Spatial patterns of homeobox gene expression in the developing mammalian CNS

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1984 was a good year for those interested in the molecular genetics of development. In March and July, two groups[1,2] independently reported the discovery of the homeobox, a 180 base-pair DNA sequence found in *Drosophila*. The sequence is associated with a number of genes that are known to affect body pattern, and that are expressed in characteristic regions during the development of the fly. What was more surprising is that the homeobox turned out to be highly conserved in vertebrates[3]: it is remarkable, for example, that the homeo-domain associated with the *Drosophila* gene *Antennapedia* and the human gene *HHo.c10* differ by only three of the terminal 53 amino acids[4]. An editorial review that accompanied the original papers called the homeobox '*A Rosetta stone* . . .', betraying the hope that some fundamental principle about the control of gene expression during pattern formation was shortly to emerge. Since then, molecular biologists have looked hard for regional expression patterns of the homeobox genes in vertebrate embryos, while developmental biologists have started to cut sagittal or coronal sections of their embryos instead of transverse sections.

Homeobox gene expression in the epidermis and nervous system of the fly

In *Drosophila*, the genes involved in the establishment of body pattern are classified[5] into five distinct classes: (1) the selector (homeotic) genes, from which the homeobox takes its name, are those that specify segment identity – when mutated, they convert a whole epidermal segment, or a part of a segment, into another (for example, the gene *Antennapedia*, which in the mutant generates a leg instead of an antenna in the head); (2) the segment polarity genes, which affect the polarity of individual epidermal segments (for example, the gene *engrailed*, which is expressed in the posterior portion of each segment); (3) the pair-rule genes, which affect alternate metameres along the length of the animal (examples are the genes *fushi tarazu*, *odd-skipped* and *even-skipped*); (4) the gap genes, which are expressed in a group of contiguous epidermal segments, an example of which is the gene *Krüppel*, which in the mutant deletes a group of segments in the middle of the fly; and (5) the maternal effect genes, which affect development but are transcribed in the mother (an example is the gene *bicoid*, which when defective produces a fly with no head and two caudal ends). With the exception of the gap genes, there are homeobox genes in all these classes.

Although the effects and the patterns of expression of these genes have been studied mainly in the epidermis of *Drosophila*, many are also expressed inside the animal, in regions that include the nervous system (e.g. Refs 6–11). The mutant phenotypes have been harder to recognize here, being invisible from the exterior of the fly.

Vertebrate homeobox genes

Homeobox genes have now been found in most vertebrate classes, and those in the frog, the mouse and the human have been studied most intensively. In the mouse, some 25 or so genes have been identifed, falling into two distinct sub-groups: one with close sequence homology to the homeobox associated with the *Drosophila* gene *Antennapedia*; and the other with close homology to the homeobox of the *Drosophila* gene *engrailed*. The former genes appear as clusters, named *Hox 1*, *Hox 2*, *Hox 3* and *Hox 4*, on chromosomes 6, 11, 15 and 1, respectively. At least two more clusters (*Hox 5* and *Hox 6*[33]) have been identified (Ruddle, F. H., pers. commun. and Ref. 33). The two *engrailed*-like homeobox genes identified in the mouse have been designated *en-1* (chromosome 1) and *en-2* (chromosome 5) (Ref. 12 and Frohman, J. and Martin, G. R., pers. commun.).

It should be emphasized that there are other *Drosophila* genes that have roles during development and also have vertebrate homologues, but which do not possess a homeobox. For example, the *Drosophila* gene *wingless* is homologous to the mouse proto-oncogene *int-1*; like the mouse homeobox genes (see below), *int-1* is regionally expressed in the developing neural tube[13–16] (Fig. 1).

Patterns of expression of vertebrate homeobox genes

Despite the fact that the mesoderm imposes segmental organization on the vertebrate peripheral nervous system (PNS)[17,18], the central nervous system (CNS) is also segmented morphologically[19]. Although we do not yet know if segmentation in the CNS has any functional significance, it is interesting that all the mouse homeobox genes studied to date are transcribed in the developing CNS. As in *Drosophila*, each gene has a characteristic regional pattern of CNS expression, summarized in simplified form in Fig. 1. While many of the genes begin their expression along the major portion of the neural tube, transcription quickly becomes more restricted: with *Hox 2.1*, for example, the cranial border between non-expressing and expressing regions remains fixed, and the caudal border moves cranially at 13.5 days[25]. In some cases (e.g. *Hox 1.3* and *Hox 3.1*[27,28]) expression is also found in the adult CNS; interestingly, regional expression of *Hox 1.3* in the adult CNS includes the forebrain[28], an area that apparently fails to express the gene during embryonic and fetal development[20].

Most of the mouse homeobox genes are also expressed in other organ systems, which may be derived from ectoderm or mesoderm. It may be significant that no homeobox gene has yet been found to be expressed in organs of endodermal origin; for example, where expression is found in the gut (e.g. *Hox 1.3* and *Hox 2.1*), it is restricted to the cells of mesodermal or neuroectodermal origin, such as muscle or parasympathetic ganglia. Some genes are expressed very early in development. *Hox 1.5*, for example, starts to be transcribed during gastrulation[17]; others are transcribed only at later stages[25].

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What is the functional significance of homeobox genes?

A portion of the amino acid sequence of the homeo-domain (amino acids 31–50) has some homology with certain DNA binding proteins in bacteria[29]. In immunohistochemical studies, antibodies raised to the conserved peptide bind with a punctate pattern to the nucleus of mammalian cells[28,30,31], suggesting that proteins containing this sequence may have DNA binding properties in vertebrates. In turn, this means that the homeobox protein is likely to be involved in the regulation of gene expression in vertebrates, as it appears to be in invertebrates.

Although mouse and other vertebrate homeobox genes have been mapped in terms of their chromosomal locations, the portions of these genes outside the box itself have yet to be fully defined. *en-1* is an exception, in that it has been partially sequenced and found to have some homology (63 nucleotides) to the *Drosophila engrailed* gene outside the box region[12], but we know little about the function of this protein, even in flies. The most direct way forward would be to produce mutations at these loci, a task which has, to date, proved impossible. Meanwhile, there are two indirect ways of addressing the problem: first, correlating the location of a homeobox gene with that of a known mutation, and second, inferring function from regional patterns of gene expression.

It may be relevant that no mouse homeobox gene has been mapped to any region corresponding to a known mutation, and no mutations affecting regional differentiation in the neural tube have yet been identified. However, it may be more than coincidental that both *engrailed*-like genes of the mouse, *en-1* and *en-2*, map fairly closely to two dominant hemimelia genes, *Dh* (0.25 centimorgans away from *en-1*) and *Hm/Hx* (about 1.25 centimorgans away from *en-2*) (Frohman, J. and Martin, G. R., pers. commun.). This is a considerable distance, nevertheless, and the phenotypes of these mutations do not correspond to the observed patterns of expression of the *engrailed*-like homeobox genes. The correlations between other mouse homeobox genes and the chromosomal positions of other mutations of body pattern are even weaker. This may imply that mutations of these loci are lethal. Alternatively, perhaps because of redundancy, individual genes may not be so important as to produce identifiable phenotypes when mutant.

Because we cannot rely on known mutations to establish the function of homeobox genes, and site-directed techniques have proved unsuccessful, we are forced to make inferences entirely from regional patterns of expression. Since 1984, molecular biologists and embryologists alike have wondered whether a vertebrate homeobox gene would prove to be expressed in a segmental, striped, manner in the mesoderm or CNS. To date, however, the transcription patterns have failed to yield stripes. The patterns described do suggest that some of these genes may be concerned with the specification of regional identity within the embryonic axis, although this is by no means proven. If functional parallels with the epidermis of *Drosophila* can be drawn, these patterns are most analogous to those of the 'gap' or 'selector' gene classes.

Of the genes studied so far, it is striking that none is expressed rostral to the developing hindbrain. Moreover, only one gene (*Hox 3.1*; Fig. 1) has a rostral boundary of expression caudal to the hindbrain, in the cervical spinal cord. The hindbrain is segmented morphologically[19,32], and it will be interesting to correlate precisely the boundaries between these seg-

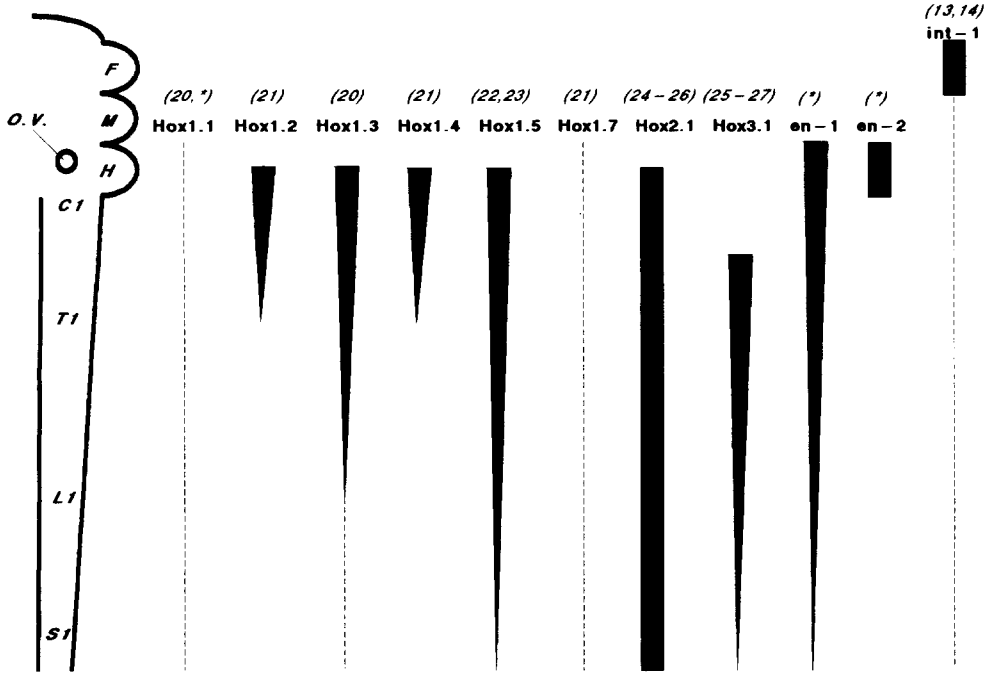

Fig. 1. *Schematic diagram of the patterns of expression of homeobox genes and* int-1 *(homologous to the* Drosophila *gene* wingless*) in the mouse CNS, at about 12.5 days of gestation. F, forebrain; M, midbrain; H, hindbrain; O.V., otic vesicle. C1, T1, L1, S1 indicate approximate positions of the first spinal roots of the cervical, thoracic, lumbar and sacral regions, respectively. The dotted vertical lines under* Hox 1.1, Hox 1.7 *and* int-1 *indicate that the limits of expression of the genes concerned have not yet been determined precisely. The numbers in brackets above the name of each gene refer to the references on which this diagram is based. *,Frohman, J. and Martin, G. R., pers. commun.* **Notes:** Hox 1.2 *also expressed in thoracic pre-vertebrae.* Hox 1.3 *is expressed, additionally, in lung, stomach and gut (not in the endodermal portions of the above organs) and in myenteric plexus, kidney, somites and derivatives.* Hox 1.4 *is also expressed in gonads, spermatocytes (not spermatogonia), and in* postmitotic *neuroblasts. In the spinal cord, the mantle layer shows greater level of expression than the ependymal layer.* Hox 1.5 *is additionally expressed in adult spermatocytes and spermatids, and also appears to be expressed in the somite.* Hox 2.1 *is expressed in the dorsal portion of the spinal cord, dorsal root ganglia, myenteric plexus, nodose ganglion, granulocytes caudal to the otic vesicle, mesonephros, metanephros, lung and gut (not in endodermally derived portions of the last two).* Hox 3.1 *is expressed ventrally in the spinal cord, and in pre-vertebrae below the level of T4. It is not expressed in the dorsal root ganglia.* en-1 *is expressed ventrally in the spinal cord and is also expressed in the dorsal root ganglia. The data for* en-1 *are based on the binding of an antibody to the protein product of this gene.* en-2 *is expressed in 'posterior brain'. We have interpreted this to mean hindbrain.* int-1 *is expressed dorsally in the neuroectoderm.*

ments (neuromeres) with those between expressing and non-expressing regions. In one case, the expression boundary appears to coincide with a morphological boundary (*Hox 1.5*; Ref. 22). In most cases, however, they do not correspond either to the boundaries between neuromeres or to the middle of each neuromere (Ruddle, F. H., pers. commun.). Unlike *Drosophila*, therefore, there is no clear connection between the pattern of homeobox gene expression and the process of segmentation.

We can expect many more anatomical descriptions of homeobox gene expression in vertebrate embryos, but considerably more knowledge about these genes, particularly of a functional kind, will be needed before we can tell whether homeobox genes indeed represent a 'Rosetta stone' for elucidating the mechanisms responsible for regional specification in vertebrate embryos.

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The lateral geniculate nucleus strikes back

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For many years the dorsal lateral geniculate nucleus of the thalamus has been regarded simply as a relay station between the retina and the visual cortex. Where the lateral geniculate nucleus was merely a colony ruled by the retina, the cortex, it appeared, had declared its independence by generating novel receptive fields that were highly selective for the orientation, contrast, velocity, size and depth of the stimulus in visual space. The lateral geniculate was clearly a nucleus in search of a function.

For the lateral geniculate nucleus at least, 1984 was a happy year, because a possible function was found for it. The nucleus was picked out of the Orwellian gloom by a searchlight manned by Francis Crick[1], who proposed that transmission through the thalamus to the cortex could be selectively controlled by inhibitory neurones in the reticular complex that surrounds the thalamus. The role of the reticular neurones was to highlight the activity of specific small groups of thalamic cells that projected to cortex. This mechanism, he suggested, was the neurophysiological substrate for the internal attentional searchlight proposed by Anne Treisman[2,3], Bela Julesz[4] and their co-workers on the basis of cognitive and psychophysical theory and experiments. However, Crick's proposal, and its subsequent neurophysiological elaboration[5,6]. could be comfortably accommodated within the long tradition that the thalamus was a gate on the path leading to cortex, whose width of welcome could be 'modulated' by a long list of ascending and descending projections, as suggested by Wolf Singer[7] for the visual system.

Crick made no suggestion that the stimulus selectivities of cortical cells had their origins in the lateral geniculate nucleus. But this is exactly the radical proposal that has emerged from the recent work of Penelope Murphy and Adam Sillito[8], who studied the property of 'end-inhibition' in geniculate cells. Cells that show end-inhibition respond well to a short stimulus, but decline markedly in responsiveness when the stimulus is lengthened. David Hubel and Torsten Wiesel[9] first observed this phenomena in single cells recorded in visual areas 2 and 3 of the cat and called these cells 'hypercomplex'. They suggested several wiring diagrams to explain how inhibitory cells in the cortex could generate the property.

One wiring diagram (Fig. 38C in Ref. 9) was given flesh through the painstaking morphological and physiological work of the Rockefeller group[10–12]. They first provided ultrastructural evidence that some pyramidal cells in layer 6 connect preferentially to the smooth cells in layer 4, rather than the spiny cells that form about 80% of the layer 4 population. The smooth cells, thought to be inhibitory in function, connect to neighbouring spiny cells in layer 4. Functionally, the circuit they found (Fig. 1) operates as follows: as the stimulus bar is lengthened, the layer 6 cells (p) respond more strongly, thus activating the inhibitory cells (i) in layer 4 more strongly, which in turn inhibit the

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