The homeobox in vertebrate development

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Introduction

Embryonic development is regulated by a co-ordinated programme of intra- and inter-cellular signals which combine to shape the final body. Each organism contains within its genome a developmental programme allowing the faithful reproduction of the species. It is known that many of the cellular processes involved in organizing the embryo are conserved amongst vertebrates, suggesting that widely divergent species may share similar strategies for pattern development. One genetic element underpinning these strategies may be the homeobox, a conserved regulatory domain that encodes a 60-amino acid helix-containing motif [1] originally found in many *Drosophila* genes which control pattern development. Consequently, the identification of similar motifs in higher animals leads to the belief that the homeobox may also be an important regulatory domain for vertebrate development. The evidence implicating homeobox-containing genes in pattern formation in vertebrates, while still preliminary, is clearly consistent with this role.

Homeoboxes as development determinants

A number of vertebrate *Hox* genes have been isolated and their expression during embryogenesis characterized. While many of the Antennapaedia-type homeobox genes are expressed in the central nervous system (CNS) and in mesodermal structures of the mouse embryo, each gene has a unique region of expression that partially overlaps the expression region of other homeobox-containing genes. The overlapping expression of these genes in the neural tube and adjacent mesoderm may indicate a combined action in these tissues.

Homeobox-containing genes may regulate pattern development in the vertebrate embryo in the following ways:

(1) Homeobox-containing genes may direct specific cellular differentiation programmes within a discrete tissue in the developing embryo. Hence, they would act directly in concert with other genes in processes such as organogenesis.

(2) The overlapping of expression in a particular region of the embryo may provide information on positron, possibly co-ordinating the fates of different cell types within a defined region. For example, a particular pattern of gene expression might inform a cell of its position in the embryo or ensure migrating cells find their correct targets. Obviously, the overlapping expression of a small number of these genes would provide a very precise set of position determinants.

The fact that some *Hox* genes are also specifically expressed in certain adult tissues indicates a possible role in cellular differentiation. For example, the expression of the murine homologue of the caudal gene (*Cdx-1*) in the mouse embryo is consistent with a role in cell differentiation rather than determination of position. *Cdx-1* is expressed primarily late in murine embryogenesis and in the adult. Moreover, expression of *Cdx-1* is restricted to the epithelium of the large intestine during formation of the intestinal villi [2]. A role in differentiation is also consistent with *Cdx-1* expression in adult gut epithelium because of the continual proliferation and differentiation of gut epithelia that occurs in the adult. The next step in analysing the role of *Cdx-1* will be to determine whether it is expressed in stem cells or in one of the four types of differentiated epithelium found in the intestine.

The expression pattern of the mouse engrailed gene *En-2* suggests a role in both localization and cellular differentiation. The mouse *En-2* gene contains a homeobox of the engrailed-type and is expressed from early embryogenesis throughout development into adulthood [3]. In the 8–12 day embryo, *En-2* is expressed in a band of neural tissue in the metencephelon, and subsequently in the structures derived from this region, namely, the developing cerebellum, pons, periaqueductal gray and collicoli. In the neonate and adult strong expression of *En-2* is still observed in the granule layer of the cerebellum and in the pons. While the region-specific expression of *En-2* in the early mouse embryo suggests that it has a role in delineating a specific subdomain in the neural tube and brain, later expression in the cerebellum of the neonate may reflect an alternative role in cell differentiation. Interestingly, the restricted regional expression of the *En-2* gene at the junction of the hindbrain and midbrain has also been conserved in zebra fish (Njolstad and Fjose, *Biochem Biophys Res Commun* 1988, 157:426–432) and in the chick [4].

The expression of the *Antp*-type homeobox-containing genes in vertebrates indicates that they may have a

Abbreviations

Cdx-1—caudal homeobox gene; **CNS**—central nervous system; **mRNA**—messenger RNA; **OTF**—octamer-transcription factor.

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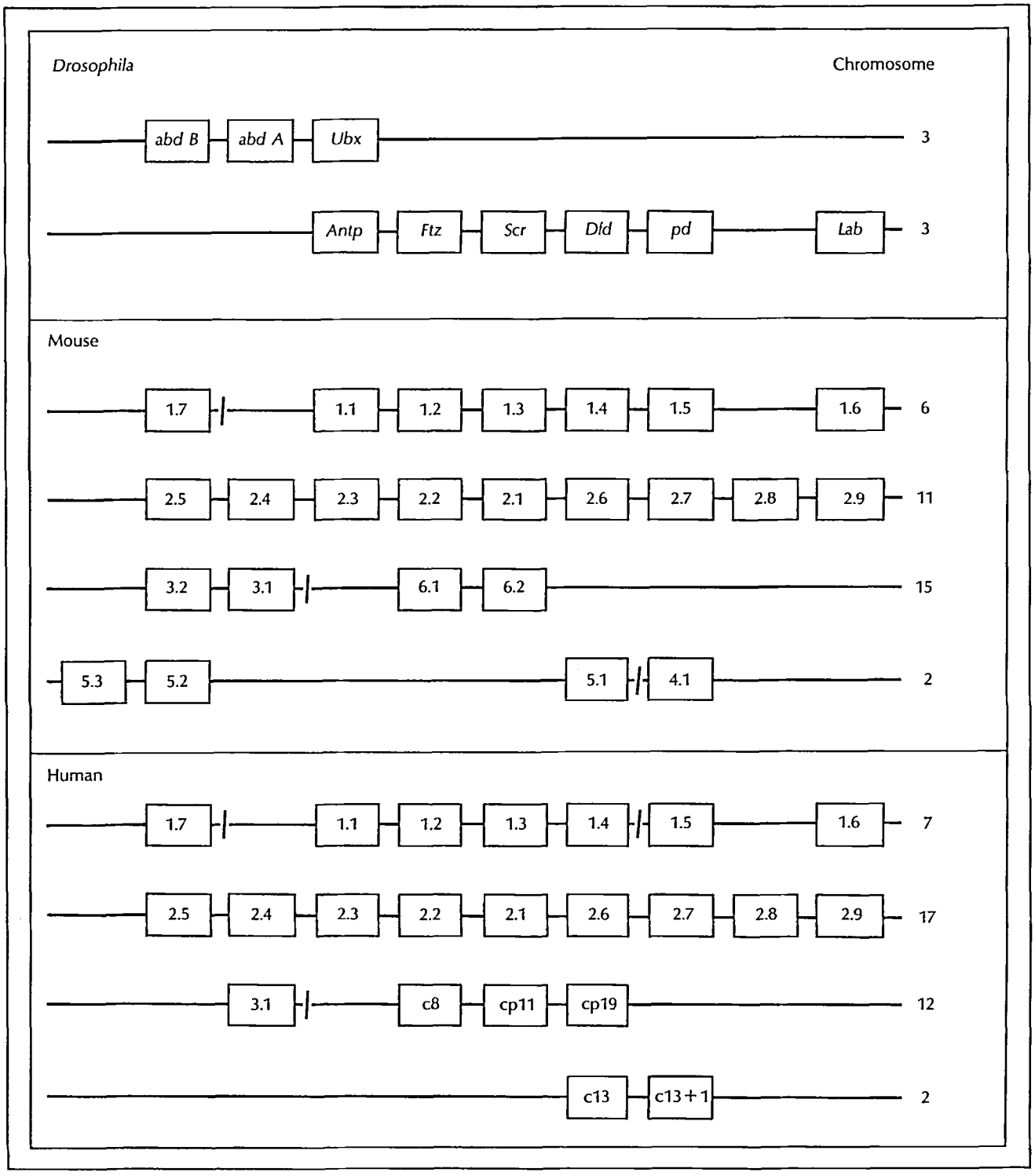

Fig. 1. Structure of the homeotic gene and *Hox* gene loci in *Drosphila*, mouse and human. Genes that have been identified in each cluster are aligned according to position in the locus and with those members of the other loci sharing the greatest homology. Reproduced with the kind permission of Dr M. Kesse.

role in determining region-specific events. In the mouse and human genome, four clusters of the *Antp*-class of homeobox-containing genes have been identified [5–8]. Recently, attention has been drawn to the fact that within these vertebrate *Hox*-gene clusters each gene shares considerable homology with its putative *Drosophila* counterpart on chromosome 3 (reviewed in [9], see also Fig. 1). Furthermore, in *Drosophila*, the arrangement of *lab-pd-DFd-Scr-Antp-Ubx-abd A-abd B* genes on chromosome 3 parallels their anterior–posterior sequence of expres-

sion in the *Drosophila* embryo. It now appears that genes within the *Hox*-gene clusters of the mouse show a similar relationship. Thus the nearer to the 3′ end a gene is in a cluster, the more anterior its border of expression is in the developing embryo. For example, in the murine *Hox-2* cluster, *Hox 2.7* is expressed in hindbrain regions, while the anterior borders of *Hox 2.4* and *Hox 2.5* expression extend only to the cervical region of the CNS [7]. This relationship also holds for the *Hox 5* cluster [8]. *Hox 5.2* and *Hox 5.3* are expressed in the lower thoracic and lumbar regions of the neural tube with the border of *Hox 5.2* being slightly anterior to that of *Hox 5.3*. In contrast, *Hox 5.1*, which occupies a more 3′ position in the cluster, is expressed in the neural tube as far forward as the myelencephalon [10]. These results support the argument that the vertebrate *Antp*-type homeobox-clusters and their *Drosophila* counterparts are derived from a common ancestral locus. Furthermore, the arrangement of the *Hox* gene clusters and their concomitant overlapping pattern of expression seems to have been conserved as a mechanism for determining position along the rostral–caudal axis of the vertebrate embryo.

The expression of many *Hox* genes in the neural tube and adjacent mesoderm of vertebrates is not surprising, since the general body plan of vertebrates would appear to necessitate co-ordination of positional cues between these tissues. One gene that is expressed with similar boundaries in neural and mesodermal tissue is the *Xenopus XI Hbox 1* gene whose expression is confined within a narrow anterior–posterior band [11]. Expression is restricted to neuroectoderm and mesodermal tissue with the same anterior and posterior boundaries. De Robertis *et al.* [12] argue that at the early neurala stage of *Xenopus* the position of mesoderm is determined and can induce similar positional cues in the adjacent neuroectoderm. This process, termed 'homeogenetic induction', may be a mechanism for co-ordinating positional signals in different tissues within the embryo. In contrast to *Xenopus*, there is little evidence for homeogenetic induction in the mouse. While expression of the *Hox 1.5* gene in the neuroepithelium and mesoderm is in close register in the early mouse embryo (Gaunt, *Development* 1987, 102:51–60), expression of the endogenous *Hox 1.1* gene and a transgene containing lac Z under the control of *Hox 1.1* regulatory sequences are not in register 7–9 days post-coitus (Dressler and Püschel, personal communication). Certainly, later in embryogenesis, nearly all the known *Hox* genes are clearly expressed 'out of register' in these two tissues.

Pax 3, a new member of the paired-type homeobox gene family has been identified and its expression suggests that it may regulate morphogenetic gradients along the ventral–dorsal axis of the embryo. Analysis of *Pax-3* in the developing neural tube shows that it is expressed in the neuroepithelium only in the dorsal half of the neural tube. Restriction of *Pax-3* transcripts to those neuronal progenitor cells along the entire dorsal half of the neural tube raises the possibility that *Pax-3* plays a role in dividing the CNS into sensory and motor compartments (Goulding *et al.*, unpublished observations).

The development of extremities such as the limb requires a unique set of positional determinants, some of which may be shared by body and limb alike, whilst others might be region specific. Homeobox-containing genes are among the potential candidates for regulating positional cues in the developing limb. In *Xenopus* and mice, the homologue of the human *Hox 5.2* gene is expressed in all four limb buds as a gradient as well as in caudal regions of the neural tube and somitic mesoderm. Maximal expression of *Hox 5.2* is observed in the distal and posterior mesenchyme of the limb bud in contrast with *X1 Hbox 1* which is expressed only in the anterior mesenchyme of the forelimb [13]. The *Hox 7.1* gene shows a more limited pattern of expression in the mouse. In 12½-day embryos, *Hox 7.1* is expressed predominantly in the most distal region of the limb bud, but also in the maxillary and mandibular processes that are derived in part from neural crest cells [14,15]. Interestingly, *Hox 7.1* expression is restricted to neural crest cells prior to and during neural tube closure. Whether *Hox 7.1* acts to influence the fate of these neural crest cells needs to be clarified. The fact that *Hox 1.1, X1 Hbox 1, Hox 5.2* and *Hox 7.1* are all expressed in characteristic gradients during limb bud formation may be indicative of an involvement in processes that polarize the limb bud. It may be now possible, by manipulating their expression pattern in limb bud cultures, to test whether these genes act as morphogenetic gradients in the limb.

Functional analysis of homeobox genes in the vertebrate

In *Drosophila* the functional analysis of homeobox-containing genes in pattern formation has been greatly facilitated by the plethora of genetic mutants available. With the advent of molecular biology many of these mutants have been identified and characterized. Data from genetic analyses of the *Hox* genes in vertebrates are limited and as a result their exact role in vertebrate development remains unclear.

Altering the normal morphogenetic gradients by over-expression of *Hox* genes at ectopic sites in the embryo is one approach to testing their role during embryogenesis. Such studies may resolve the question of whether the observed patterns of *Hox* gene expression impart positional information to the developing embryo.

In *Xenopus* the functions of two homeobox-containing genes have been analysed by injecting synthetic messenger RNA (mRNA) encoding them into *Xenopus* embryos. Using this approach, it has been shown that over-expression of the *Xbox-1A* gene, which is expressed predominantly in the somitic mesoderm, can disrupt somitogenesis. In particular, over-expression of *Xbox-1A* resulted in dysplasia of somitic muscle tissue with the consequent loss of the metameric pattern of muscle bundles adjacent to the neural tube. The differentiation of somitic mesoderm into muscle cells was not grossly altered, but the myotome on the affected side of the embryo appeared to be incorrectly orientated on the anterior–posterior axis. This supports the argument that *Xbox-1A* acts as a posi-

tional signal during somitogenesis rather than a differentiation signal [16].

A second *Xenopus* homeobox-containing gene has also been analysed using this approach. *Xbox-3* is particularly interesting, since there is a gradient of expression of *Xbox-3* along the anterior–posterior axis of the *Xenopus* embryo. This means that it is probable that *Xbox-3* has a role in positional determination. Injecting *Xbox-3* mRNA into prospective anterior regions of the early embryo resulted in alterations to the normal gradient of *Xbox-3* mRNA during gastrulation and neuralation. The anterior region, which normally has a low level of *Xbox-3* mRNA, had morphological defects in embryos injected with *Xbox-3* [17]. Interestingly, the phenotype observed with *Xbox-3* over-expression mimicked the defects caused by treatments that prevent prospective anterior mesodermal cells from migrating to their correct position during gastrulation. Both these studies support the theory that *Hox* genes act as positional determinants for mesodermal cells during *Xenopus* development.

The roles of two developmentally regulated homeobox-containing genes have also been analysed in mice by causing over-expression during embryogenesis. In one study, the murine *Hox 1.4* gene was over-expressed in transgenic animals by introducing multiple copies of it under the control of its putative endogenous promoter [18]. Although very little expression of the transgene was detected in testes, which normally express *Hox 1.4*, there were elevated levels of the transgenic mRNA with the correct spatial distribution in the CNS and lung. More importantly, a high level of *Hox 1.4* mRNA was observed in the gut mesenchyme, a site where no expression of *Hox 1.4* had been detected previously. The consequence of the over-expression of *Hox 1.4* in the gut mesenchyme was that newborn mice developed a fatal condition known as congenital megacolon. Previously congenital megacolon has been associated with a severe deficiency in myenteric ganglia, neural crest derivatives that migrate into the gut mesenchyme. It is, therefore, possible that over-expression of *Hox 1.4* in these mice interferes with the positional signals that normally guide the neural crest progenitors of myenteric ganglia to their target. This would suggest that *Hox*-gene expression may act as a positional cue for migrating neural crest cells.

Neural crest cells also appear to be a target for ectopic expression of the *Hox 1.1* gene in transgenic mice. When ubiquitous expression of the *Hox 1.1* gene in mice was achieved using the β-actin gene promoter, a number of abnormalities were found in newborn transgenic mice, i.e. cleft palate and non-fused pinnae (Balling *et al., Cell* 1989, 58:337–347).

It should be noted that the mesenchyme tissue that contributes to all the affected structures in these transgenic mice is derived at least in part from first arch neural crest cells. Morphological abnormalities in structure derived from the occipital somites are also observed in these mice (Kessel, unpublished results). This may be a consequence of ectopic expression of *Hox 1.1* in the anterior somitic mesoderm and the subsequent alteration of the normal morphogenetic gradients in this region. The evidence to date is consistent with a role for homeobox-containing genes as positional determinants in the vertebrate embryo. Not only are they expressed in restricted patterns during embryogenesis, but altering these patterns of expression also has severe effects on development.

Loss of gene function by homologous recombination to inactivate a particular gene is an alternative approach to the study of the function of homeobox-containing genes in development (Thomas and Capecchi, *Cell* 1987, 51:503–512). While much progress has been made in developing these potentially powerful techniques, germ line transmission still remains a problem [19,20].

Homeoboxes are transcriptional factors in vertebrates

Recently, homeodomains have been identified in known eukaryotic transcription factors. The ubiquitous octamer-transcription factor (OTF)-1 and the B cell specific *OTF-2* each contain a functional homeobox domain [21–24]. Both *OTF-1* and *OTF-2* bind to a conserved octamer motif (ATGCAAAT) present in a variety of promoter/enhancer regions. The homeodomains in *OTF-2* and *OTF-1* are part of a larger conserved region known as the POU domain. While the homeodomain in each of these transcriptional factors is only distantly related to the *Antp*-type homeodomain, the OTF-1 and OTF-2 proteins share a high degree of homology (87%) over the entire POU domain. At present, it is unclear exactly how the POU box works in these proteins, although, at least in the case of OTF-1, it is important in DNA binding [25]. In other homeobox-containing proteins, the homeodomain appears to be sufficient for DNA binding. Two other proteins, unc-86 and Pit-1 or growth hormone factor-1, also contain the POU domain [26–28]. The function of unc-86 is in development in *Caenorhabditis elegans* to regulate neuronal cell differentiation, while Pit-1 is a rat-pituitary-specific transcriptional factor, regulating transcription in lactotrophic and somatotrophic cell types. It is possible that Pit-1 acts in these cell types to regulate certain aspects of their development, since expression of Pit-1 is seen in the rat brain during embryogenesis [29]. *OTF-2* is also expressed in the complete CNS during embryogenesis [29] (Hatzopoulos *et al.*, unpublished observations) with high levels in the diencephalon at 12 days post-coitus as well as in discrete regions of the adult brain. A family of octamer-specific binding factors has been identified in the mouse embryo (Scholer *et al., EMBO J* 1989, 8:2543–2550), and their characterization will yield further insights into the mechanism of homeobox action during development. At present, structural analysis of the homeodomain only strengthens the conclusion that the homeobox is an integral part of these transcriptional regulators. When the predicted consensus structure for a number of known homeobox domains is analysed, it shows striking structural homology with the λ-repressor protein (Tsonis *et al., Biochem Biophys Res Commun* 1988, 157:100–105) [30]. These structure predictions indicate that the homeodomain forms three α-helices, very similar to bacterial repressor molecules.

The circle has closed, but with a small twist. While it is clear that the homeobox is a functional domain in transcription factors, not all these transcription factors are cell-type specific. For a while we believed that the homeobox might be a unique motif encoded by developmental control genes. Rather the reverse now appears to be true: the homeobox is a conserved protein domain that has been used to good effect by a number of proteins to regulate development.

Conclusions and future prospects

Many gaps remain in our knowledge of how homeobox-containing proteins act during development. Clearly, we are dealing with a complex group of transcriptional regulatory proteins; however, it remains to be determined how they function in embryonic development. Analysis of their role in development will require not only a mechanistic approach to their mode of action, but also an understanding of how they interact with and influence the biological processes that shape the embryo. The structural similarity of the homeodomain to the α-helical motifs in the λ repressor and cro proteins provides an important clue to how homeo-proteins might work. It is well known that in λ phage, cro and the λ repressor (cI) play a pivotal role in executing the lysis–lysogeny decision by acting as a transcriptional switch mechanism. The subsequent transcriptional activation or repression of early lytic genes determines the growth pathway λ will follow. It is noteworthy that many of the *Drosophila* homeodomain proteins activate or repress transcription of potential candidate genes [31,32], although it is unclear whether these observations can be extrapolated to vertebrates. Nevertheless, if homeodomain proteins are important components of transcriptional switching in vertebrates, then the λ model allows us to understand how differing local concentrations of homeodomain proteins might activate or repress the transcription of their developmental target genes.

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- • Of interest
- •• Of outstanding interest

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