Zoology 117 (2014) 77–80

Contents lists available at ScienceDirect

Zoology

journal homepage: www.elsevier.com/locate/zool

The origin of developmental mechanisms underlying vertebral elements: implications from hagfish evo-devo☆

Kinya G. Ota[a,*], Yasuhiro Oisi[b], Satoko Fujimoto[b], Shigeru Kuratani[b]

[a] *Laboratory of Aquatic Zoology, Marine Research Station, Institute of Cellular and Organismic Biology, Academia Sinica, No. 23-10, Dawen Road, Jiaoxi, Yilan 26242, Taiwan*
[b] *Laboratory for Evolutionary Morphology, RIKEN Center for Developmental Biology, Kobe 650-0047, Japan*

ARTICLE INFO

Article history:
Received 9 August 2013
Received in revised form 23 October 2013
Accepted 25 October 2013
Available online 11 December 2013

Keywords:
Axial skeleton
Cyclostomes
Sclerotomes
Cartilaginous vertebral elements
twist

ABSTRACT

The origins of the vertebral elements and the underlying developmental mechanisms have so far remained unclear, largely due to the unusual axial skeletal morphology of hagfish, one of two extant jawless vertebrate clades. Hagfish axial supporting tissue is generally believed to consist of the notochord and cartilaginous fin rays only. However, careful investigations of whether vertebral elements are truly absent in hagfish are scarce, and it is also unclear whether the axial skeletal morphology of the hagfish is an ancestral or a derived condition. To address these questions, we re-examined the axial skeletal morphology of the Japanese inshore hagfish (*Eptatretus burgeri*). Based on a report published a century ago which implied the existence of vertebral elements in hagfish, we conducted anatomical and histological analyses of the hagfish axial skeletal systems and their development. Through this analysis, we demonstrate that hagfish possesses sclerotome-derived cartilaginous vertebral elements at the ventral aspect of the notochord. Based on (i) molecular phylogenetic evidence in support of the monophyly of cyclostomes (hagfish and lampreys) and jawed vertebrates (gnathostomes), and (ii) the morphology of the vertebral elements in extant gnathostomes and cyclostomes, we propose that the embryos of the common ancestor of all vertebrates would have possessed sclerotomal cells that formed the segmentally arranged vertebral elements attached to the notochord. We also conclude that the underlying developmental mechanisms are likely to have been conserved among extinct jawless vertebrates and modern gnathostomes.

1. Introduction

Vertebral elements are segmentally arranged cartilaginous or bony nodules associated with the notochord and are considered to be one of the most important morphological features of vertebrates (Janvier, 1996; Liem et al., 2001; Kardong, 2008). Such axial skeletal systems are observed in all extant vertebrate species (Janvier, 1996; Liem et al., 2001; Kardong, 2008). However, it has long been believed that the axial skeletal system, in the form of vertebral elements, is absent from hagfish, with its axial skeletal connective tissues considered by certain textbooks to consist of only notochord and cartilaginous fin rays (Liem et al., 2001; Kardong, 2008). Assuming a monophyletic relationship for hagfish and lampreys (which is widely accepted in the fields of both molecular evolution and paleontology; see Stock and Whitt, 1992; Mallatt and Sullivan, 1998; Kuraku et al., 1999; Ota and Kuratani, 2006; Gai et al., 2011), it is unclear whether the exceptional axial skeletal morphology of hagfish reflects a derived or an ancestral condition. This enigmatic morphology impedes attempts to determine the evolutionary origin of the vertebral elements.

This question has been largely overlooked in recent years. Ayers and Jackson (1900) described segmentally distributed cartilaginous nodules on the ventral aspect of the caudal notochord in an *Eptatretus* hagfish species. However, their descriptions have not been carefully verified, perhaps due to the following two reasons. The first reason concerns interspecies variations in whether the segmental cartilaginous elements are present or not. For example, the skeletal system of the Atlantic hagfish (*Myxine glutinosa*) is known to lack segmental cartilaginous nodules (Cole, 1905); the frequent use of *M. glutinosa* in early morphological studies (Müller, 1834; Cole, 1905) may account for the widely held view that segmental cartilaginous nodules are absent from hagfish (Liem et al., 2001; Kardong, 2008). The second reason pertains to the ambiguous description of hagfish vertebrae in certain textbooks (Goodrich, 1930; Wake, 1992). Although early studies briefly described the presence and unusual morphological features of the vertebrae in hagfish, no sources or figures were provided (Goodrich, 1930; Wake, 1992). Presumably due to their ambiguous nature (Goodrich, 1930; Wake, 1992), these descriptions have

---

☆ This article is part of a special issue entitled “Axial systems and their actuation: new twists on the ancient body of craniates”.

* Corresponding author. Tel.: +886 3 9880544x35; fax: +886 3 9871035.
E-mail address: otakinya@gate.sinica.edu.tw (K.G. Ota).

0944-2006/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.zool.2013.10.010

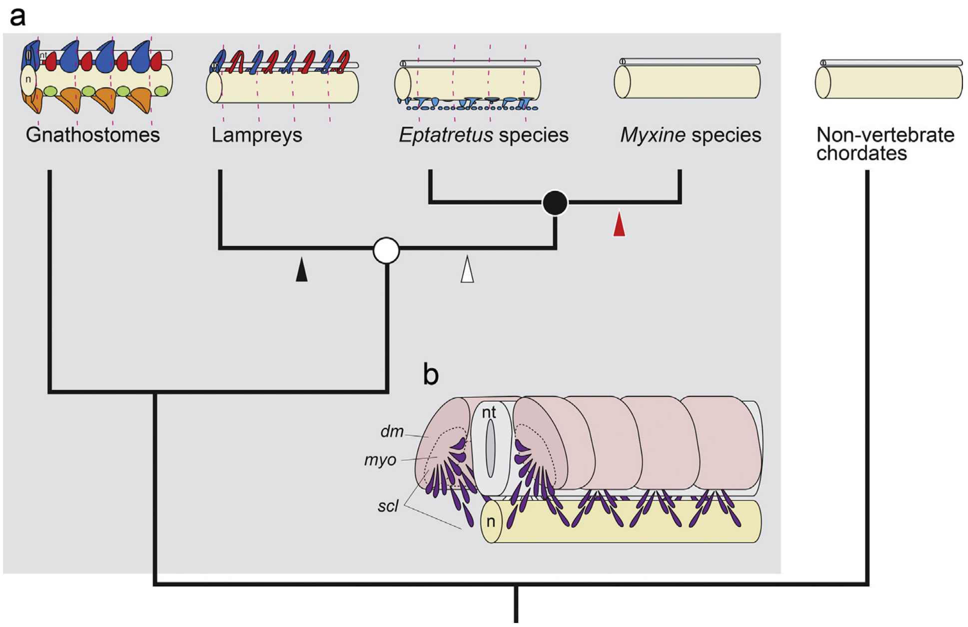

Fig. 1. Hypothetical scenario for the evolution of vertebral elements and the underlying developmental mechanisms. (a) The phylogenetic relationship between extant vertebrates and non-vertebrate chordates. This phylogenetic tree is based on molecular data. The hagfish clade, consisting of *Eptatretus* and *Myxine* species, is clustered with lampreys to form the monophyletic cyclostome group. The vertebral elements of gnathostomes and lampreys are color-coded: basidorsal (blue), interdorsal (red), basiventral (orange), and interventral (green). Dashed lines indicate myosepta, which correspond with the segmental patterns of the color-coded vertebral elements. *Eptatretus* hagfish species possess cartilaginous, segmental ventral vertebral elements at post-cloacal levels. Their segmental patterns do not coincide with the myosepta. *Myxine* hagfish species lack segmental vertebral elements. We hypothesize that the common ancestor (white circle) of hagfish and lampreys possessed cartilaginous, segmental vertebral elements at the dorsal and ventral aspect of the notochord; secondary loss of the ventral element may have occurred in the lamprey lineage (black arrowhead), while loss of the dorsal elements occurred in the hagfish lineage (white arrowhead). The common ancestor of hagfish species (black circle) may have had segmental vertebral elements at (at least) the ventral aspect. The absence of segmental vertebral elements from the *Myxine* hagfish species may be a consequence of the secondary loss of the vertebral elements in their immediate ancestor (red arrowhead). (b) Hypothetical derivative of the somite common ancestor. The fossil data suggest that the common ancestor of all vertebrates possessed the developmental mechanisms required to form vertebral elements at the dorsal and ventral aspect of the notochord (gray background). *Abbreviations*: dm, dermomyotome; myo, myotome; n, notochord; nt, neural tube; scl, sclerotome.

frequently been ignored by subsequent accounts addressing vertebrate morphology (Janvier, 1996; Liem et al., 2001; Kardong, 2008). To confirm that segmental cartilaginous nodules exist in the hagfish as early findings suggest (Ayers and Jackson, 1900), we re-examined the axial skeletal systems of hagfish (Ota et al., 2011, 2013).

2. Rediscovery of hagfish vertebral elements

Based on a description published a century ago (Ayers and Jackson, 1900), we subjected the Japanese inshore hagfish (*Eptatretus burgeri*) to comprehensive anatomical and histological analyses (Ota et al., 2011); in this analysis, we identified segmental cartilaginous nodules in a hagfish species. As noted by Ayers and Jackson (1900), hagfish cartilaginous nodules are distributed only at the ventral aspect of the notochord in the post-cloacal region (Ota et al., 2011) (Fig. 1a). More noteworthy is that there is no clear topographical correlation between myosepta and cartilaginous nodules in hagfish (Fig. 1a). A classic schematic divided the vertebral elements of the jawed vertebrates (gnathostomes) into four elements (Goodrich, 1930; Gadow, 1933). The skeletal elements located at the same level as the myosepta are referred to as basidorsal and basiventral, while the remaining elements are designated as interdorsal and interventral (see the topographical relationship between the color-coded skeletal elements and the dashed line in Fig. 1a). This classic schematic can also be applied to the skeletal systems of lampreys, but not to the cartilaginous nodules of hagfish, since the latter exhibit no overt segmental pattern consistent with myotomes (Fig. 1a). Furthermore, the majority of cartilaginous cells in hagfish do not express the gene encoding collagen type 2 alpha 1 (*col2A1*), a major component of the vertebrate cartilaginous extracellular matrix (ECM), suggesting differences in the composition of ECM between hagfish and other vertebrates (Ota and Kuratani, 2010).

Our observations indicate that hagfish possess cartilaginous elements that resemble, and are similarly positioned to, ventral vertebral elements in gnathostomes. However, these observations also indicate that a number of differences exist between hagfish cartilaginous nodules and the generally accepted morphology of vertebral elements (Goodrich, 1930; Gadow, 1933; Ota and Kuratani, 2010; Ota et al., 2011, 2013). These differences prompted us to investigate the developmental process underlying cartilaginous nodules in hagfish. We hypothesized that if hagfish vertebral elements are truly homologous with the vertebral elements of the other vertebrates (despite their divergent adult morphologies and ECM compositions), their developmental processes and patterns should also be comparable.

3. Development of hagfish vertebral elements

It is well accepted that the vertebral elements in gnathostomes are derived from the sclerotome, a ventromedial component of the embryonic somite (Christ et al., 1998). During differentiation, the ventromedial portion of an epithelial somite delaminates to form a mesenchymal sclerotome expressing the *pax1/9* and *twist* genes; this sclerotome then migrates to populate the lateral side of the notochord and the neural tube. Sclerotomal cells are distributed in a segmental pattern and differentiate into the vertebral elements (Christ et al., 1998; Morin-Kensicki et al., 2002). To determine whether hagfish cartilaginous nodules arise through a similar process, we analyzed gene expression patterns in a series of hagfish embryos.