Genomes of diploblastic organisms contain homeoboxes: sequence of eveC, an even-skipped homologue from the cnidarian Acropora formosa

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SUMMARY
We report the nucleotide sequence of eveC, a cnidarian eve-class homeobox; this is the first homeobox to be identified in any diploblastic organism, and is only the second eve-class in an invertebrate. Similarity between the predicted amino acid sequence of the eveC homeodomain and its insect and vertebrate equivalents was approximately 75—80%, but, in the case of eveC, a role in segmentation can be ruled out.

Our findings thus support the ‘co-option’ hypothesis: homeoboxes were an early feature of metazoan genomes, corresponding to the DNA-binding domains of more general transcription factors.

In triploblastic organisms, such as insects and vertebrates, homeobox-containing genes are thought to be involved in cell-fate specification and segmentation processes; however, they have not previously been detected in diploblastic metazoans, such as cnidarians and cnemaphores. Indeed, early reports (McGinnis et al. 1985) implied that cnidarians lacked homeobox-genes, which were known to be highly conserved throughout the Triploblastica. If cnidarians do have homeobox-containing genes then these cannot be involved in the development and specification of repeated patterns, as there is no evidence for segmentation in these radially symmetric organisms. The presence or absence of specific homeobox-containing genes may also provide criteria with which to address problematic relationships between the triploblasts and various lower metazoan phyla (Cnidaria, Porifera, Placozoa, etc.).

To determine if homeobox-containing genes exist in cnidarians, we used genomic DNA from the coral Acropora formosa (Anthozoa; Scleractinia) as a template, and the redundant polymerase chain reaction (pcr) primers which had previously identified homeoboxes in annelids (the hom3 and en5 primers) (Wedeen et al. 1990). The pcr products obtained included sequences distinctly resembling several of the known classes of homeoboxes, including Antennapedia (Antp) and fushi-tarazu (A. Miles & D. J. Miller, unpublished observations). By screening a JEMBL3 genome library (Miller et al. 1992) with an A. formosa Antp-type pcr product, a single clone containing a homeobox closely resembling the even-skipped (eve) box was identified (figure 1a). This is the first homeobox to be identified in any diploblastic organism, and is only the second eve-class in an invertebrate; eveC resembles eve in lacking the intron in the region corresponding to the recognition helix, a feature that is characteristic of vertebrate homeoboxes of this class.

The similarity between the predicted amino acid sequence of the cnidarian eve-like homeodomain and its Drosophila and vertebrate equivalents is approximately 75—80% (figure 1b); as cnidarians are often considered to be only distantly related to the (triploblastic) eu-metazoans (Field et al. 1988; Ghiselin 1988; Bergstrom 1991; Christen et al. 1991), this is a remarkable degree of sequence similarity; eve-class homeoboxes were thus already present before the diploblast—triploblast divergence in the Cambrian.

Note that eve is a pair-rule gene, intimately involved in the segmentation process in Drosophila (Lawrence et al. 1987; Lawrence & Johnston 1989); eveC is clearly a member of the eve-class of homeoboxes, but in this case a role in segmentation can be ruled out. Our findings give further support to the co-option hypothesis (Patel et al. 1989; Holland 1990); namely, that the homebox gene classes, and the clustered pattern of organization of Antp-class homeobox genes, predate their co-option, or ‘capture’, to fulfil roles in segmentation by several phyla independently. Several other lines of evidence support the co-option hypothesis; for example, the ancestral engrailed gene apparently had a function in neurogenesis, and has been co-opted to a role in segmentation in arthropods, but not in annelids or chordates (Patel et al. 1989). Furthermore, in addition to its role in segmentation, eve is later expressed in a defined subset of neurons in the Drosophila CNS (Doe et al. 1988), which probably represents a more ‘primitive’ role. The presence of a cluster of Antp-class homeobox genes in the apparently unsegmented roundworm Caenorhabditis elegans (Burglin et al. 1991; Kenyon & Wang, 1991) is often considered as confirmation of the co-option hypothesis. However, the epidermis of newly hatched C. elegans consists of repeated sets of cells, and...
the only C. elegans Antp-like gene whose function is known, mab-5, is involved in specifying the fate of some of these cells. Thus, in the case of C. elegans, a role for homeobox genes in ‘segmentation’-like processes cannot be ruled out. However, the detection of eveC and the presence of Antp-like sequences in Cnidaria unequivocally confirms that homeobox genes arose and diversified in unsegmented metazoans.

Homeoboxes were clearly an early feature of metazoan genomes, probably corresponding to the DNA-binding domains of more general transcription factors. This hypothesis is supported by the presence of diverged homeodomains in the POU transcription factor family and the similarity between the homeodomain and various yeast transcription factors (Scott et al. 1991). Percentage similarity to eveC is stated on the right of each sequence, in parentheses.

REFERENCES


Bastian, H. & Gruss, P. 1990 A murine even-skipped homologue, Evx 1, is expressed during early embryogenesis and neurogenesis in a biphasic manner. EMBO J. 9, 1839–1852.


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