Body building exercises

In vertebrates, regional variation in mechanisms of axial segmentation may reflect sequential modular construction of the trunk and head during evolution.

Definition of the vertebrate *bauplan* is a zoological problem of classic pedigree; it is an issue in which extreme, and often contradictory, views have been maintained even up to the present day. One of the most striking features of the body plan is its metamerism — the repeating 'segmental' nature of much of the anatomy, including components of the skeleton, musculature, vasculature and nervous system. For many years it was believed that, as in the protochordate *Amphioxus*, vertebrates are fundamentally segmented from anterior to posterior extremities. However, it was also assumed that segmentation at the anterior end had become obscured by the evolution of increasing head specialization (cephalization) and, within individual ontogenies, by the increasing anatomical complexity that accompanies development and maturation. This traditional view, encapsulated in the writings of Goodrich [1], is upheld by modern-day anatomists such as Bjerring [2] who claim not only that virtually the entire head is segmented but that its metamerism is identical and continuous with that of the trunk.

Over the years, various contrary views have been posited, but perhaps the most influential has been the evolutionary scenario postulated by Gans and Northcutt [3]. They propose that the appearance of the neural crest as a developmental 'novelty', together with a complex transition in feeding mechanisms, underpinned the evolution of vertebrates from a primitive chordate ancestor and facilitated their subsequent explosive evolution. Because the notochord extends to the anterior tip of the organism in *Amphioxus* (unlike vertebrates, where its anteriormost limit is at approximately mid-brain level), and given that in vertebrates the most anterior part of the head is largely comprised of neural crest- and placode-derived cells, Gans and Northcutt reject any inherent segmentation to the bulk of the head, at least to that part anterior to the notochord. This unsegmented and evolutionarily 'younger' part of the head is in this way viewed as an 'add on' module — an anatomical extension appended to the front of an *Amphioxus*-like ancestor.

These approaches utilizing descriptive morphology have been insufficient to resolve the metamerism debate. Molecular biology, however, may provide the tools with which to resolve this seemingly intractable problem. Three advances make this possible: identification of 'periodic' patterns of expression of *Antennapedia* class regulatory genes; the exploitation of cell-type-specific differentiation markers; and the application of more rigorous and objective criteria for defining segmentation. Four recent studies provide dramatic new insights into the pattern of axial segmentation in neural, mesodermal and ectodermal components of the *bauplan*, and in doing so, offer molecular evidence of fundamental differences in segmental patterning mechanisms along the body axis.

Segmentation within the nervous system is an integral feature of vertebrate metamerism (for example, spinal nerves). Typically, the first indication, often preceding neuronal differentiation, is the appearance of periodic bulges in the early neural tube; these 'neuromeres' are termed 'rhombomeres' in the rhombencephalon or hindbrain, and 'myelomeres' along the length of the trunk neural tube. There are fundamental region-specific differences in how this organization is created. Segmentation might be a primary feature of the cephalic neural primordium as early as neural-plate stage, but trunk segmentation, evidenced by the myelomeres, seems to be acquired secondarily. Certainly the evidence for an inherent metameric character in the hindbrain is well established; rhombomeric organization reflects a metameric distribution of proliferation centres in the neuroepithelium, the rhombomere boundaries coincide not only with periodically distributed cell lineage restrictions but also with the anterior limits of expression of a number of homeobox-containing genes, and there is a segmental pattern of neuron differentiation [4].

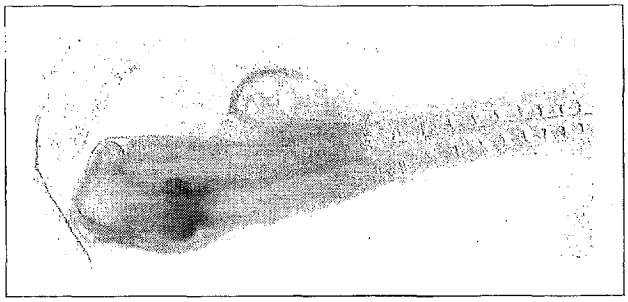

Fig. 1. Immunostained chick embryo, revealing the distribution of En2, a nuclear protein found in posterior mid-brain and rhombomere 1. (Photograph courtesy of C Stern.)

Two recent papers using chick embryos focus directly on these issues. The first adopts a classic experimental embryology strategy — ectopic grafting of the primary 'organizer' (in amniotes, Henson's node) — to induce a secondary axis, including a supernumerary neural primordium. In the past, such work has relied largely on morphological criteria by which to identify the regions of the induced neural primordium. Interpretation of structures as simply 'neural' has been acceptable but identification of discrete regions has often been subjective. But by using specific markers that characterize regions within normal neural primordia, interpretation can be precise and objective (Fig. 1). By grafting Henson's nodes of different ages into an extraembryonic site, different regions of the neural primordium were induced ectopically [5]. Younger nodes tended to elicit the formation of anterior structures such as fore and mid brain, whereas older

nodes elicited progressively more posterior central nervous system (CNS) structures. It is important to remember that Henson's node, like its homologue in amphibians, the dorsal lip, is a dynamic structure whose cellular composition changes as gastrulation of presumptive mesoderm progresses. Accordingly, the authors conclude that normal regionalization of the CNS is the result of different signals emanating from the mesoderm, and they even raise the question of how many signals might be necessary to elicit the formation of a full length CNS from the overlying ectoderm.

This result essentially confirms previous work but also extends it significantly because interpretation is based on the use of region-specific markers. It is consistent with the idea that different signals may underlie the induction of head and trunk CNS regions and even that different signals elicit the differentiation of the various regions of the brain itself. It is not yet clear, however, if this finding reflects a qualitative difference or a gradient in some quantitative influence, with threshold effects eliciting multiple differentiation pathways.

A second paper from the same laboratory compares the nature and origin of the myelomeres of the trunk neural tube with the rhombomeres of the hindbrain, and shows a number of fundamental differences [6]. The use of a mitotic inhibitor revealed that myelomeres are not the consequence of local proliferative centres in the neuroepithelium of the trunk neural tube. Furthermore, there is no evidence for any inherent segmental differentiation of motor neurons; the earliest detectable spinal cord neurons differentiate at apparently random intervals along the anterior–posterior axis. The authors conclude that there is no inherent segmentation of the trunk neural tube, at least according to the rigorous criteria by which metamerism in the hindbrain is recognized. Instead, metamerism of the spinal cord primordium in birds seems to be secondarily acquired from surrounding tissues. The authors suggest that one of the simplest, yet nevertheless important, influences may be mechanical moulding by somites formed as segmentation proceeds in the adjacent, or paraxial mesoderm.

The two remaining papers both use homeobox-containing genes of the *Antennapedia* class to investigate segmental organization in the mouse. In the mouse embryo, the *Antennapedia* class homeobox genes are located in four chromosomal clusters related by duplication and divergence from a common ancestral gene complex. Because of their common origin, genes related by virtue of their sequence homology are found in equivalent positions within the four clusters. Such related genes are referred to as 'paralogues' and the sub-families so recognised, as 'paralogous' genes. In the trunk mesoderm, anterior limits of expression of the *Hox* genes align with somite interfaces, such that each gene has an anterior limit of expression that coincides with the anterior face of a particular somite pair. Moving from anterior to posterior along the axis, one finds a progression in the combination of *Hox* genes expressed such that small groups of somites (and thus their respective axial levels) are characterized by the expression of a unique combination of *Hox* genes.

The vertebral column comprises a small number of discrete anatomical regions (for example, cervical, thoracic); within each region, vertebrae display shapes characteristic of each axial level. Kessel and Gruss [7] analyse metamerism of the paraxial mesoderm and establishment of trunk segment identity by perturbation of this system. Treatment of embryos with retinoids such as retinoic acid (known as a teratogen but recently recognized as a morphogen or mimic of a morphogen) results in transformation of vertebrae. These changes are not simply random alterations or chaotic dysmorphology but constitute precise and actual homeotic transformations into other identifiable vertebral forms that vary according to stage of development at the time of exposure (Fig. 2). Because each pre-vertebra normally has a precise *Hox* gene combination according to axial level, and because retinoic acid effects transformation from one vertebral form into another, it is logical to ask if this *Hox* 'code' changes accordingly. In fact, it is altered — a code is expressed appropriate to the new vertebral form. This relationship between the expression of a particular combination of regulatory genes and a particular morphogenesis of a segment suggests a causal role for *Hox* genes in the establishment of regional axial identity along the trunk.

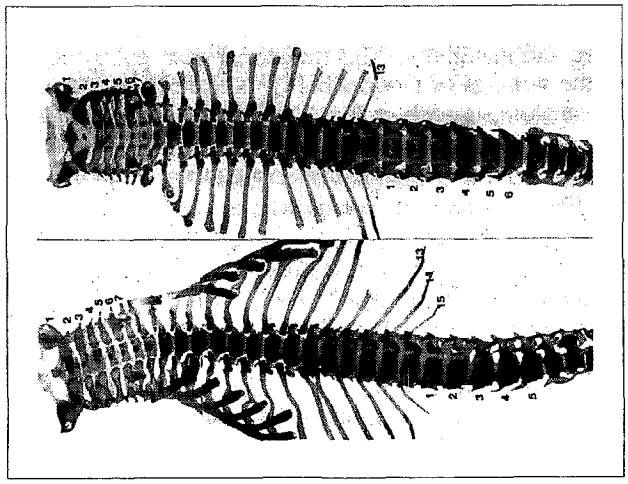

Fig. 2. Vertebral patterns in wild-type (above) and retinoic acid-treated (below) mice; note transformation from 13 to 15 thoracic vertebrae and associated ribs. (Photographs courtesy of M Kessel and P Gruss, reproduced with permission from *Cell* [7].)

The fourth paper, by Hunt *et al.* [8] examines whether a comparable *Hox* code is detectable in the embryonic mouse head. In the head, mesoderm-derived mesenchyme is sparse and largely replaced by the neural crest. Furthermore, only part of the head — the hind brain and branchial arches — is obviously metameric in its organization. (Segmental organization of cranial paraxial mesoderm, in the form of pre-somitic somitomeres, has been claimed for many taxa [9] but these claims remain to be assessed by the rigorous criteria applied to the CNS.) In fact, there is no periodic expression of regulatory genes detected so far in head mesoderm and *Hox* gene expression in this tissue is only seen secondarily, after these regions have been colonized by the neural crest migrating from the hindbrain. Work from several laboratories, most notably those of Robb Krumlauf and David Wilkinson, has demonstrated not only that the rhombomeres display a neuromere-specific expression of the *Antennapedia* class genes but also that the neural crest migrating from them retains the same axial

level-specific pattern of gene expression [10]. These results are especially significant in the light of the putative role assigned to the crest in patterning the branchial arch derivatives. A *Hox* code might be a means of implementing the transfer of positional information from the hindbrain to peripheral head structures such as the arches. It is also significant that no *Hox* genes are expressed in mesenchyme (whether crest- or mesoderm-derived) in the anterior part of the embryonic head.

Recently, these laboratories have examined the relationships between the various regulatory genes involved, to see if there is any resemblance between the manner in which they are deployed in mesodermal cells of the trunk — as evidenced by the *Hox* code, and the combinatorial expression in the hindbrain neuroepithelium and the crest cells it produces. A striking difference emerges. It is clear from the Kessel and Gruss '*Hox* code' [7] that regions of expression of paralogues are not necessarily co-incidental in the trunk. Thus, part of the specificity of the trunk code is apparently the consequence of offset in expression limits (that is, paralogues have different anterior expression limits). In marked contrast, Hunt *et al.* [8] find that, with the exception of the *labial* cluster, offsets between paralogous members of the remaining three clusters do not occur in the head tissues. In fact paralogues display identical expression limits amongst the rhombomeres and the neural crest that is derived from them. If the *Hox* code concept is applicable to the segmented regions of the head, then clearly the head code is fundamentally different from that of the trunk. Not only is it initially expressed in a different germ layer (mesoderm in trunk, neuroectoderm in head) but the *Antennapedia* class regulatory genes are combinatorially deployed in a different fashion.

It is tempting to use these studies to formulate a general model of segmentation of the body axis in vertebrates, its genetic control and its morphogenetic implementation, but any such model would be incomplete and probably simplistic at this time. First, it is likely that only some of the regulatory genes involved have been identified; new genes and gene clusters undoubtedly remain to be isolated even from these model systems. Second, it remains to be seen if the mechanisms of segmentation in chick and mouse — both amniotes — are representative of segmentation mechanisms in vertebrates generally. If we are to understand how the head evolved and what made vertebrate evolution possible, then we must study the appropriate taxa and this means analysing the genetic basis of axis specification in fishes, amphibians and reptiles. Two examples illustrate this dramatically. The trunk neural tube in teleost fishes, unlike that of amniotes, is segmental from the earliest stage, as evidenced by the metameric differentiation of the motor neurons [11]; mechanical moulding by somites seems unnecessary to elicit segmental organization. Might this represent the actual primitive vertebrate condition? And consider the branchial basket of *Amphioxus*. It is usually considered to be homologous with the branchial arch apparatus of vertebrates, but *Amphioxus* has more than 90 pairs of double branchial bars developing in the absence of a neural crest, whereas jawed fishes typically have 6 gill clefts and tetrapods have 4 or 5 gill pouches! Although the *Amphioxus* condition is clearly a specialization for filter feeding, it raises a number of questions about its relation with the branchial region in vertebrates.

Despite such reservations, one general message emerges from these studies: segmentation is displayed in different tissues and specified by genes operating in different ways along the body axis. Given that the vertebrate body plan, especially the head, probably evolved sequentially and possibly in a modular fashion, perhaps it is not surprising that disparate developmental strategies have been adopted at critical points in our evolutionary past. Furthermore, it is probably simplistic to think in terms of head versus trunk. The body axis probably comprises at least three regions — trunk, hindbrain, anterior head — definable with respect to several key aspects of segmentation. Is metamerism present? If so, in which germ layer is it first expressed, and precisely how are regulatory genes used to specify regional identity?

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