Was the tail bud the ancestral centre where the fin developmental program evolved in chordates?

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Abstract

The structural origin of the vertebrates’ paired limbs is still an unsolved problem. Historically, two hypotheses have been raised to explain the origin of vertebrate limbs: the Archipterygium Hypothesis and the Fin Fold Hypothesis. Current knowledge provides support for both ideas. In the recent years, it has been also suggested that (1) all appendages correspond to body axis duplications and (2) they are originated by the ventralization of the developmental program present in the median fins. The tail bud is also a relevant structure in the attempt to understand the origin of the vertebrates’ limbs. Due to their similarities in gene expression and general organization, both structures should be studied more closely to understand their potential evolutionary link. Interestingly, in non-vertebrate chordates such as Amphioxus, it is possible to find a tail fin that during development expresses several genes that are conserved with other vertebrates’ limbs and tails. This shared gene expression could be considered as an evidence of potential co-option of the same genetic tool kit from the tail to the extremities. This observation is congruent with the hypothesis of Axis paramorphism, which previously suggested similarities between the tail and limb buds.

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Introduction

The structural origin of the vertebrates’ paired limbs is still an unsolved problem. In the 19th century, morphologists proposed two explanations for the origin of the limbs/fins: The ‘Archipterygium Hypothesis’ and the ‘Fin Fold Hypothesis’ (both reviewed by Cole and Currie, 2007). Later, it was suggested that the extremities were related with side folds in the Cambrian vertebrates Myllokunmingia and Haikouichthys. This has been rejected due to lack of evidence of a skeletal and muscular support, which are the distinctive features of true limbs (Coates, 2003).

The oldest known paired fin-like appendages were present in the jawless Thelodontia, however it is not obvious they had an endoskeletal support (Coates and Cohn, 1998) and their homology to gnathostome paired fins is uncertain. The first unquestioned pectoral fins possessing an internal skeleton arise later in the Osteostraci and Pituriaspida (Janvier, 2008). While the pelvic fins are first observed in the jawed Placoderms. They are considered a case of serial homology with respect to the pectoral ones (Coates, 2003).

The paleontological and anatomical evidence do not provide a definitive answer to uncover which is the ancestral structure of vertebrates’ extremities. However, the analysis of genes involved on fins/limbs development as well as the detailed developmental mechanisms could provide new data to evaluate old and new hypotheses.

In conjunction to the ‘Archipterygium hypothesis’ (Fig. 1, right side), ‘Fin fold hypothesis’ (Fig. 1, centre) and new ideas related with Hox gene patterning, we will examine the tail bud as a structure from which potentially the developmental mechanism for the appendage development was co-opted. This idea builds up on previously suggested similarities between the tail and limb buds (Grüneberg, 1956; Freitas et al., 2006) and the hypothesis of Axis paramorphism (Minelli, 2000, 2003).

The aim of this study is to examine gene expression and developmental data in order to evaluate which of the proposed hypothesis about the origin of vertebrates’ extremities is better supported. On this paper
we show that the shared gene expression through development between tail bud and extremities could be considered as an evidence of potential co-option of the same genetic tool kit. The fact that the tail appears in the fossil record before any kinds of fins or limbs, opens to the possibility of the tail bud as the ancestral structure where this genetic mechanism first appeared.

The Archipterygium hypothesis

In the middle of 19th century, Carl Gegenbaur (1876, 1878) proposed that the limbs might be derived from the gill arches based on observations in Chondrichthyes’ fins and in the archipterygial fins found in the Australian lungfish Neoceratodus forsteri (Krefft, 1870) (Kardong, 2012). He proposed that the archipterygial axis present in the fins corresponds to the extended gill radial and its gill arch would give rise to the pectoral girdle. It has also been reported that during the breeding season the male pelvic fin of Lepidosiren paradoxa (Fitzinger, 1837), also a lungfish, becomes a gill-like organ (reviewed by Foxon, 1933). Despite this being a seasonal change, it may reveal a developmental relationship between those structures.

Many genes are expressed in both gill arches and limbs in tetrapods. O’Rourke and Tam (2002) published an extensive review of the genes expressed in limb and branchial arch in the mouse. There are a numerous genes expressed in both organs belonging to different signaling pathways such as Fgf (fgf4, fgf8, fgf9, fgf10 and fgf2), Shh (shh, gli1, gli2, gli3 and patch), Wnt (wnt5a and wnt11) and Bmp (bmp2, bmp4 and bmp7). In addition, there is a long list of shared transcription factors, which include: twist, dlx1, dlx2, dlx3, dlx5, dlx6, msx1, msx2, alk3, alk4, cart1, pitx1, gsc and mtsh.

Other examples are R-fng, bmp2 and fgf4, which are expressed in the ectoderm of the gill arch and in the apical ectodermal ridge (AER) of the limb (Tabin et al., 1999). The gene goosecoid (gsc) is expressed in the mesenchyme of both structures. Sonic hedgehog (shh), on the other hand, is expressed in the ectoderm of the branchial arch and in both the mesenchyme and ectoderm of the limb (Bouldin et al., 2010). Additional interesting cases are dlx1 and dlx2, these genes are expressed in the mesenchyme of the branchial arch and in the AER of the limb (Tabin et al., 1999).

Studies in the little skate Leucoraja erinacea (Mitchill, 1825) (Gillis et al., 2009), verified that gene expression patterns typical of the limb are found in the gill arches. shh is expressed in the epithelium covering the gill arch and its receptor ptc2 is expressed in the underlying mesenchyme. Meanwhile, fgf8 is expressed in the posterior region of the epithelium and has a regulatory feedback with shh. These patterns bear simi-
larity with the expression of these genes during limb development. Furthermore, the exogenous application of retinoic acid (RA) or shh generates mirror duplication on the gill arch skeleton as it happens in the extremities. On the other hand, the gill arch of the ray has a ridge of pseudostratified epithelium, which closely resembles the AER of limb buds.

Shared gene expression between gill arches and developing extremities in different vertebrates supports the anatomical based hypothesis of Gegenbaur. However, a more systematic survey across different developmental pathways and vertebrate species is required.

**Fin fold hypothesis**

Francis M. Balfour (1881) and J.K. Thacher (1877) independently developed the Fin Fold Hypothesis; it says that paired fins evolved from ribbon-like fins, which extended along the sides of basal vertebrates. This idea correlates with the observations in early vertebrates (e.g. *Myllokunmingia* and *Haikouichthys*; Coates, 2003), as well as the presence of metapleural folds on the sides of the body of amphioxus (*Branchiostoma floridae* (Pallas, 1774); Brusca and Brusca, 2003). However, conceptually it is problematic because there is no evidence, which supports the existence of muscle and endoskeleton, so they are not considered true appendages.

During the larval stages of development in many fish species it is possible to recognize a single continuous median fin, which, later partially degenerates and forms the median fins (Mabee et al., 2002). It does not match the structure suggested by the Fin Fold hypothesis, but demonstrates a link between a continuous fin and median fins. In fact, the positional symmetry between the dorsal and anal fin has been interpreted as a modular system, which ancestrally could have been a single structure (Nelson, 1984; Mabee et al., 2002).

In zebrafish, *Danio rerio* (Hamilton-Buchanan, 1822), the median fin grows starting at 16 hours post fertilization (hpf), from caudal towards anterior. Its growth is accompanied by the expression of *dlx5a* and *sp9*, both gene families are also expressed in the pectoral fins (Abe et al., 2007). At 30 hpf the expression of *msxa*, *msxb*, *msxd* and *msxc* can be found along the whole fold (Akimenko et al., 1995). All of these *msx* genes, except *msxc*, are expressed in the ectodermal and mesodermal portion of the median fin fold and in the pectoral fin bud. *Msxc* appears only in the mesodermal tissue (Akimenko et al., 1995). Additionally, expression of the adhesion protein Laminin α5 has been found in pectoral fins as well as in the median fin fold (Webb et al., 2007).

Studies in chondrichthyes have also demonstrated gene expression shared between unpaired (median) and paired fins (Freitas et al., 2006). These comparisons are also valid between other vertebrate species. For example, the median fins of the small spotted catshark, *Scyliorhinus canicula* (Linnaeus, 1758), express Hoxd (hoxd9, hoxd10, hoxd12 and hoxd13) and tbx18 genes. All of the Hoxd genes are also expressed in different stages of development in chicken limbs (Nelson et al., 1996), while tbx18 is expressed in the forelimbs and somites of chicken (Tanaka and Tickle, 2004).

Perhaps the most compelling line of evidence supporting this hypothesis is the existence of lateral bands of ectoderm competent for AER induction. They correspond to territories that exposed to certain stimuli, such as the presence of fgf10 or fgf7, produce an ectopic AER and limb. In the chicken three bands of competent ectoderm have been reported: one in the dorsal midline of the body from neck to tail, while the other two are on the flanks between the anterior and posterior limbs. Induction of ectopic limbs is restricted to a particular time window (Tamura et al., 2001). Something similar has been found in newts (Balinsky, 1933). An extreme case corresponds to the common skate (*Raja kenojei* (Müller and Henle, 1841)) where a continuous band of *msx1* suggests that its pectoral fins use the whole lateral stripe of competence (Yonei-Tamura et al., 2008).

Because of all these gene expression and anatomical evidence, the median fin fold has been considered as a possible ancestral fin (Cole and Currie, 2007). However, explicit mechanism of how a single median fin fold duplicated to create the paired fins is not fully clear. An alternative explanation corresponds to the ventralization of the developmental program present in the median fins into the formation of the lateral fins which would have happen through the differentiation of the lateral plate mesoderm (Freitas et al., 2014). As a requirement for the fin formation it seems necessary the ventralization of the expression field of *Engrailed-1*, which has a most dorsal limit at the lateral plate mesoderm in gnathostomes (Tanaka and Onimaru, 2012). Then, the evolutionary transition between median fins and paired fins occurred within the somitic mesoderm (Freitas et al., 2014).
Hox genes and axis paramorphism

Another important insight about the origin of limbs is based on the observation that they are placed in the transition zones between different kinds of vertebrae: forelimb in the cervical-thoracic transition and hindlimbs in the dorso-sacral transition (Mabee et al., 2002). Coates and Cohn (1999) argue that these domains could have evolved in relationship to the regionalization of the gastrointestinal tract by Hox genes. Later Tanaka and Onimaru (2012) proposed a more comprehensive model where not only an anteroposterior patterning, related with Hox genes, is required for the origin of paired fins. They also included the need of dorsoventral differentiation, subdivision of somitic and splanchnic mesoderm and different initiation signals (as Tbx4/5).

Independently, Minelli (2000, 2003) pointed out the similarity between limbs and the main body axis due to the presence of sexually dimorphic traits on both structures. Another similitude corresponds to the fact that, all appendages develop from ‘buds’ devoid of endoderm. Examples from vertebrates include the tail and the paired fins, as well as the fleshy posterior dorsal and anal fins of Latimeria, which are considered to be median fins homeotically changed into a paired fin identity (Tabin and Laufer, 1993).

Which of all the appendages is the ancestral vertebrate appendage? The tail bud is present in all vertebrates, even before the origin of the extremities. Actually, the oldest fin known is the caudal fin of the Burgess Shale fossil *Pikaia gracilens* (Walcott, 1911) (Morris and Caron, 2012). Therefore, we argue that it is possible that much of the developmental pathway involved in the formation of this type of fins may have been co-opted later in evolution, for the development of paired appendages.

The tail bud

The tail bud can be defined ‘as the caudal region of the embryonic axis immediately distal to the posterior neuropore’ (Hall, 2000). Its development has been studied since the 1920’s, but not without debate. A good understanding of the formation of this structure is essential in order to compare it on an evolutionary context with respect to other extremities.

Walther Vogt (1926) suggested that tail development differentiates directly from primary germ layers established during gastrulation, without a new organization centre (as the Zone of Polarizing Activity (ZPA) in limb development). Meanwhile, Holmdahl (1925) suggested that the tail develops from the terminal area (for references see Handrigan, 2003) called the ventral ectodermal ridge (VER). The tail bud and VER replace the Hensen’s node through an epithelial-mesenchymal transition that happens during the early stages of tail development. This phenomenon has been described for chicken and mice (Ohta et al., 2007).

On the other hand, traditionally it has been suggested that the tail of Xenopus develops directly from primary germ layers, however in recent years it has been considered that this would be a derived condition and not widespread among vertebrates (Hall, 2000). The overall development of the tail in lampreys, zebrafish, frogs, chickens and mice to some degree combines the ideas of Vogt and Holmdahl, because they mix development directly from primary germ layers.

<table>
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<tr>
<th>Gene</th>
<th>Teleost</th>
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Table 1. Summary of genes expressed on fin/extremities and caudal fin/tail. *Indirect evidence (for details see on section: Similarities between the tail and the extremities). In bold, genes expressed consistently in all the discussed structures.
and secondary development from some kind of organization center (Handrigan, 2003).

**Similarities between the tail and the extremities**

The idea that limb and tail buds present similar development was first mentioned by Hans Grüneberg (1956) and later suggested again by other authors (Schubert et al., 2000). At the same time it also matches the idea of Axis paramorphism as long as the tail is considered as an appendage itself.

Histologically the VER and the AER correspond to an ectodermal epithelial tissue that covers proliferative mesenchyme. In both cases the epithelium/mesenchyme interaction is important for the proliferation of mesodermal cells (Ohta et al., 2007).

In zebrafish there is a ‘tail organizer’, in the sense of being the source of signaling pathway components such as Wnt, Bmp and Nodal (Liu et al., 2004), in a similar way that the ZPA is a source of Shh in the tetrapod limb bud (Bouldin et al., 2010). Additionally, in mouse, a mutation in the gene stratifin produces the phenotype called repeated epilation in which the VER and the AER are very thin and there is an abnormal development of both limbs and tail (Salzgeber and Guénet, 1984; Herron et al., 2005).

Concerning the development of limbs, the Shh pathway has been studied extensively and it is associated with the antero-posterior polarization processes (Bouldin et al., 2010). The presence of Shh was detected in the posterior regions of tetrapod limbs (Riddle et al., 1994), teleost fish (Reifers et al., 1998), the little skate (L. erinacea) and sharks (Chiloscyllum punctatum (Müller and Henle, 1838) and S. canicula) (Hadzhiev et al., 2007; Sakamoto et al., 2009) (Table 1). The caudal fin of zebrafish expresses transcripts of several genes (e.g. ptc and shh) present in the Shh signaling pathway (Krauss et al., 1993; Hadzhiev et al., 2007). In mouse there is expression in the caudal region, however it is in the future spinal cord area (Solloway and Robertson, 1999). As this expression occurs later in development (day 9.5) it is probably not related with the formation of the tail itself.

Elements from the Bmp pathway are expressed recurrently in both structures (Table 1). For example, Bmp2 is expressed in the chicken AER (Akita et al., 1996), the mouse limb bud (Moon et al., 2000) and the zebrafish pectoral fin (Neumann et al., 1999). It is also present in the mouse VER from the earliest stages until the growth of the tail finishes. Another gene from this pathway is bmp4, in mouse it is present in the AER (Akita et al., 1996), but not in the VER (Catala et al., 1996). In addition, many BMPs have been detected in the caudal fin primordium of zebrafish (Hadzhiev et al., 2007). A final example is Bmp11, which is present in the tail bud and also in the limb bud of Xenopus (Gamer et al., 1999).

Several proteins of the Wnt pathway are found in vertebrate limbs and tails (Table 1). Wnt3a is expressed in mice limbs (Visel et al., 2004), as well as the most caudal portion of the tail bud (Takada et al., 1994). Mice carrying null alleles for wnt3 have truncated tail bud development, but there was no major effect on the extremities (Greco et al., 1996). It could suggest the expression of other genes with redundant functions or the fact that wnt3 is actually involved in other developmental processes on the limb. For chicken wnt3a has been reported in the AER (Kengaku et al., 1998). In zebrafish, Wnt3a is expressed in the AER (Ng et al., 2002) and morpholino knockdowns of wnt8 and wnt3a completely blocked the formation of the tail (Thorpe et al., 2005). Consistent with this phenotype, wnt8 expression is detected at the tip of the tail in zebrafish (Kelly et al., 1995). Moreover, the exogenous application of Wnt8c on the flank of chicken embryos induces the formation of an ectopic limb (Kawakami et al., 2001).

Wnt5a and wnt5b are also expressed in the chicken AER (Loganathan et al., 2005). The first one has a role related with the growth of the underlying mesenchyme (Dealy et al., 1993). The same gene is expressed in the pectoral fins of medaka, Oryzias latipes (Temminck and Schlegel, 1846) (Yokoi et al., 2003). In mouse, wnt5a is involved in the proliferation of branchial arches, facial protrusions, limb bud, VER (Goldman et al., 2000), fingers and genitals. In the mutant wnt5a-/ mouse many of these tissues, including the tail and limbs, present a truncation in their growth (Yamaguchi et al., 1999). The expression of this gene in the branchial arches could also be considered as an argument in favor of the Archipterygium Hypothesis. In addition, wnt5b has a pattern of expression in the tail that is very similar to the one observed for wnt3a (Takada et al., 1994). On the other hand, during the regeneration process of the Xenopus tadpole tail, it is possible to detect the expression of wnt3a and wnt5a (Lin and Slack, 2008).

Another example is wnt11, which is expressed in the tail bud of zebrafish (Makita et al., 1998), chicken (Tanda et al., 1995) and Xenopus (Ku and Melton, 1993), as well as in the limbs of chicken (Tanda et al., 1995) and mouse (Christiansen et al., 1995). Finally, the effector of the Wnt pathway, Lef1, is expressed in...
the mouse limbs and tail (Oosterwegel et al., 1993).

A very important gene family for limb development corresponds to the Fgf genes; interestingly very few of these genes are expressed in the tail (Table 1). On mouse AER the genes fgf4, fgf8, fgf9 and fgf17 are expressed, but only the latter is present in the VER (Goldman et al., 2000). In zebrafish, fgf10 is expressed in the pectoral fin, tail and gill arches (Thisse and Thisse, 2004). Another gene in this family, fgf24, is expressed in the mesenchyme of the pectoral fin (Draper et al., 2003) and in the tail bud of zebrafish (Abe et al., 2007). No orthologues were found for this gene in tetrapods, but it is present in Chondrichthyes (Draper et al., 2003).

Functionally in mice, the maintenance of the AER depends only on Fgf10 (Norton et al., 2005) and there is no presence of this transcript in the VER (Goldman et al., 2000). The mutant mouse for this gene lacks lungs and anterior and posterior limbs (Sekine et al., 1999). Along the same line, during the regeneration process of the Xenopus tadpole tail there is expression of fgf8, fgf9, fgf10 and fgf20 (Lin and Slack, 2008).

The Sprouty family of proteins is antagonist of receptor tyrosine kinases, including FGF receptors. Spry1, spry2 and spry4 are expressed in the mouse extremities and in the VER (de Maximy et al., 1999; Goldman et al., 2000) (Table 1). In addition, spry2 is expressed in the zebrafish pectoral fin (Fürthauer et al., 2004).

Also, there are a number of common transcription factors between the two structures (Table 1). Several genes of the Msx family (msxA, msxB, msxC and msxD) are expressed in pectoral fins and the fin fold, including the caudal fin of zebrafish (Akimenko et al., 1995). In mice, msx1, functionally related to msxb in zebrafish (Akimenko et al., 1995), is expressed in the VER (Lyons et al., 1992) and AER (Tribioli et al., 2002). Other example is evx1, which is expressed in the mouse limb and the zebrafish fin (Bruflert et al., 1998), as well as in the tails of both organisms (Beck et al., 2001). Another transcription factor that is found in a wide variety of appendages is dll (Panganiban et al., 1997).

The Tbx transcription factors are also important in limb and tail development (Table 1). In chicken, tbx3 is expressed in the AER and in the tail bud, among other structures (Gibson-Brown et al., 1998). In the Japanese newt, Cynops pyrrhogaster (Boie, 1826), ctbx2 is expressed in the tail and the limb (Sone et al., 1999).

The Hox genes are usually related with segmental differentiation, but they also present shared expression between tail and limbs (Table 1). In Mexican axolotls, Ambystoma mexicanum (Shaw and Nodder, 1798), there is expression of hoxb13 and hoxc10 (short transcript) in the tip of the tail as well as in the hindlimb and in lower levels of the forelimbs (Carlson et al., 2001). In mice, hoxd11 (Gérard et al., 1997) and hoxd13 (Dollé et al., 1991) are expressed in the limb and tail bud. In zebrafish, the genes hoxc6a, hoxd12a (Thisse and Thisue, 2004), hoxc8a (Thisse et al., 2001), hoxd13 and hoxa3b (Thisse and Thisue, 2005) are expressed in the pectoral fin and the tail bud. Finally, in S. canicula there is also expression of hoxd in the tail fin (Freitas et al., 2006).

Ledent (2002) proposed a possible relationship between the adult caudal fin of fishes and the autopod of tetrapods. The author suggests that the Hox genes could be responsible for the axis bending which causes the heterocercal condition in fishes in the same way as they are responsible for the proximodistal finger specification of the tetrapod limb. In this scenario, Hox genes would have been recruited secondarily for limb development.

All these similarities between the genetic mechanism involved in limb and tail formation are also congruent with the Axis paramorphism idea (Minelli, 2000, 2003). On this conceptual framework, both structures could be considered as repetitions of the main body axis. Note that the tail is also a structure that presents sexual dimorphism. It has been documented on the tail length of birds (Winquist and Lemmon, 1994) and snakes (King, 1989); number of vertebrae in salamanders (Ficetola et al., 2013); and colours on birds (Dakin and Montgomery, 2013) and fish (Goddin and McDonough, 2003).

While it is often possible to identify mutations with a limb phenotype having no consequence in the tail or vice versa, this could be explained by the existence of functional redundancies in one of the tissues.

**Importance of retinoic acid in the development of limbs and tail**

Retinoic acid (RA) is a regulator of the Shh pathway (Dahn et al., 2007). In zebrafish, it is secreted from the somites into the lateral plate mesoderm starting with fin development (Neto et al., 2012). Experiments of exogenous application generate mirror duplications of the tetrapod limb (Tabin, 1995). In zebrafish and mummichog (Fundulus heteroclitus (Linnaeus, 1766)), the exposure to exogenous RA during gastrulation produces multiple pectoral fins. The same effect with
shortening of the tail and deletion of brain and craniofacial structures is observed when the treatment is at 50% of epiboly (Vandersea et al., 1998). If the tail were related evolutionarily and/or mechanistically to the development of the limbs it would be expected that similar effects would result from the same stimulus.

In nature and under breeding conditions spontaneous cases of tail duplication in fish have been reported. The explanation for this phenotype is not always clear or unambiguous, though among the possible causes genetic factors and/or the effect of RA pathway interference are mentioned. For example, in cultured gilt-head sea bream, Sparus aurata (Linnaeus, 1758), it has been reported the presence of osteomalformations in the tail fin. Among them, it is the formation of a second hypoplastic fin in the dorsal region of the main caudal fin. Environmental contaminants have been mentioned as potential causes, as they are known to alter the expression of homeotic genes in turn regulated by RA (Koumoundouros et al., 1997).

On the other hand, for centuries in China different varieties of goldfish have been cultivated, Carassius auratus (Linnaeus, 1758), some of which have double caudal fins. The experimental removal of yolk material of double caudal fin goldfish produces an adult with a single caudal fin (Nan’er, 1989). A similar result was found by treating the eggs with polyethylene glycol or UV light (Nan’er, 1993). The yolk sac stores vitamin A, which is a precursor of RA (Lampert et al., 2003); thus; these treatments lead to a reduced availability of vitamin A and to a decreased synthesis of RA. It is assumed that this reduction would not be strong enough to cause other developmental abnormalities. Probably somehow double-tailed varieties have managed to accumulate higher concentrations of vitamin A in the yolk. By removing this excess, the animal reverts to the ancestral condition.

Thus, though it has not been demonstrated, there is indirect evidence that RA could be involved in tail duplication, an intriguing parallel to what has been shown in limbs, branchial arches and radials in caudal fin regeneration (White et al., 1994).

Another suggestive link between limbs and tails is related to a documented homeotic transformation of the tail into legs in different frog species (Mahapatra et al., 2002; Mohanty-Hejmadi and Crawford, 2003). These effects were obtained after vitamin A treatment of larvae whose tails had been amputated (Mahapatra et al., 2002). The effect of vitamin A, a precursor for RA, could suggest a potential role of RA on this homeotic transformation.

In summary, functional experiments show that RA has a similar role in tail and limb development on different kinds of vertebrates, corresponding to another piece of evidence of the potential evolutionary link of these structures.

**Tail similarities between vertebrate and non-vertebrate chordates**

If the gene tool kit associated to the formation of the tail was co-opted into the extremities development, then it would be expected that this gene tool kit will be present on a non-vertebrate chordate possessing a tail. The tail of the cephalochordate amphioxus (B. floridae) in the early stages of development corresponds to an epithelium without mesenchymal components and the rays are groups of cilia (Flood, 1975; Crowther and Whittaker, 1994). However, after the metamorphosis abundant dermal matrix is accumulated producing a predominantly dermal tail fin (Mansfield and Holland, 2015). Then, the amphioxus tail in later stages of development is composed by mesodermal and ectodermal tissue as the one in vertebrates.

From the gene expression aspect, the amphioxus tail bud does express genes shared between the tail and limbs of vertebrates: AmphiWnt3, AmphiWnt5, AmphiWnt8, AmphiWnt11 and AmphiEvxA (ortholog of evx1) (Schubert et al., 2000, 2001; Ferrier et al., 2001; Holland, 2002). On the other hand, genes from families of the RA pathway (RAR, raldh, cyp26 and aldh) are present in its genome (Marléta et al., 2006) and, in fact, Aldh1a is expressed caudally near the developing tail (Dalfó et al., 2002). Complementarily, Koop et al. (2011) showed that a high RA concentration induces tail regression.

The Urochordate Ciona intestinalis (Linnaeus, 1767) (Asciacea) on its larval stage presents a tail that retracts during the metamorphosis. It is created by cell rearrangements and not by a posterior growth zone as in vertebrates (Takatori et al., 2007). However, sequences belonging to the RA pathway such as raldh2, aldh, RAR and cyp26 have been also found in its genome (Kanda et al., 2009). In particular cyp26 (Nagatomo and Fujiwara, 2003) and adh3 (Cañestro et al., 2003) show expression in the tail bud area. Also, exposure to RA produces malformations in its tail (Nagatomo et al., 2003). Moreover, the regionalization of the tail tip epidermis occurs through Fgf signaling (Takatori et al., 2007). But, another Urochordate, Oikopleura dioica (Fol, 1872) (Appendicularia), does
not have any of the RA related genes within its genome (Marléťaz et al., 2006).

The expression and presence in the genome of non-vertebrate chordates of many relevant genes in extremities/tail development, suggests that they were already required for tail development prior to the origin of vertebrates. Therefore, the mechanisms in which they are involved could have been co-opted for the formation of paired limbs (Schubert et al., 2000; Fig. 2).

Conclusions

Based on the current state of knowledge, is very difficult to assess whether the gill arch or a fin fold gave rise to the extremities (Fig. 1). Both hypotheses – the Archypterygium and the Fin Fold Hypothesis – are partially supported by gene expression data. On the other hand, the hypothesis of the ventralization of the zones of competence could be broadly grouped with the Fin Fold Hypothesis, because in both cases the original genetic mechanism is present in a ribbon-like fin present in the outer body of the organism. Here, as an alternative hypothesis (Fig. 1, left side), we presented evidence that similar genes are involved in the formation of the tail and the limb. Part of this evidence is the common role of RA in the duplication of limbs and tails on different lineages of vertebrates. Moreover, other genetic elements related with extremities and tail development are present in non-vertebrate chordates. This suggests that the genetic tool kit involved in tail development could have been co-opted by the extremities (Schubert et al., 2000).

This observation is congruent with the hypothesis of Axis paramorphism (Minelli, 2000, 2003). We think that further studies including gene expression analysis of the tail bud and gill arches in amphioxus and the larval tail of Ciona will help to confirm or disprove this idea.

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