Pattern of transcription of the homeo gene *Hox-3.1* in the mouse embryo

Hervé Le Mouellie, Hubert Condamine, and Philippe Brûlet

Unité de Génétique Cellulaire du Collège de France et de l'Institut Pasteur, 75724 Paris Cedex 15, France

A cDNA from the *Hox-3.1* locus, isolated from a 10.5-day postcoitum (p.c.) mouse embryo cDNA library, and the putative encoded protein are described. The spatial distribution of *Hox-3.1* gene transcripts from late gastrulation to embryonic day 14.5 p.c. was monitored by in situ hybridization, using a cDNA probe. When first detectable in 8.5-day p.c. embryos, the transcripts are distributed in all the tissues of the posterior end. At later stages, the distribution becomes progressively spatially restricted and tissue specific. By 12.5 days p.c., transcription is localized most intensely in the neural tube region lying above the heart. The early transcription pattern thus appears to be compatible with a regionalizing role for the *Hox-3.1* gene.

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Years of classical genetics and mutagenesis studies have led to the characterization of numerous genes involved in the development of *Drosophila melanogaster* (Martinez-Arias and Ingham 1986; Gehring 1987; Gergen 1987), many of which have recently become amenable to molecular analysis. A 183-bp protein-coding sequence, known as the homeo box, has been found in several homeotic and segmentation genes (Laughon and Scott 1984; McGinnis et al. 1984) and also in a maternally expressed gene (Mlodzik et al. 1985; MacDonald and Struhl 1986). The 61-amino-acid homeo domain, which shows homologies with regulatory and DNA-binding proteins (Laughon and Scott 1984; Shepherd et al. 1984), has been found with a high degree of conservation in many widely separate phylogenetic groups (McGinnis et al. 1984; Holland and Hogan 1986). This has led to speculation that the conservation of structure reflects analogous functions for these genes in embryogenesis of different species. If this is true, it would provide an opportunity to identify molecules involved in the development of those species in which access to developmentally meaningful genes is difficult.

Recent observations made on vertebrates support this hypothesis, as they have revealed further similarities with *Drosophila*. Homeo boxes have been found to be arranged in clusters in the genomes of man and mouse (Colberg-Poley et al. 1985b; Hart et al. 1985; Hauser et al. 1985; Duboule et al. 1986), a mouse homeo domain has been shown to bind DNA (Fainsod et al. 1986), and a *Xenopus* homeo protein is localized in the nucleus (Harvey et al. 1986). In addition, the transcription of homeo genes in man, mouse, and *Xenopus* embryos, as revealed by Northern blots, RNase protection assays, and in situ hybridization, appears to be spatially and temporally restricted in all cases studied so far (Gaunt et

al. 1986; Carrasco and Malacinski 1987; Joyner and Martin 1987; Krumlauf et al. 1987; Utset et al. 1987; reviewed by Snow 1986; Schofield 1987).

This paper describes the cloning and sequencing of a cDNA from a previously identified mouse homeo gene, *Hox-3.1* (Awgulewitsch et al. 1986; Breier et al. 1986). We also provide a description of *Hox-3.1* transcription patterns between days 8.5 and 14.5 post coitum (p.c.) of mouse embryogenesis. We show that although *Hox-3.1* transcripts eventually become specifically located in neural tissue, they are initially found in all tissues of the posterior region in 8.5- and 9.5-day p.c. embryos. We suggest that *Hox-3.1* might be involved in embryo regionalization rather than in the determination of a particular cell lineage.

Results

Hox-3.1 genomic and cDNA cloning

Using a *Drosophila* homeo box probe from the *Antennapedia* (*Antp*) gene, we screened a mouse genomic DNA library under reduced hybridization stringency (see Materials and methods). We obtained 20 genomic DNA recombinant molecules. In one of them (Sou 6, Fig. 1), the homeo box was localized and identified as the *Hox-3.1* homeo box by its nucleotide sequence and the restriction map of the genomic molecule (Awgulewitsch et al. 1986; Breier et al. 1986). An assay based on protection from RNase A digestion, using a mouse genomic 300-base RNA probe containing the homeo box, showed that transcription of the *Hox-3.1* locus is detectable in 10.5-day p.c. whole embryos and visceral yolk sac but not in brain extracts of the same age (data not shown). A cDNA library was constructed in λ gt10 using poly(A)⁺ RNAs extracted from 10.5-day p.c. embryos. Screening with

Figure 1. Partial restriction map of the mouse *Hox-3.1* locus, including two genomic clones (Sou 6 and DB 9), the three sequenced cDNAs (c26, c1, and c21) and the open reading frame (ORF) with the homeo box in black. The dotted lines position the 5' and 3' ends of the cDNAs and the common intron on the genomic map (E) *EcoRI*; (H) *HindIII*; (P) *PstI*; (S) *SstI*; (SI) *SalI*.

the same genomic probe yielded several cDNAs molecules. Three of them (Fig. 1) were analyzed and sequenced further.

Structure of the cDNA and the encoded protein

The nucleotide sequence of the largest cDNA (clone c21, 1470 bp long) is shown in Figure 2 with some flanking genomic sequences, together with the conceptual protein encoded by the longest open reading frame (ORF), which includes the homeo box. The two smaller cDNA clones have a nucleotide sequence identical to that of c21. They were found to be primed at an internal poly(A) stretch (973–979). Comparison with genomic sequences showed the same 1.35-kb intron, located 2 codons upstream from the homeo box, in all three cDNAs. The poly(A) tail of c21 is added to a G residue instead of the more usual C or T. There is no classical upstream polyadenylation signal (Birnstiel et al. 1985); the first such signal is found 54 bp downstream from the 3' end of the c21 cDNA. Thus, it is conceivable that synthesis of c21 was initiated at an internal A-rich region able to pair with an oligo(dT) primer, rather than at the proper terminus of *Hox-3.1* mRNA.

The largest ORF begins with an initiation codon perfectly matching the vertebrate consensus sequence CANCAUG (Cavener 1987). It is preceded by several stop codons in the three possible reading frames and codes for a putative protein of 242 amino acids (27,710 daltons). The *Hox-3.1* protein contains the homeo domain close to its carboxyl terminus like other vertebrate homeo proteins characterized thus far, which also have similar sizes (see Fig. 3A and references in the legend). The deduced protein contains several regions homologous to other homeo proteins. The 61-amino-acid homeo domain has already been reported (Awgulewitsch et al. 1986; Breier et al. 1986). It clearly belongs to the Antennapedia class (74% and 82% homology with *Antp* homeo box at the DNA and protein sequence levels, respectively). Two short sequences in the region amino-terminal to the homeo box display a substantial degree of conservation among several homeo proteins: The first 8 amino acids of the amino terminus are well conserved

(Fig. 3B), and another 6-amino-acid region just upstream from the homeo box, although coded by a different exon, is strongly conserved in the putative *Hox-3.1* protein (Fig. 3C) when compared with the consensus sequence (Mavilio et al. 1986; Krumlauf et al. 1987). The amino-terminal end of the postulated protein does not show any further significant homology with other homeo proteins, except in its overall composition, which is mostly hydrophilic with high percentages of serine (16%) and proline (8%). The most noticeable feature of the carboxy-terminal sequence is a stretch that is very rich in glutamate residues (15 out of 21 amino acids). An acidic region of this type has already been reported for the *Hox-1.1* locus, as judged from the genomic sequence downstream from the homeo box, where 15 glutamate codons were found just before a stop codon (Colberg-Poley et al. 1985a).

Transcription of the *Hox-3.1* gene during mouse embryogenesis

The cPS probe A 636-bp cDNA fragment, referred to as cPS, was selected as a probe for transcription studies. It extends 5' from the *SalI* site to a *PstI* site and, therefore, does not contain the conserved homeo box sequence (Fig. 4C). Under stringent hybridization conditions (see Materials and methods), a single band was detected in Southern analysis of genomic DNAs (Fig. 4A). At lower reduced stringency, a partially homologous locus was detected using the cPS probe or using probes located 3' to the homeo box or inside the intron (data not shown). Northern blots of total RNAs extracted from embryos aged from 9.5 to 12.5 days p.c. reveal a broad band of transcript(s) with an apparent 3.2-kb size (Fig. 4B). An apparent 5-kb transcript is, in fact, due to probe trapping by the 28S rRNA. An RNA species with the same mobility was also detected at the same stages using an antisense RNA probe generated from the same *PstI*–*SalI* cDNA fragment (data not shown), using a combination of stringent hybridization and washing conditions, and digestion of blots by RNases A + T₁, according to Melton et al. (1984). No signal was detected using the sense probe either by Northern analysis or by RNase protection assay (data not shown).

In situ hybridization To gain an insight into the spatiotemporal pattern of *Hox-3.1* gene expression during mouse embryogenesis, we performed in situ hybridization experiments on cryostat sections of 7.5- to 14.5-day p.c. embryos. Both antisense and sense single-strand RNA cPS probes were used under stringent conditions of hybridization and washing (see Materials and methods). As no labeling was ever obtained using the sense cPS probe, which has the same polarity as the mRNA, the results described below all refer to the antisense probe.

Hybridization on parasagittal sections from 11.5- and 14.5-day p.c. embryos revealed an intense signal restricted to the posterior cervical region of the neural tube, directly above the heart in the 14.5-day embryo

Figure 2. The nucleotide sequence of the c21 cDNA clone, flanking genomic regions, and the predicted amino acid sequence of the protein. The cDNA sequence is in uppercase letters, and the flanking genomic sequences is in lowercase letters. Numbering starts at the first 5' nucleotide of the cDNA. (Δ)The position of the intron, whose splicing sites (ACG ↑ GTGAGA TTCTGTTCAG ↑ CT) fit well with the known consensus sequence (Cech 1983). The sequence of the putative Hox-3.1 protein is shown above the ORF. Two conserved peptide regions are underlined (see text and Fig. 3B,C). The homeo domain is printed in boldface type. Note the two long (CT) repeats in the 5'-genomic sequence, a potential stable secondary structure between nucleotides 59 and 241 (− 141 kcal/mole), and a [CA]₁₀ repeat in the 3' UT cDNA sequence (1290–1328).

Similar transverse sections made of 10.5-day embryos cross the neural tube twice owing to the curvature of the embryo (Fig. 7III). Figure 7, I and II, shows two transverse sections, each with two neural tube regions. In both of them, the labeling is present only on the more anterior section of the neural tube, where it is not distributed

Figure 5. Localization of *Hox-3.1* transcripts in 11.5- and 14.5-day p.c. embryos. The anterior end of the embryo is on the right. (a1,b1) Phase-contrast; (a2,b2) dark-field illumination. (a1) Parasagittal section of a 14.5-day p.c. embryo shown at the level of the heart. (b1) On this parasagittal section of a 11.5-day p.c. embryo, the neural tube, dorsal aorta, heart, and brain are shown from left to right. The arrows in a1 and b1 point to intensely labeled regions of the neural tube shown in a2 and b2, respectively. The blood cells in the dorsal aorta give an artifactual luminescence in the right part of b2, which does not represent true labeling. Magnification in a1, 340 $\times$; a2, 1150 $\times$; b1, 140 $\times$; b2, 400 $\times$.

Cross sections of 8.5-day p.c. embryos yielded similar results. The signal appeared to be located in the posterior region and distributed there on all tissues, that is neural tube and hind gut as well as mesenchyme surrounding coelomic cavities, caudal veins, and aorta. However, there seemed to be less difference of labeling intensity between the various tissues (Fig. 8d2) than with 9.5-day p.c. embryonic tissues.

When sections made through a 7.5-day p.c., late primitive streak stage embryo were examined, no labeling was detectable on any embryonic or extra embryonic tissue under the conditions used (with the possible exception of the allantois, which seemed to show some labeling) (data not shown).

Discussion

The homeo gene under study here is identical to the *Hox-3.1* gene described previously (Awgulewitsch et al. 1986; Breier et al. 1986), both by the nucleotide sequence of its homeo box and the restriction map of the genomic locus. We present the structure of a putative protein coded for by this gene and provide a description of *Hox-3.1* transcript distribution at the time of early organogenesis.

The transcripts revealed on Northern blot or by in situ hybridization under high stringency conditions, including treatments with RNases A + T₁, should be specific for the *Hox-3.1* locus. This is because the cPS probe reveals only the *Hox-3.1* locus upon stringent Southern blot hybridization. However, it cannot be excluded that the mRNA species detected in 9.5- to 12.5-day p.c. embryos is heterogeneous. We are currently investigating the *Hox-3.1* expression at the RNA and protein levels. In any case, the general distribution of stop codons, partic-

ularly those upstream to the initiation codon, implies that the translation product described here, from the sequence of three different cDNAs, could be the main, if not the only, *Hox 3.1* protein synthesized during this period of embryogenesis. This putative translation product shares extensive structural homologies, not only with other vertebrate homeo proteins but also with three truly homeotic *Drosophila* gene products (*Antp*, *Ubx*, *Dfd*). This suggests that these homeo proteins might be involved in molecular interactions that are more highly conserved than was thought when considering the homeo box alone.

The transcription pattern of the *Hox-3.1* gene, as determined by in situ hybridization with 7.5- to 14.5-day p.c. embryos undergoes gradual modifications during the course of embryogenesis. When expression is first detected, in the 8.5-day p.c. embryo, it occurs in all tissues of the posterior region, from whichever embryonic layer they are derived. A similar pattern is still observable 1 day later, although different intensities of transcript accumulation are noticeable in different tissues, the neuroectoderm of the distal neural tube being a prominent site of *Hox-3.1* expression.

Strikingly, day 10.5 p.c. of embryogenesis appears to be a transition step in *Hox-3.1* transcription. The posterior transcription region becomes more weakly labeled. In contrast, an intense signal is detected in a more anterior region, at the level of the forelimb bud (i.e., at the level of somites 8–12, Hogan et al. 1986), essentially in the ventral horns of the neural tube. The dorsal region of slightly posterior somites is also strongly labeled. In the absence of any precise fate map of the mouse embryo at that stage, it cannot be determined whether cells that are positive at day 10.5 p.c. are the direct descendants of labeled 9.5-day p.c. cells.

The pattern of transcription of *Hox-3.1* detected previously in 13.5-day p.c. embryos and in newborn mice is characterized by intense expression in a highly localized

area of the neural tube posterior to the third cervical vertebra (Awgulewitsch et al. 1986; Utset et al. 1987). Our results indicate that this pattern is established at

Figure 6. Localization of *Hox-3.1* transcripts in 12.5-day p.c. embryos. (I) Transverse sections in phase-contrast illumination. The dorsal side, recognizable by the spinal cord, is on the left. Magnification, $130\times$. (II) Dark-field illumination of the spinal cord seen in I at a higher magnification ($430\times$). (III) Schematized sagittal section of a 12.5-day p.c. embryo (redrawn from Rugh 1968), showing the level of sections shown in I and II; the two arrows bracket the region of most intense labeling in the spinal cord. (a1) Section through the spinal cord and lower head regions. (b1) Spinal cord and cervical region. (c1) Spinal cord and heart. (d1) Spinal cord, lung bud, and liver. (a2) The labeling is barely above background. (b2, c2) Intense labeling in the mantle region of the spinal cord. (d2) Positive signal with lower intensity.

Figure 7. Localization of *Hox-3.1* transcripts in 10.5-day p.c. embryo. Transverse sections shown in *I* and *II* are identified as *a* and *b*, respectively, on the diagram of an embryo in *III*. The anterior side is on the left. The two regions of labeling (see text) are also represented in *III* by four arrows. (*a1*, *b1*) Phase-contrast; (*a2*, *a3*, *b2*, *b3*) dark-field illumination. (*a2*) Labeling in the anterior part of the neural tube, especially intense on the ventral horns, and also on the somitic mesoderm shown in *a3* at a higher magnification. The coiled labeled ribbon in (*a1*, *a2*) is displaced yolk sac. (*b1*) A forelimb bud in the lowerleft corner and a hindlimb bud in the upper right corner. (*b2*) Labeling in the neural tube at the level of the forelimb bud. (*b3*) Anterior part of the neural tube seen in *b2*; the signal is the most intense on the ventral horns. Note the artifactual luminescence of the dorsal aorta blood cells at the center of *a2* and the two sides of *b2*. Magnification in *a1*, *a2* and *b1*, *b2*, 130 $\times$; *a3*, *b3*, 450 $\times$.

about day 11.5 p.c. and thus represents the outcome of a complex, gradually evolving distribution. The reorganization of the transcription pattern along the anteroposterior axis thus disclosed is accompanied by changes along the dorsoventral axis of the neural tube. Although the labeling is essentially confined to its ventral region in 10.5-day p.c. embryo, a more diffused pattern is seen on transverse sections of 12.5-day p.c. neural tube.

These results emphasize the importance of a chronological study for the understanding of homeo gene transcription patterns, as has already been pointed out by Krumlauf et al. (1987). These investigators showed that *Hox-2.1* transcription occurs in tissues of different embryological lineage in the 12.5-day p.c. mouse embryo.

In particular, a region of the neural tube posterior to the brain and mesenchymal cells around lung tubules are highly positive. It is quite conceivable that the transcription studies at earlier stages will reveal a very different pattern, as suggested by these workers.

Similarly, it will be interesting to have further information on transcript distribution of *Hox-1.5*, another mouse homeo gene. Gaunt et al. (1986) have shown that its expression appears to begin in different layers (ectoderm and mesoderm) of the posterior region of the embryo at a slightly earlier stage (7.5 day p.c.) than *Hox-3.1*. At 9.5 days p.c., expression of *Hox-1.5* is essentially concentrated in a region of the neural tube posterior to the hindbrain and gradually declines toward the caudal re-

gion. Trunk mesodermal derivatives are weakly positive at that stage. Although there is no information presently available regarding a possible evolution of this pattern, our results suggest that profound modifications could occur during this period and later.

Some features of the *Hox-3.1* transcription pattern are also shared with homeo genes in other organisms. For instance, *Ultrabithorax* transcripts in the *Drosophila* embryo (Akam and Martinez-Arias 1985) are first seen in all the cells of several metameric units but later show

Figure 8. (See facing page for legend.)

differential rates of accumulation in ectodermal and mesodermal tissues. Perhaps more relevant is the comparison with the distribution of transcripts of the *Xeb 1* gene in the *Xenopus* embryo (Carrasco and Malacinski 1987). It is striking that there is at first a cotranscription in neurectoderm and lateral mesoderm tissues, limited to the caudal region of the neurula. Later, the labeling in the caudal neural tube decreases in the tailbud embryo and then in the swimming tadpole, whereas inversely, the *Xeb 1* transcripts accumulate in the more anterior part of the neural tube, particularly in its ventral region, just posterior to the hindbrain.

The transcription pattern of the *Hox-3.1* gene calls for a few preliminary remarks concerning its possible role during embryogenesis. Recent cell lineage studies in the zebra fish embryo (Kimmel and Warga 1986, 1987) have provided new information on cell determination in vertebrates. The progeny of cells marked at the blastula stage were shown to populate any germ layer, unlike those of cells marked at gastrulation, which are tissue restricted. However, the progeny of a gastrula cell can be found at any anteroposterior level of a particular germ layer. Thus, it seems that cells are determined for a layer specificity at gastrulation, but only later do they undergo regional restriction. The second of these two successive commitments might correspond to the 'nonequivalent' state postulated by Lewis and Wolpert (1976), which distinguishes cells expressing similar histotypic differentiation programs in different sections of the embryo. Little is known about cell commitment in the mouse embryo, and much of the reasoning is based on the model of the chicken (Hogan et al. 1985; Keynes and Stern 1985). In the chicken, it appears that the somites of the presomitic mesoderm already carry the information required for the future somite organization, including their anteroposterior polarity (Keynes and Stern 1985). Moreover, the positional address is acquired, at the latest, at somite compaction. The first cervical somite is condensed at day 7.75 p.c., and by day 10.5 p.c., compaction of somites is completed along the whole trunk (Hogan et al. 1986, p. 17). Thus, the transcription pattern of a mouse homeo gene such as *Hox-3.1*, both in time and space, is compatible with its being involved in the regulation network responsible for this positioning phenomenon.

Materials and methods

Embryos

F₁ (C57BL/6J × DBA/2J) females were mated with the same F₁ males and inspected daily for copulation. The day a vaginal plug was found was taken as day 0 of embryogenesis. F₂ embryos were dissected at various times of pregnancy and used for RNA extraction or cryostat sections.

RNA purification

Embryos from appropriate stages were pooled and Dounce homogenized in 5 M guanidine monothiocyanate, 8% β-mercaptoethanol, and 25 mM sodium acetate (pH 5.0) in 7 ml/g of wet tissue (Cathala et al. 1983). RNA was selectively precipitated by 3 M LiCl and further purified by centrifugation through a cesium chloride step gradient (Maniatis et al. 1982).

Construction and screening of genomic and cDNA libraries

An SWR genomic library in λ47.1 (9×10^5 plaques) was screened with a *Drosophila Antennapedia* homeo-box-containing fragment (*Bam*HI–*Kpn*I in plasmid p903G, a gift from W. Gehring). Reduced stringency hybridization was carried out at 37°C for 24 hr in 43% formamide, 5× SSC, 0.1% bovine serum albumin (BSA), 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 50 mM sodium phosphate (pH 7.0), 0.1% SDS, and 250 μg/ml denatured sonicated salmon sperm DNA. Subsequent washes were in 2× SSC, 0.1% SDS, twice at room temperature and twice at 50°C for 20 min.

The cDNA library was constructed according to Maniatis et al. (1982). The first strand was synthesized using Moloney murine leukemia virus reverse transcriptase (GIBCO BRL), and poly(dC)-tailed using terminal deoxynucleotidyl transferase (GIBCO BRL). An oligo(dG) primer was used to synthesize the second strand using polymerase I and RNase H. After adding *Eco*RI linkers, the double-stranded cDNA was inserted into λgt10 (Huynh et al. 1985). Screening 10^6 plaques on nylon filters (Amersham) was done using a genomic fragment extending from the *Sal*I site in the homeo box to a *Sau*3A site 1 kb downstream. Hybridization under stringent condition differed from the reduced stringency conditions (see above) in the formamide concentration (50%), the temperature (42°C), and two 30-min washes in $0.2 \times$ SSC, 0.1% SDS at 65°C.

DNA sequencing

Nucleotide sequencing was performed by the dideoxynucleotide chain termination method (Sanger et al. 1977). The c21

Figure 8. Localization of *Hox-3.1* transcripts at early stages of organogenesis. Transverse sections of 9.5-day p.c. (a, b, c) and 8.5-day p.c. (d) embryos. (I) Phase-contrast, (II) dark-field illumination. (III) Drawing of 9.5- and 8.5-day p.c. embryos on which are localized the levels of sections shown in (I and II); the regions of labeling are enclosed by arrows. (a1) The section crosses the dorsal region almost tangentially but shows a very distal caudal region in the upper part. (a2) Enlarged view of the caudal region framed in a1; the labeling is most intense in the neural tube and hind gut but is also present on surrounding mesoderm tissues and the yolk sac. (b1) Section longitudinal to the dorsal region and transversal to the caudal region (in the upper part). (b2) No labeling in the dorsal region, including the neural tube; the tissue distribution of labeling in the caudal region is the same as in a2; bright spots on the right are due to blood cells in the placenta. (c1) The section crosses, from left to right, brain, heart, and trunk with the neural tube and a forelimb bud and, in the upper part, a caudal region. (c2) Labeling only in the caudal region, in all tissues, and on the surrounding yolk sac. (d1) From left to right, an anterior region, with the neural tube, pharyngeal cavity, and brain and, in the upper right part, a distal caudal region. (d2) Enlarged view of the caudal region framed in d1, showing the neural tube on the right; labeling on the yolk sac and on all tissues of the caudal region. Magnification in a1, b1, b2, d1, 200×; a2, 700×; c1, c2, 170×; d2, 450×.

clone was inserted into Bluescript plasmid (Stratagene cloning system) and deleted by the exonuclease III–mung bean nuclease method. Subclones of c21, c1, and c26 cDNAs and specific genomic regions were obtained in the M13 phage or Bluescript and sequenced.

Blot hybridization

DNAs from BALB/c and SWR were prepared by classical methods (Maniatis et al. 1982) and, after restriction and electrophoresis, transferred to nylon membranes (Amersham). RNAs were electrophoresed in denaturing formaldehyde gels (Maniatis et al. 1982) and transferred to NEN GeneScreen Plus membranes. The *Pst*I–*Sal*I cDNA fragment was labeled to a specific activity of 1.5×10^9 cpm/ μ g using the multiprimer labeling system (Amersham). Blots were hybridized with the denatured DNA probe at 42°C, for 17 hr, with 2×10^6 cpm/ml in 20 ml of 1 M NaCl, 1% SDS, 50% formamide, and 10% dextran sulfate and washed twice at 65°C for 30 min in 200 ml of $2 \times$ SSC, 1% SDS.

Synthesis of sense and antisense RNA probes from T7 and T3 RNA polymerase promoters in the Bluescript vector were performed according to Melton et al. (1984), using [α - 32 P]UTP (800 Ci/mmol, Amersham) and RNA polymerases from Pharmacia (T7) and GIBCO BRL (T3). Blots were hybridized with single-stranded RNA probes at 55°C for 24 hr in 50% formamide, 0.3 M NaCl, 20 mM sodium phosphate (pH 6.8), 1% SDS, 100 μ g/ml denatured salmon sperm DNA, and 300 μ g/ml baker's yeast RNA, washed twice at 65°C for 30 min in $2 \times$ SSC, 1% SDS, treated at 37°C for 30 min with RNases A (40 μ g/ml) and T₁ (2 μ g/ml), rinsed in $2 \times$ SSC, and further washed twice for 30 min at 65°C in $0.2 \times$ SSC, 0.1% SDS. Alternative washes after RNases treatments were at 55°C in hybridization buffer minus dextran sulfate for 24 hr.

In situ hybridization analysis

Cryostat sections were obtained from embryos fixed with 4% paraformaldehyde and processed as described (Brûlet et al. 1985). The sense and antisense cPS probes were synthesized using T3 and T7 RNA polymerases (see above) to a specific activity of about 1.5×10^9 cpm/ μ g with [35 S]UTP α S (1000 Ci/mmol, Amersham). The sizes of the probes were monitored on 6% acrylamide sequencing gels. Sections were hybridized (Hogan et al. 1986) for 24 hr at 55°C with 2×10^6 cpm in 20 μ l of a mixture containing 50% formamide, 10% dextran sulfate, 0.3 M NaCl, 10 mM Tris-HCl, 10 mM sodium phosphate (pH 6.8), 5 mM EDTA, 0.02% Ficoll 400, 0.02% polyvinylpyrrolidone, 0.02% BSA, and 20 mM dithiothreitol (DTT). Sections were washed for 1 hr at 55°C in the hybridization buffer, 30 min at 37°C in $2 \times$ SSC, treated with 40 μ g/ml RNase A and 2 μ g/ml RNase T₁, 30 min at 37°C in $2 \times$ SSC, rinsed in $2 \times$ SSC, and further washed for 24 hr at 55°C in hybridization buffer minus dextran sulfate. Autoradiographies were for 2–3 weeks. Phase-contrast and dark-field photographs were obtained with an Olympus IMT2 microscope with 4.0 $\times$ and 10.0 $\times$ objectives and a Zeiss Axiophot with a 2.5 $\times$ objective.

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Note added in proof

These sequence data have been submitted to the EMBL/GenBank Data Libraries under the accession number Y00215.

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Pattern of transcription of the homeo gene Hox-3.1 in the mouse embryo.

H Le Mouellic, H Condamine and P Brûlet

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