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.

Contents

Introduction

The structural origin of the vertebrates' paired limbs is still an unsolved problem. In the 19th century, mor-phologists 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 extremi-ties were related with side folds in the Cambrian verte-brates *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 Thelodonta, 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 pecto-ral fins possessing an internal skeleton arise later in the Osteostraci and Pituriapsida (Janvier, 2008). While the pelvic fins are first observed in the jawed Placo-derms. 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. How-ever, the analysis of genes involved on fins/limbs de-velopment as well as the detailed developmental mech-anisms 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 ap-pendage 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 expres-sion and developmental data in order to evaluate which of the proposed hypothesis about the origin of verte-brates' extremities is better supported. On this paper
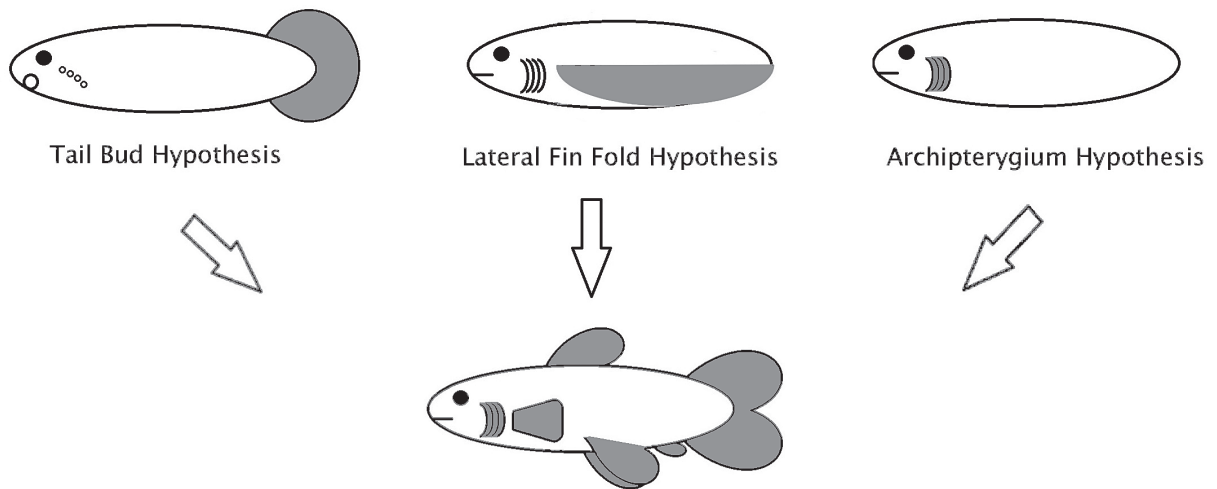
Fig. 1. Traditionally there have been suggested two alternatives as potential ancestral structures to vertebrates' extremities: continuous lateral fin folds (centre; Lateral Fin Fold Hypothesis) or branchial gills (right; Archipterygium Hypothesis). We propose that the same genes involved on tail fin development could later have been co-opted in other fins (left; Tail Bud Hypothesis). It is also possible to postulate another scenario where the genetic tool kit moves from the branchial gills to the ribbon fin or from the tail fin into the branchial gills.

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 *fgfr2*), 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 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.

Gene	Teleost		Tetrapod	
	Fin	Caudal fin and tail bud	Extremities	Tail and tail bud
shh	x	x	x	
ptc		x	x	
bmp 2/4	**x**	**x**	**x**	**x**
bmp11			x	x
wnt3a	**x**	**x***	**x**	**x**
wnt5 a/b	x		x	x
wnt11		x	x	x
lef1			x	x
fgf4/8/9			x	x
fgf10	x	x		
fgf17			x	x
fgf20				x
fgf24	x	x		
spry1/4			x	x
spry2	x		x	x
msxA/C/D	x	x		
msxB	**x**	**x**	**x**	**x**
tbx2/5			x	x
hoxa13b, hoxc6a, hoxd12a hoxc8a and hoxd13	x	x		
hoxb10/13			x	x
hoxd11/13			x	x

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 been 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-/-* 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 (Brulfert *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), *cptbx2* 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 Thisse, 2004), *hoxc8a* (Thisse *et al.*, 2001), *hoxd13* and *hoxa13b* (Thisse and Thisse, 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 Lemon, 1994) and snakes (King, 1989); number of vertebrae in salamanders (Ficetola *et al.*, 2013); and colours on birds (Dakin and Montgomerie, 2013) and fish (Godin 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 gilthead sea bream, *Sparus aurata* (Linnaeus, 1758), it has been reported the presence of osteological malformations 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 (Marlétaz *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) (Ascidiacea) 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
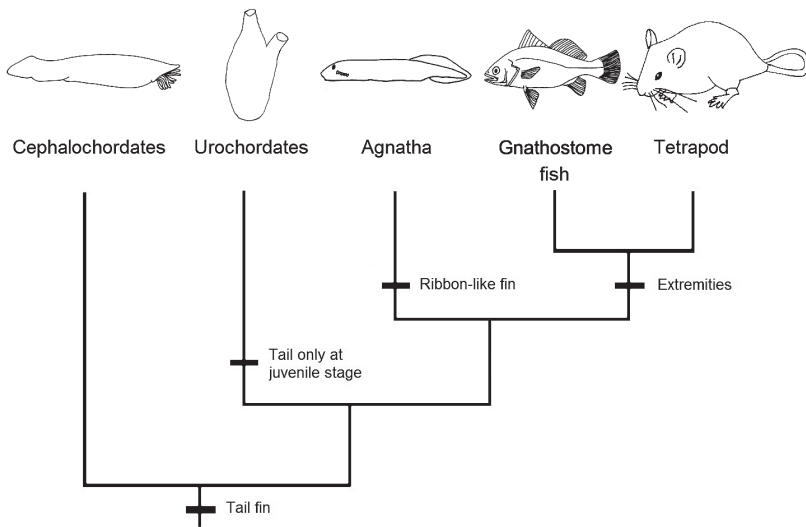
Fig. 2. Evolution of the extremities in chordates. The extremities appeared with the basal gnathostomes. Ribbon-like fins are possible to identify in agnatha and early stages of development in some fish. The tail fin appears at the base of the chordate and secondary lost in the adult stages of Urochordata. Note that the presence of tail fin is considered as a 'minimum boundary' to the estimation of existence for the tail bud. Phylogeny after Putnam *et al.*, 2008. Animal drawings after: Ljósm, Christine Walsh and ratbehavior.org.

not have any of the RA related genes within its genome (Marlétaz *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 ia 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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