Initiation of meiosis

How do synonymous mutations affect fitness?

Early mouse embryo development

Dynamic mutations as digital modulators of brain development

Developmental regulation of static allometry in insects

Kruppel-like factors in epithelial biology and pathobiology

www.bioessays-journal.com

25 Years Thinking About Biology
It’s a long way from amphioxus: descendants of the earliest chordate

Jordi Garcia-Fernández,1*† and Elia Benito-Gutiérrez2,3**†

1Departament de Genètica and Institut de Biomedicina (IBUB), Universitat de Barcelona, Spain
2National Institute for Medical Research, Mill Hill, London, UK
3Current address: Developmental Biology Unit, European Molecular Biology Laboratory, Heidelberg, Germany

The origin of chordates and the consequent genesis of vertebrates were major events in natural history. The amphioxus (lancelet) is now recognised as the closest extant relative to the stem chordate and is the only living invertebrate that retains a vertebrate-like development and body plan through its lifespan, despite more than 500 million years of independent evolution from the stem vertebrate. The inspiring data coming from its recently sequenced genome confirms that amphioxus has a prototypical chordate genome with respect to gene content and structure, and even chromosomal organisation. Pushed by joint efforts of amphioxus researchers, amphioxus is now entering a new era, namely its maturation as a laboratory model, through the availability of a large amount of molecular data and the advent of experimental manipulation of the embryo. These two facts may well serve to illuminate the hidden secrets of the genetic changes that generated, among other vertebrates, ourselves.

Keywords: amphioxus; chordates; Evo–Devo; living fossil; model system; prototype

Introduction

The year 2009 marks the 150th anniversary of the publication that many have considered to be the most influential scientific book ever, with the profound impact it has had on human society. The year 2009 is also the 200th anniversary of the birth of the book’s author. The Origin of the Species and the theory of evolution(1) changed our view of the natural world. All previous systematic classifications of living things changed to an evolutionary and historical tree of life on Earth which aims to explain current biodiversity through extinct ancestors, ancient nodes, revolutionary changes, crown and specialised groups and slow and fast appearing morphological and functional innovations.

Many animals captivated the interest of Darwin because of the amazing variety of their adaptations, such as the finches, or because they possess primitive forms of evolved characteristics in recognised animal clades. The discovery by one of the greatest ever Russian zoologists Alexander Kowalevski, of the notochord in amphioxus(2) fixed the idea that this animal might be the missing link between invertebrates and vertebrates. It also served to unify in a single phylum, the Chordata, three apparently diverse classes of animals: vertebrates, amphioxus and ascidians, with the latter two predating the origin of the former. The cephalochordate amphioxus, or lancelet, may be defined as the minimalist form of a vertebrate, and this is why it deserves its own chapter in the introduction to the vertebrates section of any zoology textbook. Amphioxus shares with vertebrates a dorsal nerve cord, a notochord, segmented mesoderm and post-anal tail, but it lacks most of the advanced morphological features of modern vertebrates, like limbs, neural crest cells, paired sensory organs and an elaborate anterior brain. If the ancestor of the vertebrates was believed to look like the modern amphioxus, it is not surprising that many distinguished zoologists of the 19th and beginning of the 20th centuries described the morphology of amphioxus and its embryology in great detail.

After several inactive decades due to the lack of understanding of the molecular basis of development, the discovery in the 1980s that animals as diverse as flies and mammals share the same developmental genes, such as homeobox genes,(3) revived interest in amphioxus and opened up the field of developmental genetics and later that of genomics.

The new amphioxus school was originally based in San Diego, where it was founded by Linda and Nicholas Holland, and in Oxford by Peter Holland; the little amphioxus became their favourite animal. We, the authors of the present article belong to these very schools, and were also, irremediably, captivated by the amphioxus.

Here we describe how the anatomy and development of the amphioxus are related to the critical evolutionary crossroad of the chordate-vertebrate transition. We further offer a take-home message from its recently sequenced genome: the undoubted demonstration that vertebrates originated from a lancelet-like genome after two rounds of genome duplication,
with conserved macro- and microsinteny with respect to vertebrates and minimal gene losses. Throughout, we highlight the progress in amphioxus research over the years, including the most recent advances towards experimental genetics in amphioxus. The ambitious goal of this road is to test, in the laboratory, the genetic changes that presumably were responsible for the origin and diversification of the vertebrates, including ourselves.

**A short history of amphioxus and its anatomy**

First described by Peter Simon Pallas as a molluscan slug in 1774,\(^4\) it was not until 1834 that Gabriel Costa brought the phylogenetic position of amphioxus closer to the agnathan vertebrates (hagfish and lampreys).\(^5\) From Costa’s misinterpretation of the amphioxus’ oral tentacles as ‘mouth gills’, we inherited the term *Branchiostoma* (branchio = gills, stoma = mouth) to designate the genus; a denomination that is still used for the most widespread type of amphioxus. Approximately 10 years later, Johannes Müller\(^6\) demonstrated the chordate nature of amphioxus, and this has been maintained ever since.

The name ‘amphioxus’ is derived from its shape (Fig. 1): sharp at both ends (amphis = both, oxys = sharp). This shape is due to its rigid (but flexible) dorsal notochord, which runs along the anterior-posterior axis, and atypically exceeds the length of the neural tube at the head. Such an unusual characteristic is imprinted in the name of the subphylum, *Cephalochordata* (kephale = head, khorde = cord) where only the different amphioxus is grouped (genera *Branchiostoma* and *Epigonichtys*). In contrast to vertebrates, the notochord is maintained for life and it is the only skeletal structure in its body. It is made up of special muscle cells arranged like a stack of coins and is highly vacuolated.\(^7\) In contrast to any other chordate, there are a number of neurochordal synaptic contacts along the anterior-posterior axis. These are at the interface between the base of the nerve cord and the notochord, which in amphioxus is under nervous control.\(^8\) Over the notochord the hollow dorsal nerve cord runs along the anterior-posterior axis; it is enlarged at the anterior tip and forms what is known as the cerebral vesicle. The nerve cord is unregionalised externally, except for the cerebral vesicle. The expression patterns of different genes that have been analysed show that most of the cerebral vesicle is comparable to the vertebrate diencephalon.\(^9\) Such amphioxus forebrain (telencephalon + diencephalons in vertebrates) without a discernible telencephalon, is followed by an extensive region similar to the vertebrate hindbrain. There is no apparent midbrain or midbrain-hindbrain boundary.\(^10,11\) True to its chordate nature, amphioxus possesses a series of V-shaped muscle blocks, myotomes or myomeres, which are arranged in segments along the lateral flanks of the body but display a moderate and characteristic left-right asymmetry (the left row being displaced half a segment with respect to that on the right side). With an average of 63 pairs in *Branchiostoma lanceolatum*, the number of myomeres has served to classify different species of amphioxus.\(^12\) Following this mild asymmetry, axons coming out from the nerve cord slide...
through the myotome boundaries with a left-right offset until they reach their final destination in peripheral tissues. As opposed to those in vertebrates, the dorsal nerves are devoid of ganglia, so there are no formal dorsal root ganglia in amphioxus. Serial exiting of the nerve cord is not a rule for motoneurons, which never abandon the nerve cord and instead synapse in serially arranged synaptic zones located in the lateral walls of the neural tube. In common with other chordates, amphioxus possesses a perforated pharynx with a number of pharyngeal slits or ‘gill slits’. Together with their reproductive organs and digestive tract, the pharynx is suspended in the atrium, which runs along two thirds of the length of the adult animal, and has a distal atrio-pore opening. Caudal to the atrio-pore, the anus delimits another typical feature of chordates, the post-anal tail. The heartless circulatory system of amphioxus is composed of large contractile blood vessels and its excretory system looks rather primitive, with about 90 pairs of nephridia dorsally located in the pharyngeal region and associated with the two dorsal aortae. Although ‘disguised as fishes’, amphioxus lacks separated eyes, paired fins, nasal openings, ears and jaws.

A widely distributed living fossil

At present, 29 species of amphioxus cohabit with us on earth. This is still quite a surprisingly large number if we consider the peculiarities that characterise these animals. Based on their primitive appearance, one could easily think that natural selection has just ignored them and exerted no pressure on them to change their ‘fossil-like’ appearance. For millions of years amphioxus seems to have remained in morphological stasis. This is evidenced by the fossil record, which has brought to light a number of Cambrian soft body fossils that closely resemble our contemporary amphioxus. About 520 million years ago marine fauna diversity was booming and there were amphioxus-like creatures, i.e. owners of a fish-like shape, a notochord, a dorsal nerve cord, pharyngeal gills, segmented muscle blocks, inconspicuous median fins and a post-anal tail. The extinct *Pikaia gracilens*, originally classified as an annelid worm, has been extensively compared to the modern amphioxus and widely considered as a stem cephalochordate after Conway Morris reinterpreted the available fossils. *Pikaia*’s anatomy, which is amphioxus-like in shape, size and inconspicuous fins, even allows room for hypothesising a similar lifestyle to that of the present-day amphioxus, swimming by eel-like undulations and possibly burrowing in the benthic sediment. Haikouella fossils and the very similar *Yunnanozoon* also recall current amphioxus morphology, with additional similarities in the segmental arrangement of the gonads. However, *Haikouella* may be a step forward in evolution compared with our contemporary amphioxus, since it probably had neural crest derivatives, which seem to be absent in amphioxus. Even though one may imagine that amphioxus remained in a ‘lost world’ for millennia, hermetically isolated from the evolving environment around it, amphioxus adults populate a broad range of sandy and shell-gravel habitats in coastal marine waters across the globe, where they can reach substantial population densities. They live in tropical and subtropical waters but also in colder waters, such as those of the North Sea. Their habitat, far from being isolated, appears to be a highly dynamic ecosystem. Some fishermen’s legends place amphioxus colonies around submarine rivers where there is probably an important mix of waters with considerable differences in composition and seasonal variation (Jean Luc Martinez, personal communication). They can be found anywhere between the shore and the continental shelf and at different depths depending on the species and location. Paradoxically, their exposed natural environment comprises pleasant beaches where a number of swimmers enjoy their holidays.

Always half buried in the benthic substrate with only their heads out, amphioxus may have been passive spectators of our sea activities over the years, feeling our presence with their cyclopean ‘eye’ or with the sensory cells that are spread over their bodies. These sensory cells have fed the curiosity of everyone who has observed them, but still after decades, opinions are divided as to their function. Whether they are mechanoreceptors, chemoreceptors or both is still unclear; however, when working in the field with these creatures one cannot fail to notice the fact that they are extremely sensitive. This was particularly evident in all the specimens we collected over the years in the south of France. Unlike in Florida, where we sieve the powdery sand to extract adult amphioxus from their natural environment, in France we dredge the coarse benthic sand with our hands and pick them out. In these circumstances their ability to detect the sand being moved by our hands at a distance is quite astonishing. They sinuously move away from us leaving no track of their path, as if they completely vanish from the sand. When uncovered in their hiding place their behaviour turns into a frenetic sinusoidal movement to escape. Few behavioural studies have been published till date but in our experience, adults are only occasional swimmers; the benthic substrate is their preferred medium for locomotion and where they spend most of their time filter feeding on small particles of plankton. Undulating their bodies they gently move back and forth as if the sand was lighter than air. Their characteristic lancet shape, from which their most famous and common appellation ‘lancelets’ is derived, helps them to glide effortlessly through the sand.

An exciting life cycle

When night falls, quietness ceases in the submarine world of amphioxus, and a limited window of activity opens up. This
liveliness increases when amphioxus are about to spawn and they keep those who observe them up through the night to collect eggs and sperm for in vitro fertilisations in the laboratory (University of South Florida, Tampa; Observatoire Oceanographique de Banyuls, France). In their natural environment they flood the marine waters with millions of gametes.

The newly formed zygotes develop inside their fertilisation envelopes, which protect them from external aggressions during early development. By the end of gastrulation they begin to rotate anti-clockwise within their fertilisation membrane and as soon as they become neurulae they impatiently hatch. As young neurulae they join the planktonic community and live in it for anything from a little over 1 month to several months, depending on the species and environmental conditions. After metamorphosis is complete, they slowly develop into juveniles and return to burrows in the benthic ground of their progenitors (Fig. 2). Unlike the adults, amphioxus embryos are tremendously active. Non-stop ciliary propulsion seems to be the dominant mode of locomotion during the entire planktonic period. In the laboratory, this frenzy is a major challenge when visualising development under the microscope or when manipulating embryos in vivo at these stages. Undoubtedly we could add several lines to this paper to explain how this problem has been taxing our imagination; the long nights spent in Florida and Banyuls considering ways to chill them out without harming them. The most successful of our methods till date, oxygen deprivation, works for quick manipulations and observations but not for long-term live imaging.

By the time they reach their late neurula stage they show an amazing positive phototropism; they swim to the surface during the day and stay there until sunset, then they disperse back down to the bottom (of the petri dish, as observed in the laboratory). Their attraction to light does not last long and hours later they have quite the opposite response. By the time they are young larvae they soon discover they can use something else other than their cilia to swim. Like babies learning how to walk, at first early amphioxus larvae weakly bend their elongated bodies to one side, as if in a single ‘wink’. This one-sided movement becomes more frequent over time and later it becomes alternated with bending of the opposite side. As development proceeds, the larvae arc to one side and then to the other until they manage to coordinate the movements of both sides. This results in an undulating wave-like movement that is nearly identical to that observed in adults, though slower. Larvae locomotion shows a variety of patterns that seem to increase in complexity as the central nervous system matures, their bodies become elongated and the density of epidermal cilia gradually decreases.

**Sexual reproduction and development**

All amphioxus species have separate sexes and show mild sexual dimorphism which is only denoted by the colour of the gonads: paler in males (Fig. 1). The zygote forms rapidly after the emergence of the fertilisation membrane. Protected by its fertilisation envelope, the zygote experiences repeated radial holoblastic cleavage until entering the blastulation phase. During this phase the blastocoel (inner cavity) progressively grows until it reaches the cells at the periphery. The result is a beautiful spherical zygote sheltered by an external monolayered cell sheet. Just before entering the gastrulation phase, the blastula loses its spherical shape by flattening out at one of their poles (Fig. 3). The flat (vegetal) pole invaginates progressively, giving rise to the characteristic cupshape gastrula. The furrow generated across the vegetal pole deepens in the direction of the opposite pole diminishing the size of the inner blastocoel and giving rise to the archenteron, which remains wide open through the blastopore. This establishes the rear end of the embryo. Up to this point, amphioxus embryonic development is, in general terms,
typical of invertebrate deuterostomes; not very distinct to the early development of echinoderms or tunicates. However, fate maps for amphioxus are scarce in contrast with those well characterised from echinoderms or tunicates. Till date, nobody has dared to re-evaluate experimentally the impressive blastomere transplantation experiments performed by Tung et al.\textsuperscript{(24)} Challenged by Conklin’s view of an ascidian-like fate map in amphioxus,\textsuperscript{(25)} Tung demonstrated the regulative capabilities of the amphioxus zygote as opposed to the autonomous development of ascidian embryos. As early as

**Figure 3.** Embryo development. A: Cell fate map proposed by Tung et al.\textsuperscript{(24)} for the 32-cell amphioxus embryo. Adapted from Ref.\textsuperscript{(27)} B: External view of an amphioxus blastula and C: transverse section of an embryo at the beginning of gastrulation. D: Transverse section of a late blastula when the embryo is completely spherical and its interior is totally occupied by the blastocoel (b). E–J: Different stages of amphioxus gastrulation. Invagination is initiated at the vegetal pole (\(\wedge\)) giving rise to the cup-shaped gastrula (external view in G). The invaginated layer progressively deepens in the direction of the animal pole, reducing the blastocoel as the archenteron (a) is generated. In late gastrulae, the vegetal pole remains open through the blastopore (bp) marking the posterior side of the embryo. Movement of tissue is indicated by arrows. K–Q: Neurulation begins with a flattening of the dorsal part of the ellipsoidal embryo, which generates the neural plate (np). External view of the embryo before (K) and after (L) generation of the neural plate furrow. (M) Longitudinal section of the early neurulating embryo. (N) The epidermis progressively fuses dorsally at the mid-line level over the neuroectoderm (ne), which overlies the chordomesodermal plate (for clarity, dorsal lamellipodia are not represented). On the posterior side, the neuroectoderm remains separated from the endodermal sheet by the neuroenteric canal at the blastopore site, which at this point in development is closed by epidermal tissue. (O) Transversal section showing the development of internal structures: neural tube, notochord and somites, are derived from the chordomesodermal plate. (P) Longitudinal section of a mid-neurula with a closed neural tube and the first most anterior somites (s). The notochord lies behind the somites out of view. The dorsal posterior part under the epidermis is the tailbud (tb) from which posterior somites and notochord will progressively form as the embryo elongates. (Q) Longitudinal section of a late neurula. For simplicity only the somites on one side are represented to provide a view from the closed neural tube, the notochord and the tailbud. The ventral epithelia of the archenteron bends in on itself and begins to develop into the digestive tract and other endodermal derivatives.
that it was suggested that an organising centre might be coordinating the development of the animal and vegetal poles in a vertebrate-like manner. This has remained a subject of debate for a long time. Only recently have molecular data from molecular data from Yu et al. (27) provided strong evidence that amphioxus gastrulae possess an organising centre similar to that of the Spemann’s organiser in birds or mammals, which remarkably was lost in tunicates, probably as an adaptation of a quick and lineage-based embryonic development. (28)

Once the gastrulation phase is complete, the embryo flattens dorsally and enters the neurulation phase (Fig. 3). As in vertebrates, the flat dorsal surface progressively sinks along the mid-line giving rise to a V-shaped furrow, the base of which becomes the neural plate. The closing of the neural tube is quite peculiar in amphioxus embryos and occurs by dorso-lateral epidermal tissue overgrowing the furrow at each side and ultimately fusing along the dorsal mid-line. The notochord and mesoderm develop in parallel from the neural plate underlying the epithelia, the chordomesodermal plate, which also remains and becomes the roof of the archenteron. Like a blooming flower, the chordomesodermal plate forms three separate parts: one central, giving rise to the notochord primordia and two lateral sections that give rise to the somites or future myomeres. Later, the ventral epithelium of the archenteron bends round on itself to form the digestive tract, pharynx and other endodermal derivatives. The first anteriorly located somites are exclusively formed through enterocoelously, while those located more caudally are derived from the tailbud by schizocoely. The posterior undifferentiated tailbud remains as a source for posterior structures for a long time during development, as occurs in vertebrates during secondary body formation but in the absence of bona fide mesenchimal cells. (29) Subsequently, amphioxus embryos elongate continuously as they develop into larvae that slowly enter metamorphosis, when they acquire the definitive appearance of tiny adults. Amphioxus larvae undergo morphological changes particularly in the anterior region. Their asymmetry is particularly obvious with the opening of the mouth on the left side of their bodies, together with the opening of the primary gill slits. During metamorphosis, massive tissue re-organisation occurs and gives rise to a practically symmetric juvenile. (30)

Jumping positions in the tree: amphioxus is the earliest chordate

For the last two centuries most biologists have viewed amphioxus as the closest living invertebrate relative to vertebrates. Amphioxus are vertebrate-like in having a hollow dorsal nerve cord, notochord, segmented muscles, pharyngeal gill slits and a post-anal tail that develops from a tailbud. These characteristics are maintained for life in amphioxus, while only retained by larvae in ascidians (urochordates). Although both cephalochordates and urochordates are central to the study of the origins and evolution of vertebrates, the complete loss of protovertebrate features in adult ascidians has generated a lot of controversy. (31) The confusing sessile adult of ascidian tunicates engendered a number of hypotheses to explain how body plans evolved from anchored organisms to free-swimming creatures with advanced chordate features, as illustrated by amphioxus, and finally developed into the first agnathan (jawless) vertebrates. Perhaps the dominant theory until recently was that amphioxus evolved by paedomorphosis of an ascidian-like larva and establish the basics for evolving vertebrates. Early molecular data did not change this overall picture since initially most of the genes analysed were nicely conserved in amphioxus with respect to vertebrates, but this was not always the case for the respective genes in tunicates (the most paradigmatic example is the single Hox in amphioxus compared to the broken or disintegrating clusters of tunicates). (32–34) After years of battling for the position closest to vertebrates, Delsuc et al. (35) finally inverted the branches of the taxon trees published over the years and established, through the analysis of a large amount of genetic data and a range of taxa, that tunicates are the closest extant invertebrates to vertebrates (Fig. 4). This explains some of the puzzling data obtained over the years regarding some vertebrate innovations. Illustrative examples are the presence of a vertebrate-like midbrain-hindbrain boundary, proposed on the expression of Pax2/5/8, and the existence of migratory neural crest-like cells in tunicates. (36–38) The absence of such features in amphioxus (39,40) could only be explained by secondary loss in amphioxus, based on the old phylogeny, while the new phylogeny provides a more rational sequence of
events in the generation of these and other key traits of vertebrates. Less secondary losses in amphioxus highlights the prototypical condition of these creatures, slowly evolving and, without the highly derived characteristics seen in ascidians, more than ever a reliable ‘snapshot’ of what may have been the common ancestor of all extant vertebrates.

The remarkable amphioxus genome

The hypothesis that major genome duplications (up to full genome polyploidisations) occurred around the origin of vertebrates originated with Susumu Ohno(41) and his visionary anticipation of comparative genomics and genome evolution. Initial molecular analyses of the Hox cluster and other regulatory genes in amphioxus,(32,42) together with the first synteny analyses of the human genome,(43) excitingly generated the modern 2R hypothesis, with two full polyploidisation events from invertebrates to mammals. For years, ‘2R or not 2R’ has been a subject of hot debate.(44–46) Even the full sequencing of the human genome(47) was not enough to resolve the controversy. In this landmark paper in biology, the authors claimed it was necessary to sequence the amphioxus genome to clearly resolve the issue (cited in Ref.47, p 911). Seven years later, the amphioxus genome finally saw the light(48) and it is indeed a remarkable genome, for the reasons summarised below. More detailed analysis may be found in the original genome article and the accompanying papers,(49–51) and also in the December 2008 special issue of Development, Genes and Evolution devoted to the amphioxus genome and in other articles elsewhere.(52–57)

2R or not 2R?

The amphioxus genome analysis undoubtedly confirms the 2R hypothesis. Among its 21 900 protein-coding loci, amphioxus has many single members for most vertebrate multigenic families. This makes amphioxus an excellent surrogate for the ancestral chordate genome, not only in gene content but also with respect to exon-intron distribution and chromosomal organisation (see below). It also demonstrates that the other non-vertebrate subphylum, the urochordates, secondarily lost many genes and scrambled many chromosomal segments. After the two genome doublings, approximately 20–25% of the duplicates were maintained in the vertebrate genome, with a strong bias towards the retention of genes involved in transcriptional regulation, signal transduction, development and neuronal processes. This was probably due to subfunctionalisation(58) of genes with complex regulatory regions, but the data also encourages speculation that the doublings also produced genomic flexibility in duplicated coding and cis-regulatory sequences, facilitating the emergence of morphological and physiological novelties in vertebrates.

Macro- and microsyneny and maintenance of linkage groups

The analysis of the amphioxus genome served to identify 17 ancestral chordate linkage groups (chordate protochromosomes): large genome segments that are conserved in both amphioxus and vertebrate genomes. Linkage groups that were tested by FISH or library screenings showed that they correspond to single amphioxus chromosomes. This supports the claim that most of the 17 putative ancestral chordate groups have been maintained in amphioxus as single chromosomes, despite more than 500 million years of evolution. Only two subsequent chromosomal fissions were then needed to account for the 19 chromosome pairs of modern Branchiostoma floridae. The macrosynteny analyses between amphioxus and vertebrates shed light on the quadruple conserved synteny in vertebrates, and allow the timing of specific chromosome breaks and rearrangements to be established. Indeed, this resolves the evolutionary origin of the human genome (Fig. 5): most of it (95% of human euchromatine) can be ascribed to 112 segments originated and scrambled through translocations and inversions. These gave rise to the 23 human chromosomes. As an example, chordate linkage group 8 was quadruplicated and the four copies ended in: human chromosomes 8 and X (copy a); two segments of human chromosome 4 (copy b); human chromosome 11 and two segments of chromosome 13 (copy c) and human chromosomes 2 and X (copy d).

Conserved non-coding elements

By comparing the human and amphioxus genomes, 56 putative chordate conserved non-coding sequences of unknown function were identified. In a pilot experiment, eight of these from the human genome were checked for functionality in mice embryos, and four were found to drive specific expressions.(49) Conversely, one of the amphioxus elements and its two human orthologous sequences were checked in both amphioxus and mice embryos and revealed specific expression in both cases. This indicates that these elements shared putative primitive enhancer functions, and showed that the phylogenetic footprinting approach identified regulatory elements back to chordate origins. The 520 million-year separation between amphioxus and humans is the largest evolutionary distance till date across which specific enhancers are conserved, and offers interesting glimpses into deeper analyses(59) to identify regulatory regions that were instrumental in chordate origins and vertebrate evolution.
The amphioxus genome and body plan: a proxy for understanding morphological evolution

Now, 150 years after the *Origin of Species*\(^1\) was published and the short but passionate flirt between evolution and development (‘commonality of embryonic structure reveals community of descent’), the recent reconciliation of the two fields has given rise to the stable, though also passionate, marriage known as ‘Evo-Devo’. The lancelet has occupied several front pages on this new honeymoon. Since the finding that amphioxus has a single *Hox* cluster, compared to four of...
mammals, the study of researchers’ favourite genes, gene networks or developmental processes has served to illuminate the presumed basis of morphological evolution in vertebrates. The literature is rich in primary articles and reviews addressing the impact of amphioxus research on the origin and evolution of axial patterning, the neural crest, the Spemann organiser, the skeleton, the origin of the tripartite brain or the increasing complexity of the nervous system among others. The general rule is that amphioxus possesses all the genetic machinery that was co-opted in evolution to generate vertebrate innovations. It is commonly speculated that the co-option in time or space of this machinery, probably facilitated by the genomic flexibility acquired after two rounds of genome duplication, was instrumental in all these evolutionary innovations.

The recent genome sequence has served to polish the evolutionary story of many gene families and developmental or physiological processes. Noteworthy are the homeobox complement, with 133 genes representing 87 gene families, which indicates that not a single homeobox family was lost in the amphioxus lineage. No or scarce gene loss is also illustrated by the presence of all 33 tyrosine kinase families including all 33 genes families and the identification of 21 out of the 23 Fox gene subclasses. Regarding processes, amphioxus possesses all the major transcription factors deployed in vertebrate neural crest cells, with the only exception being myelin protein P0. This suggests that neofunctionalisation of some paralogs may have facilitated co-option of genes into a neural crest gene regulatory network. For thyroid hormone production, all key players but not thyroid-releasing hormone (TRH) are present in the genome, as are most of the genes involved in neuroendocrine function with the exception of GH, ACTH and prolactin. These missing genes may well be vertebrate innovations. Insulin, insulin-like and the related relaxin genes have also been identified. The fundamental pathways for mesodermal segmentation are also found in the amphioxus genome.

However, the amphioxus genome also includes clade-specific expansions of particular gene families. Noteworthy are the extraordinary expansions of the innate immune complexity genes like Toll-like or NOD-NALP receptors and the vast expansion of some tyrosine kinase families. These highlight the fact that, in addition to being the most prototypical chordate genome till date, it has undergone particular expansions down through its own evolutionary history.

Conclusions and perspectives: into the future research of the past

If the origin of vertebrate limbs was due to the co-option of Hox, Tbx, FGFs or other developmental gene networks in the lateral mesoderm of a vertebrate ancestor, what would happen if this co-option was reproduced in amphioxus? If the neural crest appeared because cells at the neural border acquired migratory capacities, what would happen if amphioxus neural border cells were induced to perform epithelial-mesenchimal transition? If neural plasticity was due to subtle changes in key neural receptors, how would an amphioxus with these mutations behave? These are examples of the main Evo-Devo questions presently infiltrating the authors’ hearts and minds, as well as those of others. This is the dream that we envisage for the coming years: much work to do, many unsuccessful experiments to perform. However, we have the tools to hand: the full genome of the Florida lancelet, and the successful reproduction of amphioxus in the lab. This provides number of embryos never seen before on which to perform experimental genetics, on the experimental Evo-Devo route to the origin of chordates. Only 3 out of the 29 recorded species are commonly used for research: the Floridian-Caribbean B. floridae, the European B. lanceolatum and the East Asian B. belcheri. Genomic tools are available or are being developed for all three species. Embryo facilities, especially for the European amphioxus, are now well developed, with systematic, daily and predictable spawnings in the laboratory, at least during the spring and summer spawning season, with a slight change in the circadian rhythm, that makes spawnings at noon more amenable. Till date experimental genetics has been limited to a few morpholino oligonucleotide knock-down, qui meralacZ-derived reporters and few tracing fluorescent cell lineages. A report of the first control-fluorescent

![Figure 6](image-url)
B. lanceolatum embryo has just been published, (71) indicating that we are on the right track (Fig. 7). In conclusion, genome data, embryo availability and developing experimental techniques are the ingredients of the recipe for research in amphioxus for the coming years, which we hope will help some dreams come true. We are heading towards modifying the present to check the past; amphioxus is today and tomorrow a trip down memory lane, which is why it is our favourite animal.

Acknowledgments: One is truly lucky to interact with brilliant scientists. Only on rare occasions does this interaction become anything more; a life experience that enriches not only at a professional level but also at a personal level. For providing us with such experience we thank Peter, Nick and Linda Holland, Seb Shimeld and Hector Escrivà. We also thank Robin Rycroft and Christopher Evans for correcting our English. J.G.F. also thanks past and present members of the laboratory of J.G.F. is funded by grant and 7) and Nature Publishing group (Fig. 5) for granting figure

References