Available online at www.sciencedirect.com

ScienceDirect

www.sciencedirect.com/science/journal/16720229

GENOMICS PROTEOMICS & BIOINFORMATICS

Review

Hox Gene Clusters of Early Vertebrates: Do They Serve as Reliable Markers for Genome Evolution?

Shigehiro Kuraku*

Laboratory for Zoology and Evolutionary Biology, Department of Biology, University of Konstanz, 78464 Konstanz, Germany.

Genomics Proteomics Bioinformatics 2011 Jun; 9(3): 97-103 DOI: 10.1016/S1672-0229(11)60012-0

Received: Jan 31, 2011; Accepted: Mar 21, 2011

Abstract

Hox genes, responsible for regional specification along the anteroposterior axis in embryogenesis, are found as clusters in most eumetazoan genomes sequenced to date. Invertebrates possess a single Hox gene cluster with some exceptions of secondary cluster breakages, while osteichthyans (bony vertebrates) have multiple Hox clusters. In tetrapods, four Hox clusters, derived from the so-called two-round whole genome duplications (2R-WGDs), are observed. Overall, the number of Hox gene clusters has been regarded as a reliable marker of ploidy levels in animal genomes. In fact, this scheme also fits the situations in teleost fishes that experienced an additional WGD. In this review, I focus on cyclostomes and cartilaginous fishes as lineages that would fill the gap between invertebrates and osteichthyans. A recent study highlighted a possible loss of the HoxC cluster in the galeomorph shark lineage, while other aspects of cartilaginous fish Hox clusters usually mark their conserved nature. In contrast, existing resources suggest that the cyclostomes exhibit a different mode of Hox cluster organization. For this group of species, whose genomes could have differently responded to the 2R-WGDs from jawed vertebrates, therefore the number of Hox clusters may not serve as a good indicator of their ploidy level.

Key words: Hox cluster, Chondrichthyes, Cyclostomata, whole genome duplication, hidden paralogy

Introduction

Hox genes in eumetazoan genomes are generally organized into clusters (*1*). During development, the orders of genes comprising the Hox clusters are converted into information governing specification of different body compartments along the anteroposterior axis. In vertebrates, this scheme was originally presented for the patterning of the hindbrain and pharyngeal arches ("Hox code") (*2*). Later, similar patterns were observed in the development of limb buds (*3*) and gut endoderm (*4*). With some exceptions, Hox genes closer to the 3'-end of Hox clusters are expressed earlier in development (temporal collinearity) and also more anteriorly in embryos (spatial collinearity) (*5*). In all vertebrate genomes sequenced to date, Hox genes are found in multiple clusters, and the number of Hox clusters exactly (in tetrapods) or roughly (in teleost fishes) corresponds to their ploidy levels, indicating how many times those species experienced whole genome duplications (WGDs) (*6*).

Hox gene regulation is often introduced as one of the most striking examples of conserved molecular programs underlying conserved morphological architecture of animal body plans. However, even among vertebrate model systems serving as traditional labo-

*Corresponding author.

E-mail: shigehiro.kuraku@uni-konstanz.de

ratory animals, Hox cluster composition varies to some extent as a result of secondary gene losses and cluster doublings (*6*). Most importantly, our knowledge about vertebrate Hox cluster organization and gene regulation has concentrated mostly on traditional laboratory model systems, all of which belong to a subgroup of vertebrates, Osteichthyes (bony vertebrates). With all technical factors regarding access to animal resources, recent development in genomic and embryonic studies on cyclostomes and cartilaginous fishes is providing a new frontier of vertebrate Hox studies. In this review, focusing on these early-branching evolutionary lineages, I summarize the progress broadening our scope, which previously depended exclusively on osteichthyans. In addition, insights into evolutionary processes of Hox gene cluster organization are also presented.

Hox Genes in Cartilaginous Fishes and Cyclostomes

Hox gene repertoire in cartilaginous fishes

Among cartilaginous fishes, the first report of Hox cluster organization came from the horn shark *Heterodontus francisci* (*7*). This species is categorized in the order Heterodontiformes, and this order is regarded as the most basal among extant members of Galeomorphi. In this species, only two Hox clusters, designated HoxM and HoxN, were sequenced. Presence and absence of particular Hox paralog groups, as well as lengths of intergenic regions and molecular phylogeny, consistently supported the homology of HoxM and HoxN clusters to HoxA and HoxD, respectively (*7, 8*).

Later, in the elephant shark (also called ghost shark or elephant fish), *Callorhinchus milii*, genomic sequencing of Hox clusters was completed based on the 1.4× whole-genome shotgun reads and targeted bacterial artificial chromosomes (BAC) clone screening (*9, 10*). Importantly, this species retains four Hox clusters, containing 45 Hox genes in total (compared to 39 Hox genes in human). Also, 1-to-1 homology of these four clusters to osteichthyan HoxA-D was firmly supported. In *C. milii*, seven paralog groups (PGs) were detected with four members on clusters A-D, namely PG-1, -3, -4, -5, -9, -10 and -13, while there are only three in human, namely PG-4, -9 and -13. This allowed a more reliable analysis to investigate the process of cluster duplications. The molecular phylogenetic analysis supported the scenario with the 1-2-4 pattern (*6, 11*), namely the tree topology [(HoxA, HoxB), (HoxC, HoxD)] (*9*, *12*).

More recently, the organization of Hox genes and clusters of the lesser spotted dogfish *Scyliorhinus canicula* was revealed by transcriptome sequencing and BAC clone screening (*13*). This species has been one of the most promising systems for developmental biology because of its relatively small adult body size and oviparity (*14*). The most striking result in this study was the absence of HoxC members in both genomic and transcriptomic sequencing (**Figure 1**), while the other three clusters were revealed to have retained a comparable number of Hox genes to that in *C. milii* (*13*).

Overall, except for the possible absence of the HoxC cluster in *S. canicula*, cartilaginous fishes have retained more ancestral members of Hox genes than osteichthyans. More precisely, *HoxD2*, *HoxD5* and *HoxD14* have been identified only in cartilaginous fishes, and are thought to have been lost from the HoxD cluster in the basal osteichthyan lineage. Retention of ancestral features by cartilaginous fishes has also been shown in conservation of intergenic sequences in Hox clusters (*15*). The HoxA cluster was also recently sequenced for the little skate *Leucoraja erinacea*, and compared with that of *H. francisci* (*15*). This ray–shark comparison equates to a timespan of more than 250 million years (*16, 17*) and highlighted a higher level of conservation of non-coding sequences inside the Hox cluster than that in the osteichthyan lineage of a comparable evolutionary distance (*15*).

Hox gene expression in cartilaginous fishes

In cartilaginous fishes, investigations on roles of Hox genes have been driven by interests in the evolution of developmental programs responsible for limb/digit formation (*18*). Analyses on cartilaginous fish Hox genes have so far concentrated on species in two oviparous groups, namely those in the genus *Scyliorhinus* (*S. canicula* and *S. torazame*) and *L. erinacea*.

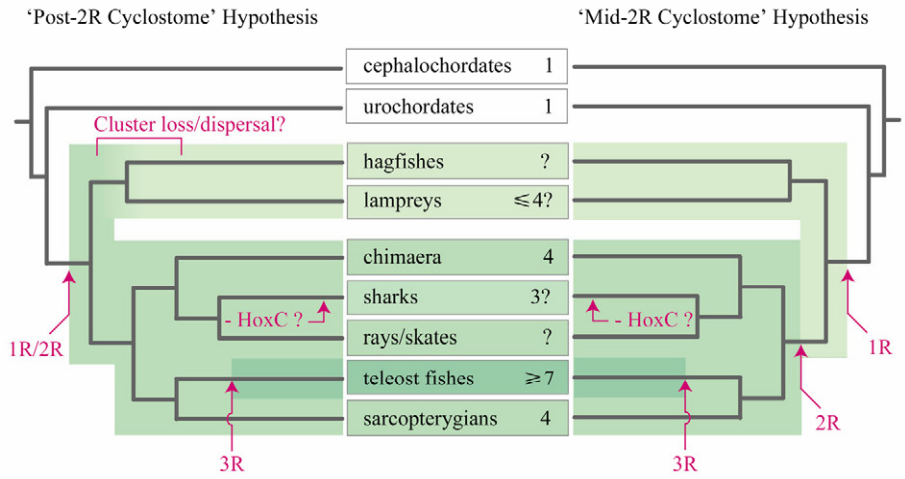

Figure 1 Timings of whole genome duplications and multiplicity of Hox clusters. Numbers of Hox clusters identified so far are shown in the box in the middle adjacent to taxon names. Exact numbers of Hox clusters are given for taxa represented by species with genomic data, while the symbol "?" is included for other taxa. Note that two urochordates were shown to possess single, but atomized Hox clusters (*1, 46, 47*). The possible loss of the HoxC cluster in the chondrichthyan lineage might have occurred even before the split between sharks and rays/skates. Based on the current understanding that cyclostomes may have a smaller number of Hox clusters than four, two possible evolutionary scenarios were presented on both sides. The "post-2R cyclostome" hypothesis (left), compatible with a large-scale phylogenetic analysis employing non-Hox genes (*36*), postulates a secondary reduction of Hox clusters. Based on the "mid-2R cyclostomes" hypothesis (right) (*48, 49*), the possibly smaller number of Hox clusters in cyclostomes is explained by their divergence before the second round of the whole genome duplication. Intensity of green color indicates the number of Hox clusters in individual evolutionary steps and taxa.

In *S. canicula*, 5' Hox genes (*HoxD9-13*, *HoxA11* and *HoxA13*) were reported to be expressed in a nested pattern, consistent with both spatial and temporal collinearity (*19-21*), as seen in osteichthyans (*22, 23*). Expression of *S. torazame HoxD14* was shown in a subpopulation of cells surrounding the hindgut, but not in tissues that would normally express Hox genes, such as the neural tube, somites and fin folds, suggesting the decoupling of this gene from the Hox code (*24*). Hindgut-associated expression is also documented for *HoxA13* and *HoxD13* of *L. erinacea*, and these genes are thought to be involved in early hindgut patterning as in amniotes (*25*).

No expression patterns of Hox genes in PG1-8 had been reported for cartilaginous fishes until very recently. As elasmobranchs have been studied as a model for craniofacial development since the 19th century (*26*), this avenue of research needed to be urgently pursued. Very recently, Oulion *et al* reported embryonic expression patterns of 34 Hox genes in *S. canicula* and concluded that their nested expression of Hox genes (Hox code) in branchial arches, hindbrain and somites was maintained in this species and is a ground plan of embryonic architecture, which underwent only small amount of changes during jawed vertebrate evolution (*27*).

Hox gene repertoire in cyclostomes

Cyclostomes are divided into hagfishes (Myxiniformes) and lampreys (Petromyzontiformes). These two lineages separated more than 400 million years ago (*28*). Each of Myxiniformes and Petromyzontiformes consists of only up to 50 species that diversified within 200 million years—a long time after the separation between Myxiniformes and Petromyzontiformes (*29*). Molecular sequence data are reported mostly for species in the northern hemisphere. In Petromyzontiformes, northern hemisphere species form a distinct family Petromyzontidae that diversified only within 50 million years (*30*).

Hox cluster organization in cyclostomes is still controversial because of some difficulties unique to this group of species (*6*). Firstly, the so-called two-round whole genome duplications (2R-WGDs)

(*31*) occurred close to the split between cyclostome and gnathostome lineages, and therefore multiple alternative scenarios for ploidy levels of this group have been postulated (*30*). Secondly, initial attempts to isolate cDNA and genomic DNA fragments were made using several different northern hemisphere lamprey species [*Petromyzon marinus* (*32*); *Lampetra planeri* (*33*); *Lethenteron japonicum* (*34, 35*)], and this made the counting of gene numbers difficult. It has been shown by a molecular phylogenetic analysis involving 55 non-Hox gene families that the 2R-WGDs probably occurred before the cyclostome–gnathostome split (*36*). Based on this scenario, if Hox cluster organization truly reflects the genomic ploidy level, cyclostome species are supposed to retain four Hox clusters. However, genomic sequencing of Hox-containing regions on *P. marinus* resulted in fragments containing up to only five Hox genes inside (*37, 38*). This situation does not allow a reliable conclusion on the number of clusters, although it is highly likely that the lamprey has multiple Hox clusters (Figure 1).

In lampreys, members of all paralog groups except for PG12 have been identified. Because of the ambiguous timing of the 2R-WGDs, the 1-to-1 orthology of a lamprey (and also hagfish) gene to either of gnathostome HoxA-D is normally not reliably shown in molecular phylogenetic analyses (*36*). They sometimes rather support exclusive clustering of lamprey sequences, suggesting independent gene duplications in the lamprey lineage (*39*). The same feature was also observed for hagfish Hox genes (*40*). These issues should be scrutinized more intensively by obtaining complete cyclostome genomes, ideally representing both Myxiniformes and Petromyzontiformes. In the genome assembly of *P. marinus* (version 3.0; http://genome.wustl.edu/), none of the available supercontigs harbors multiple Hox genes that are thought to belong to the same Hox cluster. It is notable that the secondary cluster breakage and gene loss, suggested by the currently available data of the lamprey, were proposed also for ParaHox gene organization in a hagfish (*41*).

Hox gene expression in cyclostomes

So far, no Hox expression has been described for hagfishes since their embryos are very difficult to access (*42*). Cyclostome Hox expression studies are concentrated on the Japanese lamprey *L. japonicum* (*34, 35*) and the sea lamprey *P. marinus* (*43*). The former species is used to elucidate conservation of Hox gene expression in neural crest derivatives. In this context, members of PG1-8 were characterized with *in situ* hybridization, which resulted in evidence of spatial collinearity in the neural tube and pharyngeal arches (*34, 35*). In contrast, temporal collinearity did not seem to be organized in this early-branching vertebrate (*35*).

Members of PG9-11 (namely *LjHox9r*, *LjHoxW10a*, *LjHox10s* and *LjHox11t*) as well as *Hox13α* were shown to be expressed in the tailbud of *L. japonicum* (*24, 35*). Moreover, *L. japonicum LjHox14α*, *LjHox13α* and *LjHox13β* were shown to be expressed in the hindgut (*24*). This is reminiscent of the hindgut-associated expression of *HoxD14* in the dogfish, and *HoxA13* and *HoxD13* in the little skate mentioned above, suggesting that their role in hindgut patterning was already established before the radiation of all extant vertebrates.

Lessons from Basal Vertebrate Lineages

Inclusion of basal vertebrates in the discussion of vertebrate Hox evolution broadens our appreciation of the variety of states in which vertebrate Hox gene clusters can exist. This includes not only the possible loss of an entire cluster, but also molecular phylogenetic patterns of retention and loss of particular genes. For example, in PG2, *H. francisci*, *S. canicula* and *C. milii* all retain *HoxD2* genes, while this PG2 member is absent in all osteichthyan species examined to date. In lampreys, only one *L. japonicum* sequence with sufficient length, *LjHox2*, has been identified.

Figure 2 shows how these early vertebrate members of PG2 are related to osteichthyan homologs. In *HoxA2* and *HoxB2*, cartilaginous fish sequences exhibit shorter branches, suggesting their less derived nature, compared with osteichthyan sequences. The *HoxD2* group, absent in osteichthyan species as explained above, consists only of cartilaginous fish members. In this maximum likelihood (ML) tree, the lamprey sequence is placed outside gnathostome

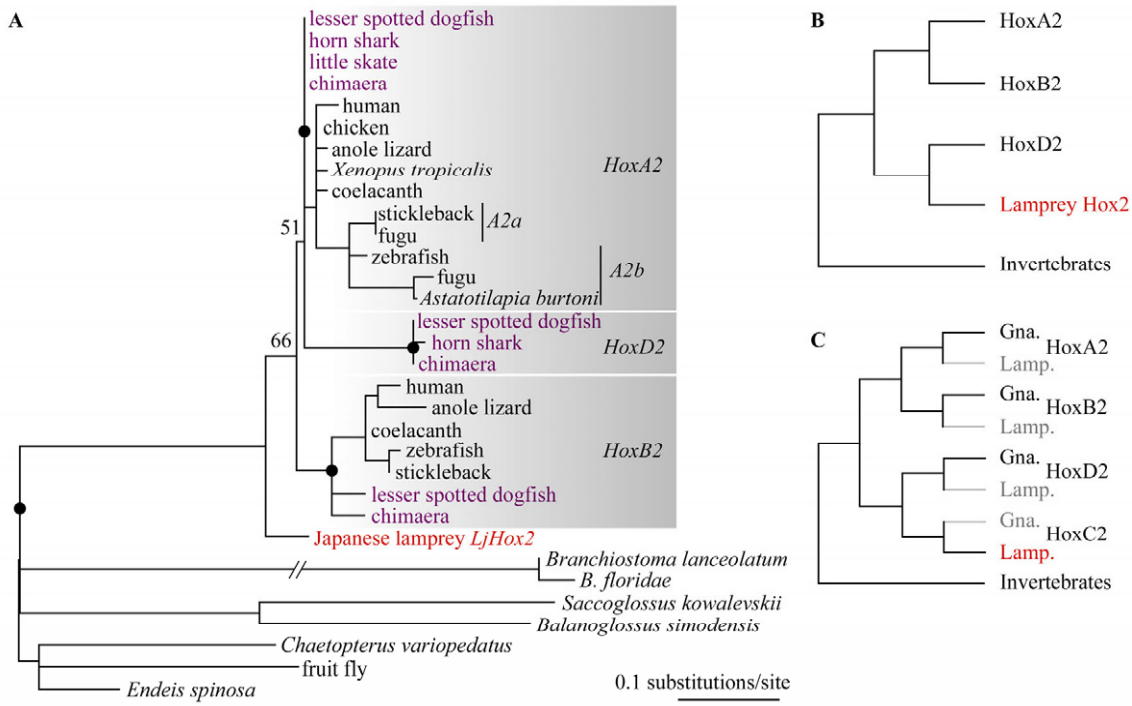

Figure 2 Molecular phylogenetic tree of vertebrate Hox2 genes. **A**. ML tree based on 104 amino acid sites. Phylogenetic relationships within black circles were constrained according to the generally accepted species phylogeny. The dataset consists of invertebrate outgroups, cartilaginous fishes (*HoxA2*, *HoxB2* and *HoxD2*), osteichthyans (*HoxA2* and *HoxB2*) and a lamprey sequence, *LjHox2* (AY497314). Other cyclostome Hox2 sequences in the public database [*P. marinus HoxE2* (AF410908), *L. planeri LpHox2A* (AF044800), and *Eptatretus stoutii Hox2_Z* (AY445839)] were not included because of their short lengths. Cartilaginous fish sequences were shown in purple, while the lamprey sequence was shown in red. Values at nodes are bootstrap probabilities based on resampling of estimated log-likelihood (*36*). **B**. One of the alternative tree topologies supported by the ML method ($\Delta\log L$=2.50±3.90). This tree topology places the lamprey *LjHox2* sequence the most closely to *HoxD2*, suggesting that they are orthologous to each other. **C**. An alternative hypothesis explaining the tree topology in Panel B. Genes lost or unidentified yet are shown in gray (Gna., gnathostomes; Lamp., lamprey). In this hypothesis, the lamprey *LjHox2* gene is the only relict member of *HoxC2*. Note that the tree topologies in Panels B and C are consistent with the order of cluster duplications recently proposed (*9*), namely [(HoxA, HoxB), (HoxC, HoxD)], whereas the tree topology in Panel A is not.

sequences (Figure 2A), which is incompatible with the "post-2R cyclostome" scenario proposed based on non-Hox genes (*36*). However, the low bootstrap support for this gnathostome group of 66 suggests that there are other possible tree topologies in which the lamprey sequence could in fact be located within the gnathostome group. Notably, one of them is the tree topology combining the lamprey *LjHox2* and the chondrichthyan *HoxD2* (Figure 2B). Based on this tree topology, the lamprey *LjHox2* is interpreted as an ortholog of *HoxD2*, or as a relict member of *HoxC2* whose ortholog was lost in the basal gnathostome lineage (Figure 2C).

In fact, both Figure 2A and 2B show tree topologies compatible with the hypothesis that the 2R-WGD occurred before the cyclostome–gnathostome split [pan-vertebrate quadruplication (PV4) hypothesis (*36*)]. The tree topology in Figure 2C is particularly striking because this suggests that different subsets of duplicates have been retained between lamprey and gnathostome lineages—paralog C have been kept in the lamprey, while paralogs A, B and D have been kept in jawed vertebrates. This is a typical situation referred to as "hidden paralogy", and more cases have been introduced for other cyclostome genes (*44, 45*). If the two lineages, namely cyclostome and jawed vertebrate lineages, evolved differently subsequent to the WGD event, it would not be surprising to detect the differential patterns of gene retention between these groups.

Conclusion

Cartilaginous fishes have well conserved ancestral Hox cluster organization except for the possible absence of a HoxC cluster in *S. canicula*. The 4-cluster state, observed at least in *C. milii*, resembles that in non-teleost osteichthyans (for example, mouse, chicken and coelacanth). In contrast, the present uncertainty surrounding our understanding of Hox cluster organization in cyclostomes prevents us from reliably deducing the number of Hox clusters and using it as a proxy for ploidy. This ambiguity could also be interpreted as a reflection of the phylogenetic landscape of non-Hox gene families in which the orthology of cyclostome genes to gnathostome genes cannot unambiguously be established. If we are based on the recently proposed PV4 hypothesis assuming "post-2R cyclostomes" (*36*), cyclostomes would be expected to have four Hox clusters, or at least had four in their ancestry. If a smaller number of Hox clusters are to be found in them, this implies additional events, such as loss of an entire cluster as a significant step in cyclostome evolution (Figure 1). This would violate the notion that Hox cluster organization serves as a reliable marker of ploidy levels. This in turn might have important implications for the evolution of the cyclostome body plan and may be consistent with its hypothesized evolution via a certain degree of simplification or degeneration.

Acknowledgements

I am grateful to Sylvie Mazan, Masaki Takechi, and Kinya G. Ota for insightful discussion. My gratitude extends to the guest editor of this special issue, Dave Ferrier. The research is supported by the Young Scholar Fund from University of Konstanz and grants from German Research Foundation (KU2669/1-1).

References

1 Duboule, D. 2007. The rise and fall of Hox gene clusters. *Development* 134: 2549-2560.
2 Hunt, P., *et al.* 1991. The branchial Hox code and its implications for gene regulation, patterning of the nervous system and head evolution. *Development* 2: 63-77.
3 Dolle, P., *et al.* 1989. Coordinate expression of the murine Hox-5 complex homoeobox-containing genes during limb pattern formation. *Nature* 342: 767-772.
4 Grapin-Botton, A. and Melton, D.A. 2000. Endoderm development: from patterning to organogenesis. *Trends Genet.* 16: 124-130.
5 Duboule, D. 1994. Temporal colinearity and the phylotypic progression: a basis for the stability of a vertebrate Bauplan and the evolution of morphologies through heterochrony. *Dev. Suppl.* 1994: 135-142.
6 Kuraku, S. and Meyer, A. 2009. The evolution and maintenance of Hox gene clusters in vertebrates and the teleost-specific genome duplication. *Int. J. Dev. Biol.* 53: 765-773.
7 Kim, C.B., *et al.* 2000. Hox cluster genomics in the horn shark, *Heterodontus francisci*. *Proc. Natl. Acad. Sci. USA* 97: 1655-1660.
8 Prohaska, S.J., *et al.* 2004. The shark HoxN cluster is homologous to the human HoxD cluster. *J. Mol. Evol.* 58: 212-217.
9 Ravi, V., *et al.* 2009. Elephant shark (*Callorhinchus milii*) provides insights into the evolution of Hox gene clusters in gnathostomes. *Proc. Natl. Acad. Sci. USA* 106: 16327-16332.
10 Venkatesh, B., *et al.* 2007. Survey sequencing and comparative analysis of the elephant shark (*Callorhinchus milii*) genome. *PLoS Biol.* 5: e101.
11 Meyer, A. 1998. Hox gene variation and evolution. *Nature* 391: 225, 227-228.
12 Lynch, V.J. and Wagner, G.P. 2009. Multiple chromosomal rearrangements structured the ancestral vertebrate Hox-bearing protochromosomes. *PLoS Genet.* 5: e1000349.
13 Oulion, S., *et al.* 2010. Evolution of Hox gene clusters in gnathostomes: insights from a survey of a shark (*Scyliorhinus canicula*) transcriptome. *Mol. Biol. Evol.* 27: 2829-2838.
14 Coolen, M., *et al.* 2008. The dogfish *Scyliorhinus canicula*: a reference in jawed vertebrates. *Cold Spring Harb. Protoc.* 13: 431-446.
15 Mulley, J.F., *et al.* 2009. Comparative genomics of chondrichthyan Hoxa clusters. *BMC Evol. Biol.* 9: 218.
16 Inoue, J.G., *et al.* 2010. Evolutionary origin and phylogeny of the modern holocephalans (Chondrichthyes: Chimaeriformes): a mitogenomic perspective. *Mol. Biol. Evol.* 27: 2576-2586.
17 Heinicke, M., *et al.* 2009. Cartilaginous fishes (Chondrichthyes). In *The Timetree of Life* (eds. Kumar, S. and Hedges, S.B.), pp.320-327. Oxford University Press, New York, USA.
18 Cole, N.J. and Currie, P.D. 2007. Insights from sharks: evolutionary and developmental models of fin development. *Dev. Dyn.* 236: 2421-2431.
19 Freitas, R., *et al.* 2007. Biphasic Hoxd gene expression in

shark paired fins reveals an ancient origin of the distal limb domain. *PLoS One* 2: e754.
20 Freitas, R., *et al.* 2006. Evidence that mechanisms of fin development evolved in the midline of early vertebrates. *Nature* 442: 1033-1037.
21 Sakamoto, K., *et al.* 2009. Heterochronic shift in Hox-mediated activation of sonic hedgehog leads to morphological changes during fin development. *PLoS One* 4: e5121.
22 Davis, M.C., *et al.* 2007. An autopodial-like pattern of Hox expression in the fins of a basal actinopterygian fish. *Nature* 447: 473-476.
23 Sordino, P., *et al.* 1995. Hox gene expression in teleost fins and the origin of vertebrate digits. *Nature* 375: 678-681.
24 Kuraku, S., *et al.* 2008. Noncanonical role of Hox14 revealed by its expression patterns in lamprey and shark. *Proc. Natl. Acad. Sci. USA* 105: 6679-6683.
25 Theodosiou, N.A., *et al.* 2007. Comparison of acid mucin goblet cell distribution and Hox13 expression patterns in the developing vertebrate digestive tract. *J. Exp. Zool. B Mol. Dev. Evol.* 308: 442-453.
26 Balfour, F.M. 1878. *A Monograph on the Development of Elasmobranch Fishes.* Macmillan, London, UK.
27 Oulion, S., *et al.* 2011. Evolution of repeated structures along the body axis of jawed vertebrates, insights from the *Scyliorhinus canicula* Hox code. *Evol. Dev.* 13: 247-259.
28 Kuraku, S., *et al.* 2009. Jawless fishes (Cyclostomata). In *The Timetree of life* (eds. Kumar, S. and Hedges, S.B.), pp.317-320. Oxford University Press, New York, USA.
29 Kuraku, S. and Kuratani, S. 2006. Time scale for cyclostome evolution inferred with a phylogenetic diagnosis of hagfish and lamprey cDNA sequences. *Zool. Sci.* 23: 1053-1064.
30 Kuraku, S. 2008. Insights into cyclostome phylogenomics: pre-2R or post-2R. *Zool. Sci.* 25: 960-968.
31 Kasahara, M. 2007. The 2R hypothesis: an update. *Curr. Opin. Immunol.* 19: 547-552.
32 Pendleton, J.W., *et al.* 1993. Expansion of the Hox gene family and the evolution of chordates. *Proc. Natl. Acad. Sci. USA* 90: 6300-6304.
33 Sharman, A.C. and Holland, P.W. 1998. Estimation of Hox gene cluster number in lampreys. *Int. J. Dev. Biol.* 42: 617-620.
34 Takio, Y., *et al.* 2004. Evolutionary biology: lamprey Hox genes and the evolution of jaws. *Nature* 429: 1 p following 262.
35 Takio, Y., *et al.* 2007. Hox gene expression patterns in *Lethenteron japonicum* embryos—insights into the evolution of the vertebrate Hox code. *Dev. Biol.* 308: 606-620.
36 Kuraku, S., *et al.* 2009. Timing of genome duplications relative to the origin of the vertebrates: did cyclostomes diverge before or after? *Mol. Biol. Evol.* 26: 47-59.
37 Force, A., *et al.* 2002. Hox cluster organization in the jawless vertebrate *Petromyzon marinus*. *J. Exp. Zool.* 294: 30-46.
38 Irvine, S.Q., *et al.* 2002. Genomic analysis of Hox clusters in the sea lamprey *Petromyzon marinus*. *J. Exp. Zool.* 294: 47-62.
39 Fried, C., *et al.* 2003. Independent Hox-cluster duplications in lampreys. *J. Exp. Zool. B Mol. Dev. Evol.* 299: 18-25.
40 Stadler, P.F., *et al.* 2004. Evidence for independent Hox gene duplications in the hagfish lineage: a PCR-based gene inventory of *Eptatretus stoutii*. *Mol. Phylogenet. Evol.* 32: 686-694.
41 Furlong, R.F., *et al.* 2007. A degenerate ParaHox gene cluster in a degenerate vertebrate. *Mol. Biol. Evol.* 24: 2681-2686.
42 Ota, K.G., *et al.* 2007. Hagfish embryology with reference to the evolution of the neural crest. *Nature* 446: 672-675.
43 Nikitina, N., *et al.* 2009. The sea lamprey *Petromyzon marinus*: a model for evolutionary and developmental biology. *Cold Spring Harb. Protoc.* 2009: pdb.emo113.
44 Kuraku, S. 2010. Palaeophylogenomics of the vertebrate ancestor—impact of hidden paralogy in hagfish and lamprey gene phylogeny. *Integr. Comp. Biol.* 50: 124-129.
45 Feiner, N., *et al.* 2009. The origin of bmp16, a novel Bmp2/4 relative, retained in teleost fish genomes. *BMC Evol. Biol.* 9: 277.
46 Ikuta, T. and Saiga, H. 2005. Organization of Hox genes in ascidians: present, past, and future. *Dev. Dyn.* 233: 382-389.
47 Seo, H.C., *et al.* 2004. Hox cluster disintegration with persistent anteroposterior order of expression in *Oikopleura dioica*. *Nature* 431: 67-71.
48 Putnam, N.H., *et al.* 2008. The amphioxus genome and the evolution of the chordate karyotype. *Nature* 453: 1064-1071.
49 Escriva, H., *et al.* 2002. Analysis of lamprey and hagfish genes reveals a complex history of gene duplications during early vertebrate evolution. *Mol. Biol. Evol.* 19: 1440-1450.