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Evolution of Developmental Control Mechanisms

Ventralization of an indirect developing hemichordate by $NiCl_2$ suggests a conserved mechanism of dorso-ventral (D/V) patterning in Ambulacraria (hemichordates and echinoderms)

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ABSTRACT

One of the earliest steps in embryonic development is the establishment of the future body axes. Morphological and molecular data place the Ambulacraria (echinoderms and hemichordates) within the Deuterostomia and as the sister taxon to chordates. Extensive work over the last decades in echinoid (sea urchins) echinoderms has led to the characterization of gene regulatory networks underlying germ layer specification and axis formation during embryogenesis. However, with the exception of recent studies from a direct developing hemichordate (*Saccoglossus kowalevskii*), very little is known about the molecular mechanism underlying early hemichordate development. Unlike echinoids, indirect developing hemichordates retain the larval body axes and major larval tissues after metamorphosis into the adult worm. In order to gain insight into dorso-ventral (D/V) patterning, we used nickel chloride ($NiCl_2$), a potent ventralizing agent on echinoderm embryos, on the indirect developing enteropneust hemichordate, *Ptychodera flava*. Our present study shows that $NiCl_2$ disrupts the D/V axis and induces formation of a circumferential mouth when treated before the onset of gastrulation. Molecular analysis, using newly isolated tissue-specific markers, shows that the ventral ectoderm is expanded at expense of dorsal ectoderm in treated embryos, but has little effect on germ layer or anterior–posterior markers. The resulting ventralized phenotype, the effective dose, and the $NiCl_2$ sensitive response period of *Ptychodera flava*, is very similar to the effects of nickel on embryonic development described in larval echinoderms. These strong similarities allow one to speculate that a $NiCl_2$ sensitive pathway involved in dorso-ventral patterning may be shared between echinoderms, hemichordates and a putative ambulacrarian ancestor. Furthermore, nickel treatments ventralize the direct developing hemichordate, *S. kowalevskii* indicating that a common pathway patterns both larval and adult body plans of the ambulacrarian ancestor and provides insight in to the origin of the chordate body plan.

Introduction

One of the earliest steps in the development of an animal's body plan is the specification of the future body axes. Although the mechanisms and timing of axis specification can vary at the species level (Prodon et al., 2004), in most metazoans, the primary egg axis, the so-called animal–vegetal (A/V) axis is established during oogenesis. This axis plays an important role in the subsequent establishment of the embryonic anterior–posterior (A/P), dorso-ventral (D/V, also called the oral–aboral (O/A) axis in larval echinoderms) and the consequent left–right (L/R) axes. Axial reorganizations may have played crucial roles in major evolutionary events, like the transition from radial to bilaterally symmetric animals (Finnerty et al., 2004; Matus et al., 2006) or the proposal that a D/V inversion (shift of the invertebrate mouth to the opposite side of the embryo) associated with the origin of chordates (Arendt and Nubler-Jung, 1994; Benito-Gutierrez and Arendt, 2009; De Robertis and Sasai, 1996; Gerhart, 2000; Lacalli, 1995). Understanding the mechanisms that drive axial patterning during both larval and adult life history stages and how they have been altered is therefore crucial for understanding possible evolutionary scenarios involved in body plan reorganization. In this study we describe the alteration of axial patterning in an indirect developing hemichordate, a representative of an early branching deuterostome with potential chordate-like features.

Chordates, a group of animals that consists of vertebrates, urochordates (ascidians), and cephalochordates (Amphioxus) are the most intensely studied group of multicellular organisms. However, the evolutionary origin of this group of animals and their unique morphological traits are largely unresolved and remain one of the more controversial questions in metazoan evolutionary biology. The chordates belong to a larger group of metazoan animals called deuterostomes. The deuterostomes consist of two major clades, the chordates and their sister group called the Ambulacraria (Swalla and

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Smith, 2008), traditionally formed by two taxa, echinoderms (e.g. sea urchins, sea stars, sea cucumbers) and hemichordates (e.g. acorn worms). A potential but highly controversial third taxon, *Xenoturbella,* has also been proposed to belong to this group, however they currently do not bear a single morphological synapomorphy with any other deuterostome (Bourlat et al., 2003, 2006; Dunn et al., 2008; Hejnol et al., 2009; Perseke et al., 2007; Philippe et al., 2007) (Fig. 1A).

Although the morphology and life history of echinoderms and hemichordates is well studied, the variation between these two groups does not provide a clear view of the origin of chordate features. For example, extant adult echinoderms provide little insight into the problem because they are highly derived pentaradial forms that are difficult to homologize morphologically with any of the defining characteristics of chordates. Although there is a large body of work regarding the development of echinoid larvae from decades of molecular work (Angerer and Angerer, 2000; Croce et al., 2006; Davidson et al., 2002a,b; Duboc et al., 2004; Ettensohn and Sweet, 2000; McClay, 2000; Röttinger et al., 2008; Smith and Davidson, 2008; Sodergren et al., 2006) including the sequencing of the purple sea urchin genome (Sodergren et al., 2006), the relationship between embryonic patterning and adult body plan formation in echinoderms relative to chordate origins remains problematic due to their radical metamorphosis of a bilaterally symmetrical larva to the pentaradial adult.

Adult enteropneust hemichordates (acorn worms) are better candidates for understanding chordate origins in that, like chordates, both their larval and adult body plan are bilaterally symmetric and the adult shares some adult chordate specific traits, such as a filter feeding pharynx with gills slits and a transient post anal tail (only in harrimaniids). However adult hemichordates also lack other defining chordate features such as a clearly homologous dorsal hollow nerve cord or notochord that runs the length of the body. Recently, the direct developing hemichordate *Saccoglossus kowalevskii* (Harrimana) has been developed as a key species for studying the origin of the deuterostome body plan (Cameron et al., 2000; Lowe et al., 2006, 2003; Rychel et al., 2006). Hemichordates contain about 100 living species, divided into three classes. Although the exact taxonomic relationship of the three hemichordate classes is currently under debate (Fig. 1A) it appears that the indirect developing ptychoderidae enteropneust worms (e.g. *P. flava*) are representatives of the basal branch of hemichordates and may retain ancestral deuterostome life history characteristics (Cameron et al., 2000; Cannon et al., 2009; Swalla and Smith, 2008).

In order to identify features crucial for the understanding of ancestral characters present in basal deuterostomes, a detailed understanding of the fate maps and early development of echinoderms, hemichordates and chordates is required. Fate mapping studies on indirect developing echinoderms (e.g. (Cameron et al., 1987)), and direct and indirect developing hemichordates (Colwin, 1953; Henry et al., 2001) revealed strong similarities between hemichordates and indirect developing echinoids (Fig. 1B). In fact, hemichordates and indirect developing echinoderms both generate their mesoderm at the vegetal pole, which differs from that in chordates, where the vegetal most region gives rise to endoderm (Kumano and Smith, 2002). Comparisons of the adult bilateral hemichordate body plan to pentaradial echinoderms are difficult, but one feature that could unite both groups is the bilaterally symmetrical "dipleurula" pelagic larval stage. An important life history characteristic of the ptychoderid hemichordates is that they undergo indirect development with the formation of a larval form that metamorphoses into a benthic adult worm after an extended pelagic phase (Hadfield, 1975; Nielsen and Hay-Schmidt, 2007; Tagawa et al.,

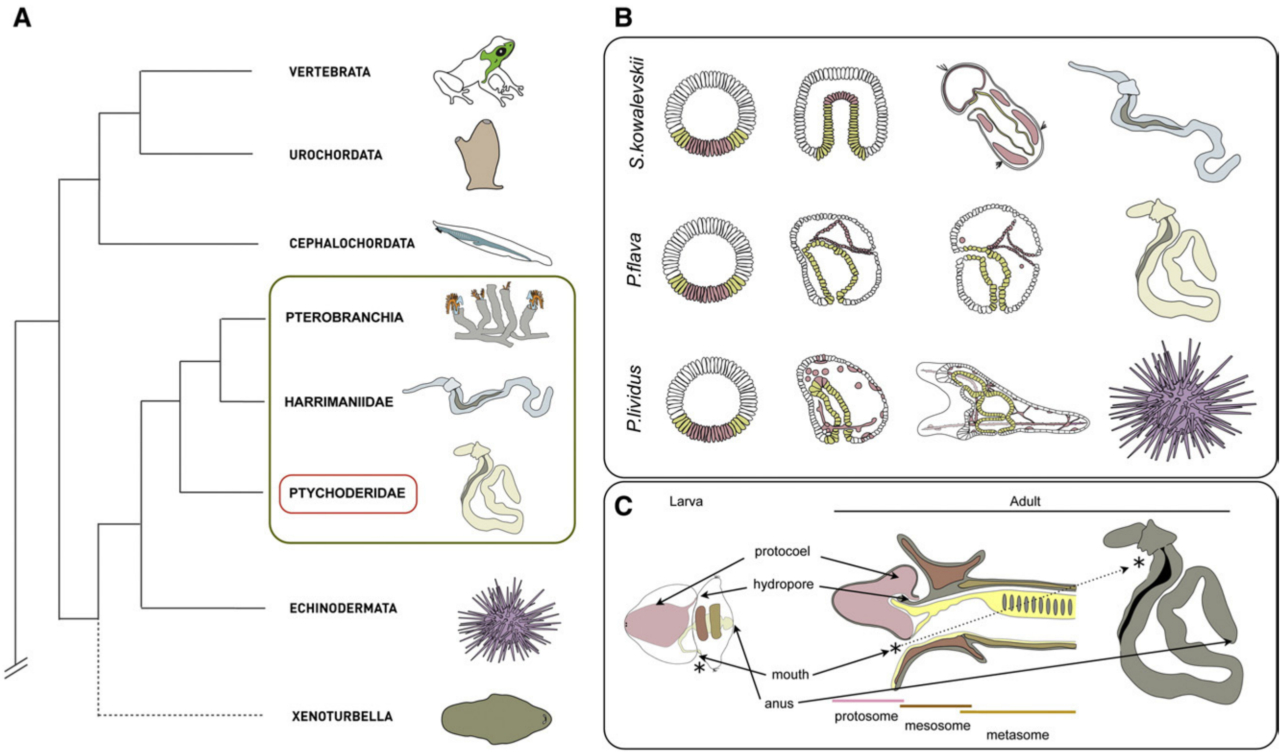

Fig. 1. (A) Phylogenetic position of the Hemichordata. Echinodermata and Hemichordata comprise the Ambulacraria, a group of non-chordate deuterostomes. Among Hemichordata (green box), the ptychoderidae enteropneust worms (red box) are representatives of the basal branch of hemichordates (Cannon et al., 2009). The dashed line connecting *Xenoturbella* indicates that their position within deuterostomes remains controversial (Hejnol et al., 2009). (B) Diagram representing the similarities in fate maps and larval morphology between echinoderms and hemichordates. *Saccoglossus kowalevskii* is a direct developing hemichordate, *Ptychodera flava*, an indirect developing hemichordate and *Paracentrotus lividus* an indirect developing echinoderm. All represented stages are oriented animal up, vegetal down, ventral (oral) to the left and dorsal to the right. Red and yellow shading represents the presumptive mesoderm and endoderm, respectively. (Henry et al., 2001; Logan and McClay, 1997; Lowe et al., 2003). (C) Schematic representation of *P. flava* metamorphosis. The adult form retains the same symmetry properties and most of the same tissues as the larval form.