	ferritin-like	prickle
	RBBP1	fibril collagen	P-selectin
	Type-III 5'-deiodinase	follistatin-like	sulfate transporter
	Zinc Finger Protein 91	globin	tropomyosin
	Zinc Finger Protein 275	hemicentin-2	tensin
	Zinc Finger protein 551	lysozyme G-like 2 precursor	tyrosine phosphatase

| Organ | Stomochord | Notochord | |
		Cephalochordates	Urochordates
	Zinc Finger Protein 626	lysyl oxidase	UBE2
	Zinc Finger Protein 681	matrix metalloproteinase	zipper
		muscle protein 20	β-1,4-galactosyl transferase (β4GalT)
		myosin heavy chain	
		myosin regulatory light chain 9-like	
		notch-2 like	
		plasminogen	
		P-selectin	
		selenoprotein P	
		transglutaminase	
		trehalase-like	
		tyrosine decarboxylase	
		thrombospondin-2	

*1*Data set-1 was obtained by comparative analysis of genes expressed in stomochord-containing tissues and those in other tissues of *Ptychodera flava* adults.

*2*Data set 2 was obtained by analysis of genes expressed in notochord isolated from *Brachiostoma belcheri* adults.

*3*Data set-3 was obtained by comparative analysis of genes expressed in notochord and those in muscle and neural tube of *Brachiostoma floridae* adults.

*4*Data set-4 was a selection of *Ci-Bra* downstream genes expressed in notochord of *Ciona intestinalis* tailbud embryos.