Bridging the gap but breaking the rule: a tunicate twists the hox puzzle

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In all bilaterians, anterior–posterior (A–P) body axis is determined by a set of genes called the homeotic or *hox* genes. These genes as well as their genomic organization are conserved during evolution. The *hox* genes exist in a cluster and their order of occurrence in the genome corresponds to the region of the body along the A–P axis, which they determine by being expressed in a spatially constrained manner in the corresponding region. This collinearity of organization and function of the homeotic genes was first discovered by Ed Lewis in fruit fly *Drosophila melanogaster*, for which he won the Nobel Prize[1]. Subsequently, this colinearity was found to be conserved in all bilaterians[2,3].

Reasons of this colinearity are not well understood, but it has been suggested that higher-order chromatin structure involved in the regulatory mechanism may be imposing this constraint of organization. Several regulatory elements that initiate, maintain and subdivide the expression pattern of *hox* genes have been isolated from fly as well as mammalian complexes[4,5]. Interestingly, if an initiator/enhancer element is combined to a heterologous reporter gene and inserted in the genome at random locations, often the reporter gene is expressed according to the property of the initiator element, suggesting that the initiator element does not need the context of the hox complex. On the other hand, if a reporter gene is inserted within the hox complex, the expression will depend upon the site of insertion and not on other regulatory elements (initiator/enhancer) that are combined with the transgene. This suggests that position in the complex is the primary determinant of spatial expression pattern.

Like the organization of the hox complex, these 'rules' of *hox* gene expression are conserved in animals possessing an A–P body axis. Remarkably though, in *Drosophila*, where the colinearity was discovered in the first place, *hox* genes are not in one complex but the complex is split into two. Furthermore, in different species of *Drosophila*, the split has taken place at different points of the complex[6,7]. Another deviation from the 'rules' in the fly hox complex is that there are other genes, unrelated to the *hox* gene, within the complex. Finally, compared to vertebrate complexes, insect hox complexes are spread out, typically ten times as big. While vertebrate hox complexes are 80–120 kb in size, insect complexes are 800 kb or more in size. The vast hox complex of insects, as the study in *Drosophila* revealed, is full of regulatory elements that are arranged in distinct domains and, most interestingly, these domains too follow the rule of colinearity[1].

While, as discussed above, in insects the hox complex seems to be more spread out, sometimes split into two, and contains non *hox* genes, the vertebrate hox complexes are more tight, devoid of non *hox* genes and no split has been observed in any vertebrate hox complex. During evolution of vertebrates, the genome is believed to have duplicated twice and accordingly, most vertebrates have four hox complexes and each one of them follows the 'rules'. Unlike in insects, in the vertebrate hox complexes all the genes are transcribed in the same direction. In addition, vertebrate hox complexes also have temporal colinearity – the most anteriorly expressed genes located at the 3′ end of the complex are expressed early in time and the relatively posteriorly expressed genes that are located more towards the 5′ end are expressed later during development[8]. In summary, all bilaterians could be said to follow some general principles of organization, which has been thought to be tightly linked to their regulation. It seems that nature has not found another way to determine A–P body axis of animals. A recent report has changed this view of uniformity!

Evolution of multicellularity, tissues and symmetry was the major landmark of the natural history of animals. All bilaterally symmetrical animals (except solid worms) possess a body cavity – coelom. Advanced coelomates have body composed of repeated segments, which allows specialization of different parts of the body by introducing specification of different segments along the body axis. Based on developmental strategy, coelomates can be divided into two groups: proteostomes (develop a mouth from near the blastopore) and deuterostomes (develop an anus from the blastopore). In order to move around, animals use the skeleton attached to their muscles. While lower animals like arthropods use exoskeleton, deuterostomes use endoskeleton. Deuterostomes are divided into echinoderms and chordates. While echinoderms have a true endoskeleton just beneath the skin that is functionally similar to exoskeleton of arthropods, chordates have different kind of endoskeleton, which is developmentally as well as functionally internal. Among the hallmarks of chordates are the nerve cord to which nerves attach and reach different body parts and notochord, and a flexible but hard rod on the back of the embryo to which muscles attach. Obviously, further recruitment of specific tools on this theme along the A–P axis provided great possibility of diversification of body form suitable to a variety of living conditions. Remarkably, from this point evolution of vertebrates is accompanied by gain of *hox* genes by two duplication events of the entire hox cluster, providing the 'tool kit' for the vast variety of body forms. Bridging the gap between invertebrates and vertebrates are the non-vertebrate chordates – urochordates (tunicates) and cephalochordates (lancelets). These animals share, in addition to features like nerve chord and notochord, a gill-like opening with the vertebrates but lack bony backbone. It is this important evolutionary position of tunicates and lancelets that makes them important in the study of evolutionary developmental biology.

In a landmark paper in *Nature*, Seo *et al.*[9] reported that the tunicate *Oikopleura dioca* has nine *hox* genes, three anterior *hox* genes (*Hox1*, *Hox2* and *Hox4*) and six posterior *hox* genes (*Hox9A*, *Hox9B*, *Hox10*, *Hox11*, *Hox12* and *Hox13*), while all the central genes are missing (Figure 1). As could be predicted, these genes are expressed according to the spatial colinearity seen in other animals. Surprisingly though, in contrast to all bilaterians studied so far, the *hox* genes in this tunicate are not clustered. Furthermore, *O. dioca hox* genes, mapped to different Bacterial Artificial Chromosomes (BACs) are surrounded by other genes with ubi-

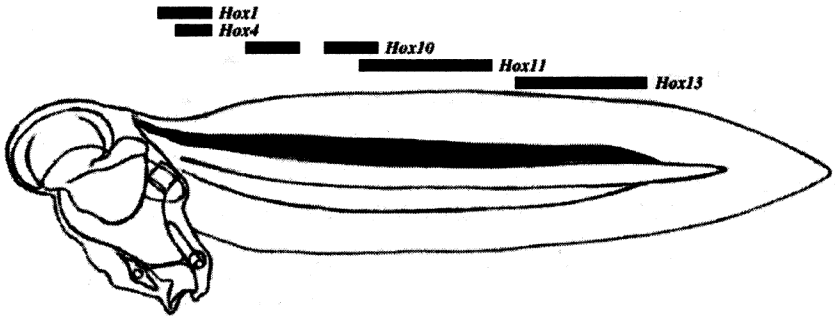

Figure 1. *Hox* gene expression pattern in nerve cord. Although, unlike in other bilaterians, *Oikopleura dioica hox* genes are not in a cluster, the expression follows the same spatial pattern.

quitous expression pattern, eliminating any possibility of coordinated expression of genes in a domain or extremely spread out cluster with gene-poor region having *hox* genes that mapped to different BACs.

Large body of evidence suggests that at least in mammals, early during development, hox clusters are in a repressed state associated with silent chromatin features in a repressive nuclear compartment and *hox* genes are released progressively for expression in a temporally colinear manner[10,11]. The order in which these genes are released for expression is the order in which they are organized in *cis*. This also explains why the genes should be in a cluster for such a restricted and coordinated pattern of expression. Being physically linked is not the only way to be in one compartment in the nucleus or being co-regulated. In *Drosophila*, long-distance influence and interaction of *Polycomb* Response Elements (PREs) have been demonstrated[12]. PREs are cellular memory elements that help the cell remember the expression state of genes (in particular the *hox* genes) by means of higher-order chromatin structure. Transgene studies have shown that PREs inserted at different locations in the genome can 'sense' the presence of other transgenes and the endogenous copy, and repress the expression of linked reporter genes[13]. Pairing of the chromosomes that brings such reporter genes in proximity, has been used as an assay–pairing-dependent silencing assay – to study the strength and genetic components of cellular memory involved in the regulation of *hox* genes[14]. One way to explain the *O. dioca* situation in *hox* genes could be that even though these genes are not linked, they could still be clustered in nuclear space, taking the clue of hox regulation based on factors that control these genes in other bilaterians. This will predict that *hox* genes in *O. dioca* may be associated with PREs and the boundary elements that insulate the spatially restricted pattern of *hox* genes from the ubiquitously expressed flanking genes. Study of chromatin elements associated with *O. dioca hox* genes will help in resolving the issue. This tunicate, interestingly, has a small genome of 70 MB with 15,000 genes, giving a high gene density, ~1 gene per 4.5 kb DNA. Such high density of genes and with possibility of differential regulation of at least some closely located genes, offer a good model system to study functional domains of gene expression and to identify DNA sequences (boundary elements) that subdivide the genome into structural and functional domains.

Why are the *hox* genes in *O. dioca* are not clustered? There are few related issues that need to be considered for a proper perspective: *hox* genes do not show temporal colinearity where the cluster is split, e.g. in *Drosophila*[6]. Genomic organization of the *para hox* genes of *Ciona intestinalis* showed that tight clustering has been lost in this ascidian[15]. Similarly, there is no temporal colinearity in the expression of *O. dioca hox* genes[9]. This may indicate that being linked is essential for temporal colinearity, while spatial colinearity can be achieved in unlinked genes as well. In such a case, *O. dioca hox* cluster could disintegrate, keeping the expression pattern intact. This recent report draws a comparison between *Caenorhabditis elegans* and *O. dioca*, as in both *hox* genes are not linked and at the same time development is 'cell lineage-driven'. In this scenario, the role of *hox* genes in specification of A–P axis has decreased and their role in specifying tissue-type has increased. This may suggest that the reason for the hox cluster to disintegrate is 'to facilitate or permit separation of expression domains'[9].

The role of *hox* genes in appendage, digit and genitalia formation is known[16,17]. It is also known that temporal and spatial colinearity is 'replayed' in early stages of development of these body parts in coordination with the spatial clues along the A–P body axis[17]. If evolutionary strategy for 'faster developmental speed and smaller organism' was opted, loss of central *hox* genes and breakdown of the complex could be one way of doing it. Such a strategy will, however, lead to loss of the tool to make digits. Perhaps, simple body features, reduction in size to a few millimetres and four days generation time indicate just that. In other words, this tunicate could bend the rule of the hox complex, obeyed by other bilaterians, to become quick and small, but has lost the possibility to 'walk' and 'grip' in bargain. One lesson that we certainly learn from this recent report is that we are not quite up to generalizing the rules of body-axis formation. The real possibility of new things waiting to be discovered certainly fuels our fascination to explore genomes and developmental rules hidden within. Most of our understanding of developmental biology comes from the study of a handful of 'model organisms'. It is clear that studies on diverse organisms will be useful in future. Fortunately, the new approaches and techniques of genomics do not need 'laboratory domestication' of an organism for studies. A large number of genome projects and free access to sequence databases promise exciting times of evo-devo to come.

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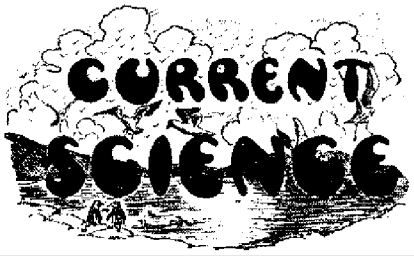

Vol. XIII] JULY 1944 [NO. 7

University College of Technology, Madras

Facilities for higher technological studies in the Madras area will henceforth be available at the new University College of Technology, which is for the present located as a temporary measure in the Government College of Engineering, Guindy. The inauguration of this College is well timed as there will be a great demand for chemical engineers and technologists in the post-war era of industrial reconstructions and developments in this country. Considerable progress has already been made under the auspices of the BSIR in various branches of Chemical Industries and a number of new processes have been developed in recent years. In order to preserve this progress, and to develop further processes in a keenly competitive world in the post-war years specialised chemical engineering talent and technological grounding and skill will be in great demand. It is, therefore, a happy augury that the Madras University has pushed forward with its arrangements for contributing to meet these very essential national demands. Parallel courses in Chemical Engineering, Leather Technology, Textile Chemistry, Electrochemistry, Fermentation Technology, etc., are under contemplation, but a beginning has been made this year with the Chemical Engineering course, through the kind courtesy of the Government of Madras, who have made available to the University the vast resources of the engineering laboratories and the workshop facilities at their College of Engineering, Guindy. The College is promised a substantial financial aid by way of a munificent grant from Dr Rm. Alagappa Chettiar, of more than Rs 3 lakhs non-recurring, and an annual recurring grant of Rs 25,000. The Government of Madras are also giving generous grants towards the building, equipment and maintenance of the College which has been so boldly ventured upon by the University in these difficult times from out of its own funds.

A good start has already been made with the appointment of highly qualified staff. Dr D. R. Nanjee, who comes out shortly to India after nearly twenty years of experience in England, both in the Universities and as a Consulting Chemist, will be the Professor and Director of the College, while Dr. M. A. Govinda Rau, who has successfully organised and conducted the courses in Chemical Engineering at the Indian Institute of Science, Bangalore, will be the Reader in charge of Chemical Engineering.

Great credit is due to the learned and enthusiastic Vice-Chancellor of the University, Dewan Bahadur Dr A. L. Mudaliar, for it was he who proposed the scheme nearly three years ago and should now be justly most happy at these successful results of his tireless efforts. We congratulate Dr A. L. Mudaliar and the University on the occasion of the Inauguration of the new College by His Excellency the Governor on the 28th of this month. The honorary degree of Doctor of Laws will be conferred upon Dr Rm. Alagappa Chettiar at a special Convocation held on the same occasion.