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Correspondence

Remodelling of the homeobox gene complement in the tunicate *Oikopleura dioica*

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Homeodomain transcription factors are involved in many developmental processes [1] and have been intensely studied in a few model organisms, such as mouse, *Drosophila* and *Caenorhabditis elegans*. Homeobox genes fall into 10 classes (ANTP, PRD, POU, LIM, TALE, SIX, Cut, ZFH, HNF1, Prox) and 89 different families/groups, all of which are present in vertebrates. Additional groups may be uncovered by further genome annotation, particularly of complex vertebrate genomes. Eight of these groups have been found only in vertebrates, but not in the genome of the tunicate *Ciona intestinalis*. The other 81 groups of homeobox gene that have been detected in vertebrates so far probably appeared during the early evolution of bilaterians or earlier, as they are also present outside the chordates. How the homeobox genes evolved during and after the main radiation of the bilaterians remains poorly understood, as only a few animal genomes have been sequenced completely. However, drastic changes have occurred at least in the lineage of *C. elegans* [2], such as loss of several Hox genes and Hox cluster fragmentation [3]. Here we report considerable alterations of the homeobox gene complement in the tunicate lineage.

Genome sequencing of *Ciona* indicated the absence of fifteen ancestral groups of homeobox genes, including at least two *Hox* genes, and the breakdown of the *Hox* cluster into five segments [4,5]. We are interested in *Oikopleura dioica*, another tunicate which belongs to the appendicularian class. Appendicularians and ascidians have been evolving separately for a substantial period of time, as is apparent from morphology and confirmed by molecular data [6]. We recently showed that *Oikopleura* has lost one anterior and all central *Hox* genes, but has more posterior *Hox* genes than *Ciona*, and that its nine *Hox* genes are dispersed in the genome [7]. Here, we take advantage of the very small and compact genome of *Oikopleura* [8], which we sequenced at a high level of coverage (14x) using the shotgun method. Alignment of 624 EST sequences with the shotgun data indeed shows a representation of 99.7% of all coding nucleotides (unpublished data).

We have identified 83 candidate homeobox genes in *Oikopleura*, and we have thus far evidence for the expression of 71 of them based on the cloning of intronless cDNA fragments. These genes fall into all known homeobox classes except the Prox class, and consist of 81 homeobox-containing genes and two non-homeobox *Pax* genes. Good candidate orthologs of 78 *Oikopleura* homeobox genes are found in the human genome, while three genes could be unique to *Oikopleura. Ciona* also has 83 homeobox-containing genes [9], but the homeobox gene complements of *Oikopleura* and *Ciona* are markedly different, both in the representation of the gene groups and in the number of genes in each group (Table 1 and Supplemental data). In addition to some *Hox* genes, several genes of other classes that are absent in *Ciona* [9] are also absent in *Oikopleura*, and another 14 homeobox gene groups present in *Ciona* are absent in *Oikopleura*. Because only three groups absent in *Ciona* were found in *Oikopleura*, the total diversity of homeobox gene groups is far lower in *Oikopleura* than in *Ciona*.

These findings indicate that the first apparent trend of homeobox gene evolution in tunicates is a substantial loss of entire gene groups (Figure 1). The second major trend is a high incidence of lineage-specific gene duplications within the remaining gene groups of *Oikopleura* (Table 1). Interpreting both trends

Table 1. Gene amplification within homeobox gene groups in tunicates.

Gene group	Expected	*Ciona*	*Oikopleura*
Pax258	1	2	1
NK2	1	1	2
BarH	1	4	1
Irx	3	4	5
Cdx	1	1	3
Otx	1	1	3
Hox9	1	1	2
Evx	1	2	1
Not	1	1	4
Prop	1	1	2
Pax37	1	1	4
Meis	1	1	2
Six36	1	1	2
Onecut	1	1	3
Prox	1	2	0

Expected number of genes in the last common ancestor compared with the number of genes in *Ciona* and *Oikopleura*, indicating an independent gain of 7 and 19 genes, respectively.

in terms of the evolution of developmental mechanisms will remain difficult until the function of tunicate homeobox genes is revealed. The body plan of chordate ancestors may have been simplified in the tunicate lineage, resulting in a relaxed pressure to conserve a full set of developmental genes [10]. The changes of homeobox gene complement in *Oikopleura* could also reflect a rapid sequence evolution. We do have several other indications for a fast evolution of intron–exon organisation and retrotransposon content of the *Oikopleura* genome [11,12], and the faster evolution of tunicate homeodomain sequences is also supported by long branches in the phylogenetic trees, especially for *Oikopleura* (Supplemental data; for the alignments used for phylogenetic analysis see: www.sars.no/ homeobox-alignments/). The high level of gene amplification observed for several homeobox gene groups in the *Oikopleura* lineage does not readily fit a simple scenario of faster genome evolution, as in this situation the gene duplicates should also disappear more rapidly. The persistence of numerous gene duplicates in *Oikopleura* could instead reflect a re-diversification of the homeobox gene complement following major group losses. The requirements driving such a secondary expansion will have to be identified through functional studies.

Supplemental data

Supplemental data, including phylogenetic trees and a comparison of the homeobox complement of *Ciona* and *Oikopleura* as well as experimental procedures are available at http://www.current-biology.com/cgi/content/full/15/1/R12/DC1/

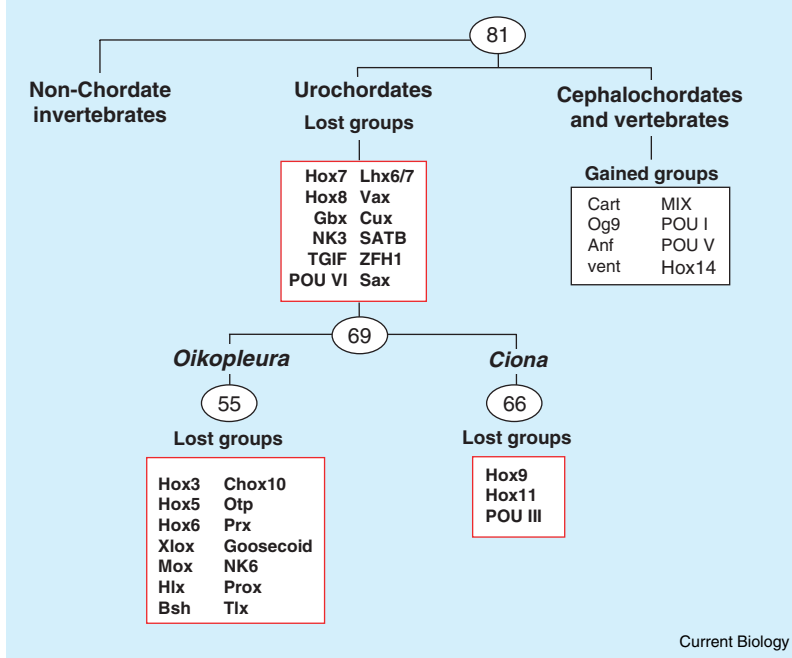

Figure 1. Loss of urochordate homeobox groups.

The numbers in ovals indicate the predicted number of homeobox groups present at a given phylogenetic position. The putative gained groups for the cephalochordate/vertebrate lineage are boxed in black. Homeobox groups predicted to be lost are boxed in red.

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