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Larval Evolution: I'll Tail You Later…

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Larval stages can be astonishingly different from their adult forms. A new study in acorn worms shows that the whole larval body is patterned only with a subset of anterior genes, revealing the intricate developmental bases that underlie the evolution of larval forms.

"From a standpoint of sheer architectural beauty
A naturalist must, I think, feel it a duty
Of all the sea-jellies that dazzle the eyes
To give to tornaria the highest prize"
–Walter Garstang

Humans are direct developers. We grow from the fertilized egg into adulthood in a progressive and steady manner. Toddlers, children or teenagers are not much different from our adult selves — in morphological terms. However, direct development is not a mainstream trend in animals. In fact, the majority of animals on this planet have one or more life stages that differ from their adult forms in the most extraordinary ways. Carl Linnaeus [1] called these 'larval stages', based on the Latin word *lārva,* which means 'a terrifying mask'. The word itself became an umbrella term to describe any embryonic stage that deviates from a blasé direct-developing life history.

Typically, larvae exhibit a disparate morphology — often with specialized feeding or locomotory structures — and occupy a distinct habitat from adults [2]. But the diversity of larval forms is so overwhelming that it almost defies biologists with questions. How do you build such contrasting larval and adult body patterns within the same ontogeny? What molecules underlie the development of larvae and how do they compare with the patterning of the adult body? How does a larval stage evolve and how can it be lost? A paper in this issue of *Current Biology* [3] approaches these questions by investigating the molecular basis of larval development in acorn worms.

Hemichordates are burrowing worm-like marine organisms closely related to echinoderms (e.g. sea stars) that occupy a strategic position, within the lineage of the Deuterostomia, for understanding the evolution of chordates [4]. Acorn worms, the largest branch of hemichordates, have direct- and indirect-developing species. While the embryogenesis of direct developers takes place inside the egg at the bottom of the sea, with the embryo only hatching as a juvenile, indirect-developing species hatch at the gastrula stage and develop into an almost transparent, balloon-shaped swimming larva — the tornaria (Figure 1A). It swims for months, capturing food with ciliary beating, and progressively grows by extending its posterior end into a worm-like juvenile that eventually sinks to the bottom for the adult life (Figure 1B). A direct-developing acorn worm develops through the staggered expression of transcription factors along the anterior-posterior axis [5,6] in a coordinate system surprisingly similar to that of chordates [7]. However, we have little idea if such molecular network is conserved in indirect-developing hemichordates. Do the same patterning genes underlie the development of the tornaria larva? Gonzalez *et al.* [3] analyzed the expression of over 20 transcription factors during the embryogenesis of the

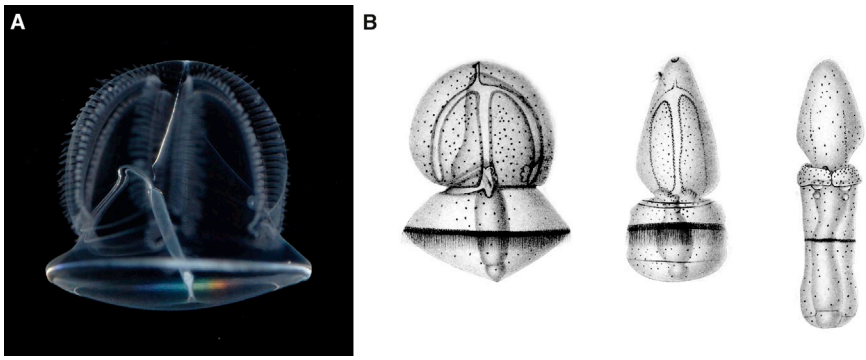

Figure 1. The tornaria larva of hemichordates.
(A) Living tornaria larva of an unidentified hemichordate species. Copyright: Eric Röttinger, Kahi Kai Images. (B) Development of the tornaria larva by growth of the posterior end. Line drawings modified from [20] with permission from John Wiley and Sons.

indirect-developing hemichordate *Schizocardium californicum*, providing the first comprehensive characterization of the anterior–posterior patterning of the tornaria.

The authors first examined post-metamorphic juveniles of *S. californicum*, a stage already with adult-like morphology, to compare with the best studied direct-developing hemichordate *Saccoglossus kowalevskii*. The genes are differentially expressed along the body regions of *S. californicum* juveniles: there are genes expressed anterior to the mouth (*dlx*, *foxQ2.1*, *foxG*, *fez*, *otp*, *rx*, *six3*, *zic*), immediately posterior to the mouth (*emx*, *irx*, *lim1/5*, *pax6*, *otp*, *otx*), in the anterior region of the trunk (*en*, *gbx*), in the middle and posterior trunk regions (*Hox genes*), and finally, at the very posterior end of the body (*cdx, xlox*). When compared to *S. kowalevskii*, the organization of these transcripts along the animal-vegetal axis in juvenile stages is the same, regardless if the hemichordate is a direct or indirect developer.

The analysis of the whole developmental series of *S. californicum*, however, revealed a different pattern. Surprisingly, the genes expressed in the early tornaria larva correspond solely to the anterior gene expression territories of the juveniles (i.e. the proboscis and collar). The larva is missing the whole set of genes expressed in the trunk region, mainly the iconic Hox genes, and only a narrow territory around the blastopore expresses anterior trunk genes, such as *gbx*. Together, this indicates that the tornaria larva corresponds — from a transcriptional perspective — to an anterior end without a trunk, or more simply put — a truncated version of the adult. While the tornaria grows, these missing gene territories are progressively activated at the posterior end of the larval body and, soon after metamorphosis, are expressed in the juvenile body of *S. californicum* in the same manner as in direct-developing species. The authors show that in hemichordates, only an anterior subset of 'adult' patterning genes are employed in larval development, while trunk-associated patterning genes, such as the Hox genes, are only activated later in the larval life. Can this finding be generalized across animals? How does it relate to other studies in which the molecular patterning of larva has been compared with adults?

The comparison of the Gonzalez *et al.* [3] findings with other animal lineages yields interesting observations about larval development. In the echinoderms — the closest relatives of hemichordates, which comprise famous marine animals such as sea stars and sea cucumbers — the set of 'adult' genes that pattern the larval tissues differ from the hemichordate tornaria. In echinoderm larvae, their mid/posterior Hox genes are expressed in the forming larval coeloms [8–10] and digestive tract [11]. Thus, the expression of Hox genes in larval echinoderms and hemichordates differ spatially and temporally. Comparisons across larger evolutionary distances show that Hox genes in annelids, brachiopods and molluscs are involved in the patterning of a diverse set of ectodermal and mesodermal structures of the larval trunk [12–14] (Figure 2). Finally, in the pilidium larva of ribbon worms (Nemertea), Hox genes are not expressed at all in the larval tissues; only in the developing juvenile [15]. The emerging picture from these comparative data is that the molecular patterning of larval structures is as diverse as the morphology of larval forms.

Despite this diversity, these larvae do show a common trait. Independent of the larval type (e.g. feeding or non-feeding) or metamorphosis (catastrophic or gradual), the Hox genes seem to be only expressed in tissues that contribute to the adult body. The somatocoels or gut in the case of echinoderm larvae, the larval trunk in annelids, brachiopods and molluscs, and the juvenile worm itself in nemerteans, which forms from imaginal discs in the larva. The comparison of hemichordates and nemerteans is particularly enlightening. Even though both groups are phylogenetically distant, we see a similar mode of direct and indirect molecular patterning. In the direct developers of both clades, the Hox genes are expressed from early stages [6,16] while indirect developers have the expression delayed until the juvenile tissues are being formed [3,15]. But what does this mean for the evolution of larval forms? A recurrently debated topic is how many times larvae have evolved and which animal taxa share homologous larvae [17]. For instance, the morphological similarities between hemichordate and echinoderm larvae raised the hypothesis that their common ancestor already had a swimming larval stage known as dipleurula (discussed in [17,18]). Sly *et al.* [19] put the argument forward that the differences between the larval deployment of adult patterning genes provide evidence for the convergent evolution of larvae. In other words: if the molecular patterning of larvae is diversified, while the adult stages are patterned in a similar manner, the larvae do not share a common evolutionary origin. Following this logic, the results from Gonzalez *et al.* [3] would speak for the convergence of the hemichordate tornaria and the echinoderm dipleurula larva, since both use different subsets of

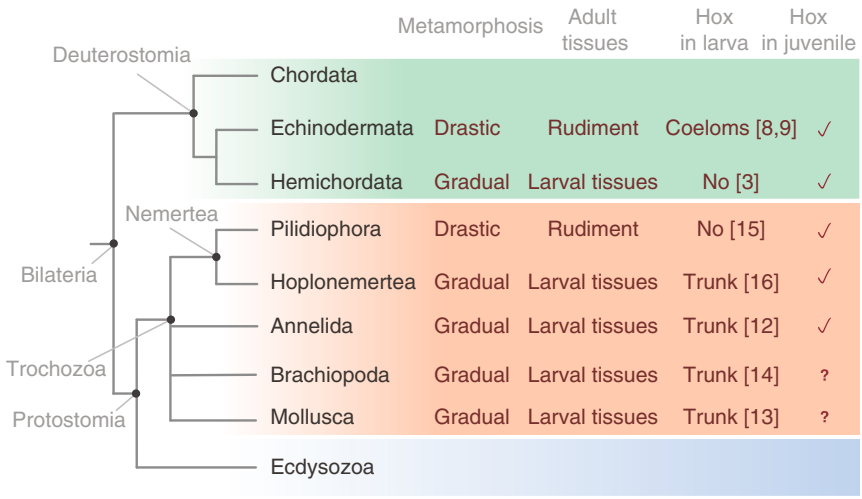

Figure 2. Simplified overview of Hox gene expression in ciliated larvae.
Only taxa with ciliated larva in which Hox gene expression has been studied are shown. The comparison suggests the expression of Hox genes is associated to the future adult tissues, independent of the type of development. Species exhibiting gradual metamorphosis express Hox genes in tissues of the larval body that will be maintained through metamorphosis (generally the larval trunk region), while in larvae with drastic metamorphosis the Hox genes are mainly expressed in the juvenile rudiments, and not in the transient larval tissues (echinoderms and nemertean pilidium). The differences between 'gradual' and 'drastic' are not as clear cut as it seems in the figure, see for example [18] for molluscs.

adult genes for the patterning of their body.

However, the answer is not that simple. Larvae themselves show many evolutionary adaptations to their lifestyle and their ecology — for example, different amounts of yolk content, and spcialized feeding apparatus and locomotory ciliation. These differences might also influence the divergent degrees to which adult tissues are present as anlagen in the larval body, and ultimately their molecular patterning. Thus, evolution could have obscured the original similarities that the larvae of the two lineages share with their ancestral form. In any case, it is relevant to note that even if hemichordate and echinoderm larvae have a common evolutionary origin, the broader comparative data on Hox expression between larval and adult stages suggests that a larval stage might be a derived condition in bilaterians. A better understanding of the larval and adult body patterning across the different bilaterian lineages, combined with a more solid phylogenetic framework, will be crucial to advance this debate for the evolution of animal life history.

What we can say is that there are as many ways of building larval bodies as there are larvae. Therefore, to understand how development evolves and larvae evolve, more studies that compare related species that differ in their larval morphology, such as Gonzalez *et al.* [3], are necessary. This approach has been successfully explored with echinoderm larvae, the group that yields a great number of insights into the intricate evolution of larval forms. Plenty of other groups could also contribute, due to their high diversity of larval forms such as nemerteans, annelids, molluscs, and even brachiopods and bryozoans. Studying these groups will lead to a better understanding about the molecular changes involved in the evolution of metazoan larval diversity, and will shed some new light onto how such different shapes develop by the genome of the same individual.

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Cell Migration: Making the Waves

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Coordinated changes of cell shape are often the result of the excitable, wave-like dynamics of the actin cytoskeleton. New work shows that, in migrating cells, protrusion waves arise from mechanochemical crosstalk between adhesion sites, membrane tension and the actin protrusive machinery.

In order to migrate, eukaryotic cells have to transduce intracellular forces to the extracellular environment. A unifying theme is that the retrograde force produced by actin filaments growing against the leading plasma membrane is transduced to the substrate, e.g. via transmembrane adhesion receptors [1]. While this principle is of broad relevance, the spatiotemporal regimes under which such mechanocoupling arises can be diverse. Most cells show some kind of oscillatory morphodynamic patterns, such as protrusion–retraction cycles or wave-like undulations of their surface curvature. These shape changes define the different strategies of how cells move and, for some amoeboid cell types, it has been suggested that coordinated deformations can even endow them with the capacity to swim within viscous media [2,3]. How such, often oscillating, dynamics arise is largely unknown, but it is firmly established that the actin cytoskeleton generates almost all intracellular forces. In this issue of *Current Biology*, Barnhart *et al.* [4] now demonstrate how an interplay between three feedback loops involving actin-driven protrusion, adhesion site formation and membrane tension can generate laterally propagating protrusion waves.

For their study, Barnhart *et al.* [4] have used the simplest known paradigm for actin-driven cell motility: the fish keratocyte. These cells, derived from fish scales, migrate spontaneously and steadily when plated on an adhesive surface. Keratocytes display a stereotypic flat, fan-like morphology, shape changes over time are minimal, and there is almost no relative slippage between the substrate and the actin network growing from the leading edge. The leading edges of these cells represent expanding actin networks surrounded by the bag of plasma membrane and thus reduce the three-dimensional and temporally complex phenomenon of cell motility to a two-dimensional, largely homeostatic problem. In other words: as long as there is no thorough understanding of keratocyte motility, there is no understanding of cell migration.

In previous work, these authors found that, when plated on highly adhesive surfaces, keratocytes often show a remarkable behavior that is rare under intermediate adhesion conditions: they switch from the steady shape to oscillatory waving, where a new lamellipodium is initiated at the front and then travels laterally until it vanishes at the side of the cell [5]. Occasionally, two waves are initiated at the front of one cell, leading to a breaststroke pattern, whereby one wave travels to the left and the other to the right. The authors took a very quantitative morphometric approach and found that the adhesiveness of the substrate not only increases the frequency of traveling wave formation but at the same time decreases the width and the lifetime of the lamellipodium. This behavioral switch was induced not only by changing substrate adhesiveness but also by modifying the stability of adhesion sites, with pharmacological stabilization or de-stabilization of adhesion sites leading to more or less waving, respectively.

Temporal oscillations of lamellipodia are seen in most cell types, and fibroblasts and epithelial cells show regular protrusion–retraction cycles [6]. It has been suggested that such cycles are coupled to retrograde transport of molecular regulators that occurs together with the actin flow. In their new study, Barnhart *et al.* [4] considered this option, but when they measured retrograde actin transport in waving lamellipodia they found that there was no slippage between actin and substrate. This allowed them to conclude that actin polymerization itself rather than coupling to the substrate defines the propagation of the protrusive wave and that a wave ultimately dies when polymerization stalls. The spatial coupling between the travelling wave front and the adjacent sections of the

