Expression of the murine homeobox-containing gene Hox-2.3 suggests multiple time-dependent and tissue-specific roles during development

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Summary

This study reports the expression pattern of the murine homeobox-containing gene Hox-2.3 during development. Using in situ hybridization, we first detect Hox-2.3 transcripts in the allantois primordium at 7.5 days post coitum (p.c.). One day later transcripts are found in embryonic ectoderm and mesoderm. In 9.5- and 10.5-day embryos Hox-2.3 expression is observed in the central nervous system (CNS) from a rostral boundary in the upper spinal cord to the caudal end. Within this anteroposterior domain, Hox-2.3 expression is also found in the peripheral nervous system, in the mesoderm and in the hindgut epithelium. The rostral boundary in the mesoderm is located at the level of the 11th somite and thus shifted posteriorwards compared to the rostral boundary in the neural tube. During subsequent development, the initially broad expression pattern in the somitic, lateral plate and intermediate mesoderm becomes restricted to structures in the urogenital system. In adults, the spinal cord and the derivatives of the Wolffian and Müllerian ducts continue to express the gene at a high level. The described temporal and tissue-specific changes in expression of Hox-2.3 are suggestive of several levels of regulation as reported for Drosophila homeotic genes and argue for more than one role of the gene during development and in adults.

Key words: mouse homeobox gene, Hox-2.3, in situ hybridization.

Introduction

The discovery of a highly conserved protein-coding sequence, the homeobox, in genes controlling development in Drosophila melanogaster (reviewed by Gehring, 1987) has led to the identification of genes containing a related sequence in higher eukaryotes including mouse and man (Levine et al., 1984; McGinnis et al. 1984). In the mouse, more than 25 genes (Hox genes), located on 4 different chromosomes, have been identified that contain a homeobox closely related to that of a homeotic gene in Drosophila, the Antennapedia (Antp) gene (reviewed in Holland and Hogan, 1988; Duboule and Dollé, 1989; Graham et al. 1989). For Drosophila, as well as for the mouse, it has been shown that the homeodomain mediates the sequence-specific binding of homeobox-containing gene products to the DNA (Fainsod et al. 1986; Desplan et al. 1988; Hoey and Levine, 1988; Odenwald et al. 1989; Treisman et al. 1989). Furthermore, Drosophila homeodomain proteins have been shown to regulate gene transcription in vitro (Biggin and Tjian, 1989) and in vivo (Han et al. 1988).

Because of the lack of developmental mutants of Hox genes, the role of these genes in vertebrate development has been addressed in a few instances by attempting to modify the distribution of the gene product in embryos: microinjection of synthetic XHox-IA mRNA into Xenopus embryos has been shown to interfere with myotome formation (Harvey and Melton, 1988); injection of anti-XlHbox1 antibodies directed against the long version of the gene product into one- or two-cell stage embryos resulted in an anterior transformation of the upper spinal cord (Wright et al. 1989); dominant gain-of-function-like mutations were obtained in transgenic mice ectopically expressing the mouse Hox-1.1 gene (Balling et al. 1989), some severely affected animals showing variation of the cervical vertebrae (Kessel et al. 1990), a phenotype reminiscent of homeotic variations in Drosophila. In addition, circumstantial arguments in favour of a role of Hox genes in mouse pattern formation has come from studies of the expression patterns in the embryo. Murine Hox genes are expressed in different but overlapping domains along the anteroposterior (AP) axis of the developing embryo in both ectoderm and mesoderm, from about 7.5 days p.c. until later stages of development. In all cases studied so far, Hox genes have a discrete anterior boundary of expression in the nervous system and, although shifted caudally, in the
mesoderm of mid-gestation embryos. Caudally, the gene expression is gradually reduced, and no clear posterior boundary is observed. Based on this region-specific expression pattern and on the homology with homeotic genes of Drosophila, it was suggested that vertebrate Hox genes provide positional information to sets of cells along the AP axis in the developing embryo (Gaunt et al. 1986; Holland and Hogan, 1988). Comparative studies on expression of murine Hox genes, located in the same or different clusters, have revealed that the more 3' the position of a gene on the chromosome, the more rostral the anterior boundary of its expression domain (Gaunt et al. 1988; Graham et al. 1989; Duboule and Dollé, 1989; Dressler and Gruss, 1989). This is also observed for the genes in the Bithorax and Antennapedia complexes in Drosophila (reviewed by Akam, 1989). Based on alignment of the clusters and on sequence comparison between genes on different clusters in the mouse and in Drosophila, subfamilies have been identified, the members of which have a higher homology to each other than to genes in other subfamilies. Therefore, it was suggested that these complexes arose by evolution from a common ancestor complex, whereby in Drosophila the BX-C and ANT-C have split, and in the mouse duplication and divergence have led to multiple clusters (Hart et al. 1987; Graham et al. 1989; Duboule and Dollé, 1989; Shugart et al. 1989; Akam, 1989).

The recent observation that the rostral expression boundaries of genes of the Hox-2 cluster in the CNS correspond to inter-rhombomere boundaries, strongly supports the view that Hox genes are involved in specifying segment-related positional identity during embryogenesis (Wilkinson et al. 1989). This demonstrates how the study of the expression patterns of Hox genes in the mouse embryo can give clues to the role of these genes during development. Comparative analysis of the expression of the individual genes in the Hox clusters is a prerequisite to understanding the regulation and function of this gene family during pattern formation. Besides, any attempt to experimentally tackle the question of the function of Hox genes by altering their expression pattern relies on the data of the normal expression patterns of these genes.

Here we present a detailed analysis of Hox-2.3 expression during embryogenesis as revealed by in situ hybridization, as well as further information about Hox-2.3 expression in adults.

Materials and methods

Animals

Embryos and postnatal and adult mice were obtained from a closed non-inbred colony of Swiss mice on a 14-10h light–dark rhythm. The postimplantation development of this strain lags up to half a day behind that described in Theiler (1972). C57Bl6 females mated to CBA males, both kept on a 12 h shifted day–night rhythm, were used to investigate some early stages of development. Gestation was assumed to have begun in the middle of the dark period.

Probes

Fragments containing Hox-2.3 cDNA sequences (Meijlink et al. 1987) were subcloned in pGEM4 or pGEMBlue vectors (Promega) and linearized with restriction endonucleases. Single-stranded, 32P-UTP labelled antisense or sense (control) RNA probes were transcribed using either T7 or SP6 RNA polymerase. One of the three antisense probes used was transcribed from a 521 basepair (bp) sequence extending from the BglII site in the homeobox to the BamHI site in the 3' untranslated region. The transcripts were partially hydrolysed to an average length of 100 nucleotides (nt) as described by Hogan et al. (1986). The second probe was 98 nt long and extended from the BamHI site in the 3' untranslated region to the polyadenylation site. The third probe was a partially hydrolysed transcript from a 296 bp subclone representing unique Hox-2.3 coding sequences just upstream of the conserved hexapeptide in the first exon (Meijlink et al. 1987).

In situ hybridization

In situ hybridization experiments were carried out on sections of paraffin-embedded embryos according to Wilkinson et al. (1987) with the following modifications. (1) Butanol was used instead of toluene before embedding in paraffin wax; (2) 6 μm sections were cut, loaded on a drop of water and dried onto slides which had been pretreated with a 2% solution of the binding silan TESPA (Sigma) in acetone for 10 s, then washed twice with acetone, once in water and dry-baked at 42°C; (3) the hybridization mixture contained 100 μm dithiotreitol (DTT) instead of 10 μM DTT; (4) after high-stringency wash and autoradiography, staining was with 0.5% toluidine blue in water.

Dissection, RNA isolation and analysis of new-born and adult male urogenital apparatus

The genital system was dissected from 1-, 3- and 6-week-old male mice, the testis being carefully separated from the epididymis, vas deferens and seminal vesicles. The uteri and oviducts of 7-week-old females were isolated separately from the ovaries. Tissues were frozen in liquid nitrogen and stored at −80°C until further use.

The isolation of RNA and analysis by Northern blot hybridization was as described by Deschamps et al. (1987b). The probe used to detect Hox-2.3 transcripts was a 32P-labelled 1.2-kb cDNA fragment, described by Meijlink et al. (1987). Washing was under stringent conditions (final wash: 0.1× SSC; 0.1% SDS at 65°C); no cross-hybridization with other homeobox gene transcripts was observed under these conditions.

Results

The expression of the Hox-2.3 gene was examined in 7.5- to 14.5-day-old mouse embryos by in situ hybridization. The first stage at which we detected Hox-2.3 expression was the late streak stage just prior to the appearance of the head fold (Thieier stage 10 to 11). As shown in Fig. 1A–B Hox-2.3 transcripts were detected exclusively in the allantois, while embryonic tissue was not labelled above background. In 8-somite embryos (Theiler stage 13), hybridization was found in neuroectoderm and presomitic mesoderm in the posterior half of the embryo as well as in the primitive streak and
Fig. 1. In situ hybridization on sections of 7.5- and 8.5-day embryos with a Hox-2.3 probe. (A,B) Bright- and dark-field photographs of a sagittal section through a 7.5-day embryo (Theiler stage 10-11). Magnification 75x; exposure was for 6 weeks. (C,D) Bright- and dark-field illumination of an 8.5-day embryo section (Theiler stage 13). Note that the embryo is in the process of turning. Magnification 30x; exposure was for 3 weeks.

Hox-2.3 expression in mouse embryos

Hindgut epithelium and mesenchyme. No expression was detected in the head, in the already formed somites, heart, foregut or amnion (Fig. 1C–D showing a partially turned embryo).

Expression in the nervous system

All known Antp-like Hox genes are expressed in the CNS, each member of a cluster with a distinct anterior boundary of expression. In 9.5- and 10.5-day embryos, the domain of Hox-2.3 expression started in the upper spinal cord and extended to the caudal end of the embryo (Figs 2A–D, 3A–H and 4A–B). By 12.5 days p.c., the anterior limit of Hox-2.3 transcript accumulation was located in the posterior myelencephalon (Fig. 5A–B; see also Graham et al. 1989) and thus seemed to be shifted rostrally compared to 2 days earlier.

From 10.5 days on, according to a process starting rostrally and progressing towards the posterior end of the embryo, dividing cells in the ventricular zone in the spinal cord will migrate laterally and generate the differentiated neuroblasts and neuroglia. The settling of neuroblasts first occurs in the ventral horns and later progresses dorsally. During this process, the ventricular zone regresses in a ventral to dorsal direction (Nornes and Das, 1974). These changes in cell population and cell type along the dorsoventral axis and the lag in time in the development along the AP axis make it difficult to relate directly Hox gene expression with neural development. Fig. 6 shows transverse sections through the neural tube between 9.5 and 14.5 days p.c. hybridized with a Hox-2.3 antisense probe. The results are representative for the thoracic and lumbar regions of the neural tube at the stages analysed. At 9.5 days Hox-2.3 expression was slightly enhanced in the dorsal region, while at 10.5 days more transcripts were located dorsally and ventrally than in between (Fig. 6A–B). In the 12.5-day embryonic spinal cord, transcript accumulation was enhanced in the dorsal-most area and in a dorsolateral strip of cells (arrowhead in Fig. 6C), while ventrolaterally no Hox-2.3 expression was found (arrow in Fig. 6C). At 14.5 days transcripts were more abundant dorsally and medially in and around the ventricular zone than in the lateralmost region (Fig. 6D). During the stages analysed Hox-2.3 expression was always detected in the ventricular zone.

Hox-2.3 expression in the peripheral nervous system (PNS) was restricted to the spinal ganglia along the entire spinal cord. Transcripts initially accumulated to high levels in the ganglia (Figs 3A–H and 6B), while at later stages the signal was decreased compared to the signal in the CNS (Figs 5C–D and 7A–B). We never observed expression in the cranial ganglia or in any sympathetic ganglia. In one instance, however, two groups of labelled cells, located laterally in the dorsal aorta in 12.5- and 14.5-day embryos (arrows in Figs 7 and 8), could possibly represent sympathetic cells on their way to the adrenals (chromaffin cells). These cells would then be of neural crest origin. Alternatively these cells might be mesodermal adrenal cortex cells.

Expression in the somitic mesoderm and derivatives

As for other Hox genes the rostral boundary of Hox-2.3 expression in the mesoderm was found more caudal than that in the CNS. In the 20-somite embryo (9.5 days p.c.) the anterior boundary in the somitic mesoderm was located around somite 11, thus at a level within the area where the forelimb arises (Fig. 2A–D and not shown) and expression extended to the caudal end of the embryo. The expression level in the somites was at this stage higher than that in the neural tube. At 10.5 days of gestation Hox-2.3 could still be detected in the somitic mesoderm (Fig. 3A–H). Around this period, cells in the ventral part of the somites start to migrate medially to form the prevertebrae (p.v.) and interverte-
Fig. 2. In situ hybridization on sections of 9.5-day embryos with a Hox-2.3 probe. (A–D) Bright- and dark-field photographs of parasagittal sections through the same embryo (Theiler stage 15; 22 somites). Magnification 65×; exposure time was 3 weeks. (A,B) the arrowhead points at the most anterior expressing somite. (C,D) the arrow points at the anterior boundary of expression in the spinal cord. (E–H) Bright- and dark-field photographs of frontal sections through a 9.5-day embryo (Theiler stage 14–15; 20 somites). (G) is a section located ventrally from (E). Magnification 85×; exposure time was 3 weeks. a, anterior; p, posterior; c, coelom; da, dorsal aorta; fb, forebrain; fg, foregut; fl, forelimb; nm, neuromeres; ma, mandibular arch; sc, spinal cord; s, somites.

Expression in lateral plate mesoderm

At 9.5 and 10.5 days of gestation Hox-2.3 expression was also detected in the unsegmented or lateral plate mesoderm (LPM) within the AP domain of expression defined in the somitic mesoderm (Figs 2A–D and 4A–B). In addition, groups of labelled cells located between the dorsal aorta and the foregut at the level of the forelimb bud were found one or two somites more anteriorly than the most rostrally expressing somite (Figs 3A–F and 4A–B). These cells possibly are part of the mesentery of the alimentary system (stomach and/or gut). Cells from the LPM contribute to several structures in the embryo among which are the trachea, lung, gut, stomach and limbs. Hox-2.3 transcripts were only detected in the more posterior organs (gut and stomach) and in the limbs. Expression in the stomach primordium was detected at 10.5 days of gestation (not shown). In the 12.5- and 14.5-day embryos, Hox-2.3 RNA is restricted to the dorsal mesogastrium and to the dorsolateral part of the mesodermal component of the stomach wall (Figs 7 and 8). In the gut, the expression pattern is more complex. At 10.5 days p.c., Hox-2.3 transcripts were found in the mesoderm surrounding...
the midgut and in the mesoderm and epithelium (presumably endoderm) of the hindgut. The tailgut mesenchyme and epithelium, both of mesodermal origin, were also labelled (Fig. 3C–H). At 12.5 and 14.5 days p.c., Hox-2.3 expression was decreased but could still be detected in some parts of the gut (not shown). Expression of the Hox-2.3 gene in the forelimb bud mesenchyme was detected in 9.5-day embryos, thus in the early phase of limb formation (see Smith et al. 1989 for discussion). At this stage, the forelimb was not homogeneously labelled, the maximal concentration of transcripts being located in the posterior half of the buds (Fig. 2E–F). In 10.5-day embryos, Hox-2.3 expression was more prominent in the posterior and dorsal part of the forelimb bud. Examination of serial transverse sections revealed that in the anterior part of the forelimb bud Hox-2.3 expression was restricted to dorsally and distally located mesenchymal cells, while in more posterior sections the boundary of expression was located progressively more ventrally (Fig. 3A–F). No expression was found in the ectoderm of the limb buds (not shown). At 12.5 and 14.5 days of gestation Hox-2.3 was no longer detected in the limbs.

**Expression in the intermediate mesoderm**

The intermediate mesoderm, which gives rise to the nephrogenic column is located between the segmented (somitic) and unsegmented (lateral plate) mesoderm and has a segmented character. A detailed description of Hox-2.3 expression in the urogenital system has been reported elsewhere (Kress et al. 1990). Briefly, Hox-2.3 expression is first detected in the mesonephric tissue at 9.5 days p.c. By day 10.5, the mesonephric ducts and tubuli show high levels of gene expression (Figs 3A–H and 4A–B). In 12.5- and 14.5-day embryos, high levels of expression were found in the Wolffian ducts, ureters and collecting tubuli, as well as in the independently arisen Müllerian ducts (Figs 5C–D, 8A–B and Kress et al. 1990). Lower levels of Hox-2.3 mRNA seemed to be present at some stages in metanephric induced tubuli as well (see Kress et al. 1990). Future experiments will tell us whether this signal is preferentially associated with an intermediate stage during development of the glomeruli. No expression was found in embryonic testis or ovary at the stages analysed. In adult gonads only basal levels of Hox-2.3 transcripts were detected, while a strong signal was observed in the structures derived...
Discussion

This paper presents a detailed analysis of the expression pattern of the murine homeobox-containing gene Hox-2.3. The earliest time that Antp-type Hox genes have been detected by in situ hybridization is at about 7.5 days p.c. (Gaunt, 1987, 1988; Holland and Hogan, 1988; Mahon et al. 1988; Dressler and Gruss, 1989; Galliot et al. 1989). As discussed by Gaunt et al. (1987) and Dressler and Gruss (1989), genes 3' in the cluster are expressed slightly earlier and more anteriorly than 5'-located genes like Hox-2.3. Expression of Hox-2.3 was first detected in the allantois of late streak stage embryos, just prior to the neural plate stage. Thus, the first cells to express the gene were in extraembryonic mesoderm. The level of expression at this stage is very low and could only be detected after long exposure of the hybridized sections. In the 8-somite embryo, the expression of Hox-2.3 is found in both ectoderm and mesoderm and is restricted to the posterior parts of the embryo. Already at this time, the expression in the ectoderm extends more rostrally than it does in the mesoderm. The anteroposterior domain of expression in the CNS of 9.5-day embryos starts rostrally in the upper spinal cord and extends caudally, while at 12.5 days p.c. the anterior boundary is located in the posterior myelencephalon. A definitive comparison between the rostral boundaries in the CNS at different stages of development is very difficult due to the absence of landmarks present in this region throughout the period considered. Therefore, although we would be tempted to speak of an anterior shift of the Hox-2.3 rostral expression boundary in the CNS between 9.5- and 12.5-day p.c., we do not think we can establish the existence of such a shift unambiguously.

Do Hox genes have multiple roles during development?

Hox-2.3 expression is very high both in embryonic and adult spinal cord. While early expression is in agreement with an involvement of the gene in specification of the positional identity of the cells along the AP axis, late expression either might be required to maintain this state or might correspond to a specific property of the maturing neural tissue. In fact, the changes in the dorsoventral pattern of Hox-2.3 expression in the neural tube between day 10.5 and day 14.5 are
Fig. 6. Transverse sections through 9.5- to 14.5-day embryos hybridized to a *Hox-2.3* specific probe. Bright-field photographs of sections through the thoracic spinal cord of 9.5-day (A) and 10.5-day (B) embryos. (C–F) Bright-field photographs of sections through 12.5- and 14.5-day embryos. (C) Higher magnification of boxed region in (E). (D) Higher magnification of boxed region in (F). The dorso-ventral distribution of *Hox-2.3* transcripts was found to be representative for the thoracic and lumbar spinal cord. Magnification 190× (A–D) and 50× (E–F); exposure time was 3 weeks. da, dorsal aorta; s, somite; sc, spinal cord; sg, spinal ganglia. For explanation of the arrow(head) see text.
suggestive of functions more related to differentiation than to positional signalling. Recent results concerning other Hox genes are in agreement with such a hypothesis. A detailed analysis of the Hox-2.5 expression in the neural tube (Bogarad et al. 1989) revealed that differentiating motorneurons ceased to express the gene. A recent study of Hox-3.1 expression (Awgulewitsch and Jacobs, 1990), using an antibody recognizing the gene product, also suggests a link between Hox expression and differentiation of certain subsets of neurons. Unlike Hox-2.3, Hox-3.1 is preferentially expressed in the developing ventral horns of the spinal cord. While stained motorneurons were identified in late gestation embryos, label was not exclusive to any functionally defined dorsoventral subregion known in the mouse spinal cord (discussed in Awgulewitch and Jacobs, 1990). Like antibody staining experiments, the selective labelling of subsets of cells by using lacZ as a reporter gene coupled to Hox gene regulatory sequences can be instrumental in the characterization of cells expressing the genes. In this way, Zakany et al. (1989) showed that lacZ, expressed under the control of a proximal promoter region of Hox-1.3, labels the brachial relay neurons, a subset of Hox-1.3-positive cells in the CNS. The dorsoventral changes in Hox-2.3 expression in the CNS that we observed between 9.5 and 14.5 days p.c. suggest some correlation as well between gene expression and differentiation of specific neuronal and/or glial subpopulations of cells. Taken together, it is possible that mammalian Hox genes are involved in positional signalling during early development, and are specifically expressed in certain differentiating or differentiated cells later on during embryogenesis and adulthood. Dynamic changes in homeotic gene expression

have been observed in Drosophila as well, reflecting several levels of regulation (Irish et al. 1989; reviewed by Beachy, 1990). The existence of two phases of regulation of segmentation genes, another class of genes controlling embryogenesis in Drosophila has also been documented: in the case of fushi tarazu (ftz) and even-skipped (eve), it appears that the genes control regional identity early at the blastoderm stage, and specify the identity of individual neurons during a later and independent phase of expression (Doe et al. 1988a and 1988b).

Hox-2.3 expression dramatically decreases in somitic and lateral-plate-mesoderm-derived structures after 10.5 days

Hox-2.3 expressing cells in the mesoderm are located within the AP domain defined by expression in the neural tube, the rostral expression boundary being located caudal to that in the CNS. In 9.5- and 10.5-day embryos, high levels of expression were detected in the 11th and all more posterior somites. At 12.5 days p.c.
lower levels of expression were detected in one of the somite derivatives exclusively, the sclerotomes; transcripts were seen in all prevertebrae and intervertebral disks posterior to and including the 7th. At 14.5 days p.c., the expression in the prevertebral column further decreases and is only detectable around the chondrification centra and at the junctions with the ribs. Thus, the initially high expression of Hox-2.3 in the somites seems to be turned off in selective subsets of cells during somite disaggregation. A decrease in Hox-2.3 expression is also observed in cells of the lateral plate mesoderm derivatives (that expressed the gene at a high level in 9.5-day embryos), as hybridization in stomach mesenchyme and gut was much weaker at 12.5 days p.c. compared to earlier stages. For these tissues as well, Hox-2.3 early expression might correspond to an early function related to the establishment of cell identity, and the decrease in expression would accompany cell differentiation and tissue morphogenesis. The observed temporal changes in the pattern of gene expression within the AP-defined domain could result from selective turning off of the gene in specific tissues. The possibility of such a biphasic regulation of a Hox-gene expression has been discussed by Püschel et al. (1990) in their report of the characterization of a regulatory region faithfully mediating Hox-1.1 expression in an early embryonic phase only.

Similarly to the situation observed in somitic and lateral-plate-derived structures discussed above, Hox-2.3 is expressed exclusively in the early phase of limb formation and no longer in the later phase when mesenchyme differentiates into the different limb tissues. Hox-2.3 expression in the 10.5-day developing forelimbs varied along the anteroposterior as well as the dorsoventral axis, the maximum accumulation of transcripts being located posterodorsally. This pattern is reminiscent of that described by Dollé et al. (1989) for genes in the Hox-5 cluster. According to the location of the gene in the aligned clusters, Hox-2.3 would fit into the Hox-5 series between Hox-5.1 and Hox-5.2; the Hox-2.3 transcript distribution along the three axes in the limb would be in agreement with the possibility that the features described for the Hox-5 complex might apply to the Hox-2 cluster as well, although verification of this hypothesis would require simultaneous analysis of the different members of the Hox-2 cluster. A difference between Hox-2.3 and the Hox-5 genes is that Hox-2.3 fades out in the limbs earlier than Hox-5.1 and Hox-5.2.

Expression of Hox-2.3 homologs in the developing limbs has been reported in human (Simeone et al. 1987) and in chicken embryos (Sundin, Pang and Eichele, unpublished data cited in Smith et al. 1989). As discussed by Dollé et al. (1989), it is tempting to speculate that the role of Hox genes during limb growth might be homologous to their putative role during establishment of cellular positional identity along the embryonic body axis. It is striking that non-ANP-like homeobox genes like Hox-7 (Hill et al. 1989; Robert et al. 1989) and S8 (Kongsuwan et al. 1988; Meijlink and Opstelten, personal communication) are expressed according to a pattern without any correlation to the AP axis both in the trunk and in the limbs.

Hox-2.3 expression in certain intermediate mesoderm derivatives may be tissue-specific

Hox-2.3 expression in the Wolfian- and Müllerian-duct-derived tissues remains high during development and in adults, in contrast with the expression pattern in somitic and lateral-plate-mesoderm derivatives. Only basal levels of transcripts were found in testis and ovaries at all stages examined. At 9.5 and 10.5 days p.c., high levels of Hox-2.3 transcripts were found in the mesonephric ducts and mesonephric tubules, and at later stages in the mesonephric duct derivatives and metanephric kidney. Fig. 3A–D shows that the most rostral expressing cells in the mesonephric duct are located anteriorly to the Hox-2.3 expression domain in the somitic mesoderm. From this observation, we can conclude that in 10.5-day and older embryos the expression domains of the gene in somitic and intermediate mesoderm do not coincide, the rostral boundary of the latter being more anterior than that of the former. One possibility is that the gene is regulated independently and performs different functions during tissue morphogenesis in these structures. In the paper by Kress et al. (1990), we discussed the possibility that Hox-2.3 expression in the mesonephric-duct-derived epithelium might be associated with a tissue-specific property rather than with AP positional signalling, as Hox-2.3/lacZ transgenic mice revealed that gene expression in these tissues is regulated at least in part independently from expression in the nervous system and other mesodermal structures.

Hox-2.3 expression in the mesonephric-duct-derived epithelium is not an exclusive property of this epithelium as Hox-2.3 transcripts accumulate at high levels not only in the structures that develop from it (ureter, metanephric collecting tubules, vas deferens and seminal vesicle) but also in structures developing from the independently arisen Müllerian duct (oviduct and uterus); nor is it a property shared by all mesodermal epithelia, as Hox-2.3 is not expressed in the testis cord epithelium derived from the lateral plate mesoderm. As Hox-2.3 expression in the epithelial cells of the urogenital system remains very high until the adult stage, it is tempting to speculate that, as discussed above, the Hox-2.3 protein has a function in these cells that is independent from the early events of positional signalling and which remains to be identified. It has been suggested previously for other Hox genes that maintainance of expression until late stages of development might be cell- or tissue-specific rather than region-specific (reviewed and discussed in Holland and Hogan, 1988). Interestingly, the expression profile of Hox-2.4 in the new-born and adult urogenital system coincides with that of Hox-2.3 (our unpublished data), suggesting that these two loci, which lie very close to each other in the cluster, are at least partially regulated in concert, and that they may perform similar functions in these structures.
The subfamily members Hox-2.3 and Hox-1.1 have clearly different expression patterns

Hox genes on different chromosomes can be divided into subfamilies based on homology in and around the homeodomain, and alignment of the clusters (Graham et al. 1989; Duboule and Dollé, 1989). Genes within a subfamily display similarities in their expression pattern as described by Gaunt et al. (1989) for the Hox-1.4 subfamily. In this regard, it is interesting to compare Hox-2.3 with its homologue on the Hox-1 cluster, Hox-1.1. While only subtle differences between the anterior boundaries of expression of the members of the Hox-1.4 subfamily were observed (Gaunt et al. 1989), Hox-2.3 expression is detected about 3 ‘metameres’ more anterior than Hox-1.1 (Mahon et al. 1988; Dressler and Gruss, 1989) in the spinal cord as well as in the mesoderm. The expression domain of Hox-2.3 thus extends anteriorly to that of Hox-1.1. Larger differences in anterior limits of expression of subfamily members seem to be a common feature for genes 5' in the cluster. Similarly to Hox-2.3 and Hox-1.1, the pairs Hox-2.4/Hox-3.1 (Graham et al. 1989; Gaunt et al. 1988) and Hox-2.5/Hox-3.2 (Graham et al. 1989; Dollé and Duboule, 1989) show relatively large differences in their anterior limits of expression in the spinal cord (discussed by Gaunt et al. 1989). An additional difference between Hox-1.1 and Hox-2.3 expression patterns concerns the level of gene expression in the somitic mesoderm relative to that in the CNS in the mid-gestation embryo: Hox-1.1 gene transcripts accumulate to much higher amounts in somitic derivatives than in vertebrates also a single gene expressed in developing limbs and gonads. EMBO J. 8, 1507–1520.


We would like to thank Drs R. Krumlauf and A. Graham for introducing us to the in situ hybridization technique, Drs K. Lawson, O. Destré and F. Meijlink for critically reading the manuscript and F. Vervoordeldonk for artwork.

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(Accepted 18 September 1990)