First publ. in: Nature 391 (1998), 6664, pp. 225-228

Developmental biology

Hox gene variation and evolution

Axel Meyer

Both developmental and evolutionary biologists try to explain patterns in the diversity among organisms, and the Hox genes encode a class of transcription factors that have provided ample material for such discussions. Because they may be pivotal in specifying regional identity in body plans, differences in their expression could (at least partly) explain the evolution of animal phyla. The Hox genes are arranged in genomic clusters and, importantly, they are expressed in a spatially colinear fashion — anterior genes are expressed early in development and towards the front of the body, posterior genes later in development and in more distal portions of the body.

In invertebrates, only a single Hox gene cluster has been found (although it is split in *Drosophila*). The common ancestor of all chordates is surmised to have had a single cluster as well. This cluster is thought to have duplicated to four clusters (A–D) on different chromosomes, accompanying the increasing complexity of body plans during the evolution of vertebrates (Fig. 1). But a report by Prince *et al.*[1], shortly to appear in *Development*, is likely to cause some questioning of this commonly held hypothesis — that genomic and morphological complexity are causally linked[2–7].

Using an experimental approach based on the polymerase chain reaction, Prince *et al.* unambiguously identified 34 Hox genes and determined their linkage with somatic-cell hybrids. Surprisingly, they found that the zebrafish has three Hox genes (*Hox*C3, *Hox*A8 and *Hox*B10), with no direct mouse equivalents (Fig. 1). Moreover, the expression domains of the anterior Hox genes are partly overlapping, and restricted to a shorter anterior region. Possibly the most important finding is that the zebrafish has at least two additional Hox gene clusters for a total of six and not, as previously thought[8], the typical vertebrate number of four. All of the genes on these additional clusters have probably not yet been discovered, and three are reported so far[1].

These two additional clusters lead us to question a simple, 'more clusters, more complexity' model of evolutionary diversification — in terms of phenotypic complexity, however measured, a zebrafish is probably not 50 per cent more complex than a mouse

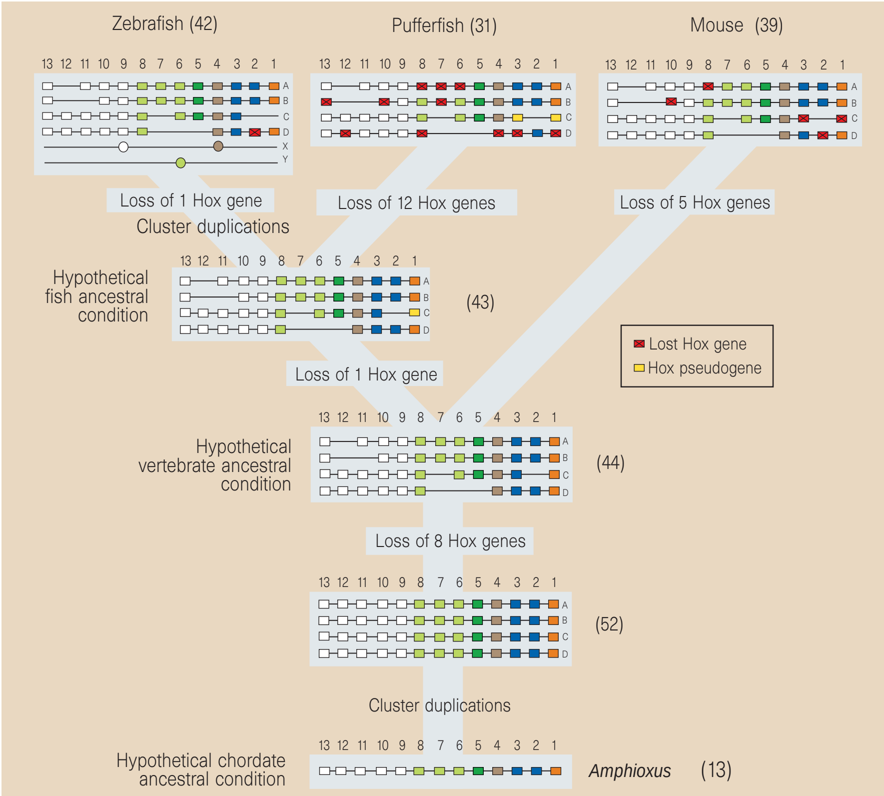

Figure 1 **Evolutionary conservation of the Hox gene clusters in zebrafish, pufferfish, mouse and *Amphioxus*. The zebrafish Hox gene clusters are shown with the 34 genes confirmed by Prince *et al.*[1], and an additional eight, for the total of 42 that the zebrafish is likely to have. Ten gene losses differentiate the mouse from the pufferfish, but there are only three losses between the mouse and zebrafish, even though fish and tetrapods shared their last common ancestor about 400 million years ago. The common ancestor of human and mouse probably lived around 60 million years ago, but the architectures of their Hox gene clusters are identical. The losses in the *AbdA (abdominal A)*-related genes (light green) in the D-cluster probably occurred early in the history of vertebrates. (Figure drawn by Heike Haunstetter.)**

or a human. The extra clusters cannot be explained by entire-genome duplications because, although polyploidy is known from other carp-like fish[9] and is common in salmonids, zebrafish are diploid. It also seems unlikely that the additional clusters are remnants of a polyploid ancestral condition. Instead, the Hox-cluster duplications in zebrafish might be a unique evolutionary event. But such events may turn out to be common, at least in fish.

Prince and colleagues' work on zebrafish[1], combined with studies on the pufferfish[10], now enables us to reconstruct the evolutionary history of the Hox gene clusters in vertebrates (Fig. 1). The initial chordate ancestral cluster of 13 Hox genes (the architecture that is still present in the cephalochordate *Amphioxus*[7]) probably duplicated in a three-step process, to form four complete clusters with a total of 52 genes. One phylogenetic study[11] indicates that the D-cluster is the most ancestral, and that the B- and C-clusters are the youngest. Hagfish and lamprey are phylogenetic intermediates between *Amphioxus* and more derived vertebrates such as zebrafish. So, if these fishes have only two or three clusters (which is not precisely known), they would be more likely to contain a D-like Hox gene cluster than a B- or C-cluster. The suggestion that the D- and A-clusters are the oldest also seems to fit the observation that the D-cluster is the most 'deteriorated' of all (Fig. 1).

Following the principle of Dollo parsimony — which assumes that losses of genes are much more common and likely than independent evolutionary origins[12] — we can speculate which Hox genes might have been present in the common ancestors of vertebrates, tetrapods and fish (Fig. 1). Based on the genomic organizations available so far, the rates of evolution of Hox clusters do not seem to be constant. For example, whereas the zebrafish is likely to have lost only one Hox gene since it shared a common ancestor with the pufferfish (probably more than 200 million years ago), the losses along the pufferfish lineage were possibly several times faster (12 Hox genes were lost, if the zebrafish really has 42 Hox genes) (Fig. 1). Ignoring the additional clusters of the zebrafish, and estimating that it has 42 Hox genes, we find that 13 differences separate the zebrafish from the pufferfish. In the pufferfish, the *Hox*C1 and C3 genes are still

100 YEARS AGO

Messrs. Swan Sonnenschein and Co. announce that they will shortly publish a work, entitled "The Wonderful Century: its Successes and its Failures," by Dr. Alfred R. Wallace, F.R.S. The object of the volume is to give a short descriptive sketch of all the more important mechanical inventions and scientific discoveries which are distinctive of the nineteenth century. … The author maintains that our century is altogether unique; that it differs from the eighteenth or seventeenth centuries, not merely as those differed from the centuries which immediately preceded them, but that it has initiated a new era, and that it may be more properly compared with the whole preceding historical period.

The January number of the *National Review* has an admirable article by Mr. Gerald Arbuthnot, entitled "In Defence of the Muzzle." The temperate spirit in which it is written, and the conscientious manner in which the statistics referred to have been collected, ought to materially strengthen the hands of those who are upholding the muzzling order for dogs, in the face of the selfish and short-sighted opposition which it is receiving from a certain section of the public.

From *Nature* 13 January 1898.

50 YEARS AGO

A symposium arranged by the New York Academy of Sciences and held in December 1946 on "Nutrition in Relation to Cancer" covered a wide field and included a number of interesting articles which have now been published. … Although it may seem disappointing that after so much study of cancer the fundamental cause or nature of it is unknown, the papers [in the symposium volume] show advances. The carcinogenic process can often be influenced by diet, which means that the process can be resolved into separate parts, and this must help in the understanding of the process. Many of the speakers at the symposium compared carcinogenesis to mutations. Both cancer and mutations can be induced in living organisms by similar agents. The hypothesis that cancer is a somatic mutation relates carcinogenesis to other biological changes and the stability of the nuclear and cytoplasmic genes.

From *Nature* 17 January 1948.

recognizable, but they are only pseudogenes (genes that are not transcribed). They might have lost their function at different points in the evolution of fish, because *Hox*C3, at least, is still present in the zebrafish.

The apparent acceleration of genomic evolution along the pufferfish lineage might be correlated with accelerated morphological evolution. Pufferfish belong to one of the most morphologically derived groups of fish, and they lack ribs, pelvic fins and the pelvic girdle. Are the missing genes those that are no longer necessary because these structures have been lost during evolution? If so, the missing Hox genes in the pufferfish might also be absent in the other groups of fish which have secondary loss of pelvic fins (such as eels) or even tail fins (for example, the ocean sunfish *Mola mola*). Moreover, the loss of Hox genes might also be accompanied by the secondary loss (or simplification) of appendages in land vertebrates, such as in limbless amphibians, reptiles and whales.

What selective forces maintain or modify genomic organizations? The observation that Hox genes are clustered, and that the architecture of these clusters is highly conserved in evolution, has led to the suggestion that the regulatory elements that control expression of the Hox genes cannot be separated from these genes without jeopardizing their proper functioning and, possibly, determination of morphology along the anteroposterior axis. For vertebrates[13,14] these ideas have been partially confirmed experimentally[15], and this tight functional linkage might be particularly strong along the lineage that leads to reptiles and mammals (Fig. 1).

The long-standing question of whether the evolution of genes or networks of interactions through regulatory elements drives most morphological diversification might, then, have different answers in different evolutionary lineages. In the most species-rich group of vertebrates — fish — organization of the Hox genes might not be completely constrained by interwoven regulatory networks, and differentiation might be driven by gene evolution. However, in the lineage that leads to reptiles and mammals, the driving force behind morphological diversification might have been newly evolving interactions in networks of regulatory elements[16]. □

Axel Meyer is in the Department of Biology, University of Konstanz, D-78457 Konstanz, Germany.
e-mail: axel.meyer@uni-konstanz.de

1. Prince, V. E., Jolly, J., Ekker, M. & Ho, R. *Development* **125,** 407–420 (1998).
2. Akam, M. *Phil. Trans. R. Soc.* **349,** 313–319 (1995).
3. Holland, P. W. H. & Garcia-Fernández, J. *Dev. Biol.* **173,** 382–395 (1996).
4. Lewis, E. B. *Nature* **276,** 565–570 (1978).
5. Krumlauf, R. *Cell* **78,** 1991–202 (1994).
6. Duboule, D. & Morata, G. *Trends Genet.* **10,** 358–364 (1994).
7. Garcia-Fernández, J. & Holland, P. W. H. *Nature* **370,** 563–566 (1994).
8. Misof, B. Y. & Wagner, G. P. *Mol. Phylogen. Evol.* **5,** 309–322 (1995).
9. Buth, D. G., Dowling, T. E. & Gold, J. R. in *Cyprinid Fish, Systematics, Biology and Exploitation* (eds Winfield, I. J. & Nelson, J. S.) 83–126 (Chapman & Hall, London, 1991).
10. Aparicio, S. *et al. Nature Genet.* **16,** 79–83 (1997).
11. Bailey, W. J., Kim, J., Wagner, G. P. & Ruddle, F. H. *Mol. Biol. Evol.* **14,** 843–853 (1997).
12. Meyer, A. in *New Uses for New Phylogenies* (eds Harvey, P. H., Leigh Brown, A. J., Maynard Smith, J. & Nee, S.) 322–340 (Oxford Univ. Press, 1996).
13. Gérard, M. *et al. Genes Dev.* **10,** 2326–2334 (1996).
14. Gould, A. *et al. Genes Dev.* **11,** 900–913 (1997).
15. Mann, R. S. *BioEssays* **19,** 661–664 (1997).
16. Davidson, E. H., Peterson, K. J. & Cameron, R. A. *Science* **270,** 1319–1325 (1995).