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Specific expression of the Hox 1.3 homeo box gene in murine embryonic structures originating from or induced by the mesoderm

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The murine Hox 1.3 homeo box-containing gene is expressed largely in mesoderm-derived or mesoderm-induced embryonal structures, as evidenced by *in situ* hybridization techniques. Expression is spatially limited to the thoracic region, specifically to components of segmental origin such as embryonal ribs and vertebrae and their precursors such as the equivalent sclerotomes, somites and somatic condensations. In addition, expression can be found in parts of embryonal lung, stomach tissue, gut and kidney, tissues whose formation is based on induction of region-specific mesoderm, as well as in some ectoderm-derived tissues. The expression is temporally controlled for the transcripts and can only be detected while the thoracic structures are being formed (days 8–13), but not at day 18 of gestation when the embryo is mature. These data suggest a role of Hox 1.3 gene in the generation of tissues derived from or induced by the embryonal mesoderm.

Key words: homeo box/*in situ* analyses/segmentation, embryogenesis/mesoderm expression

Introduction

In order to decipher the control processes involved in early murine embryogenesis it is essential to identify the relevant genes. However, owing to the complexity of the genome, the length of the reproduction cycle, and the size of the litter, genetic approaches as employed for *Drosophila* are precluded. Recently, a family of murine genes of ~15 members (for references, see Martin *et al.*, 1987), some contained in clusters (Colberg-Poley *et al.*, 1985a; Hart *et al.*, 1985; Duboule *et al.*, 1986), has been recognized, bearing a high degree of homology to *Drosophila* homeo boxes (McGinnis *et al.*, 1984b; Scott and Weiner, 1984). In *Drosophila* the homeo boxes constitute essential parts of genes controlling pattern formation (for references, see Gehring, 1985; Macdonald *et al.*, 1986b). In particular, maternal effect genes (Mlodzik *et al.*, 1985; Macdonald and Struhl, 1986a), pair-rule genes (Frigerio *et al.*, 1987; Bopp *et al.*, 1986; Macdonald *et al.*, 1986b), segment polarity genes (Fjose *et al.*, 1985; Poole *et al.*, 1985), and homeotic genes (Gehring, 1985; Regulski *et al.*, 1985) all contain homeo box sequences. The activity of some, if not all, of these genes is most likely exerted positively or negatively through direct binding to DNA sequences involved in the control of gene activities. A comparison of a number of homeo box genes with the yeast mating type genes indicated a helix–turn–helix DNA-binding motif within the homeo box domain (McGinnis *et al.*, 1984c; Porter and Smith, 1986). For en and ftz (Desplan *et al.*, 1985; O'Farrell, personal communication), *in vitro* DNA-binding assays were able to confirm the initial analysis. Recently, some indications of nuclear localization (Kessel *et al.*, 1987) and of specific DNA binding of the murine Hox 1.5 gene product have also been obtained (Fainsod *et al.*, 1986). If confirmed this would argue for conservation of a DNA-binding domain between *Drosophila* and the mouse.

In order to gain insight into a possible role specifying positional information during murine development, the activity of the murine homeo box genes has to be carefully monitored and subsequently functionally analyzed. The first indication of differential activity of murine homeo box genes was provided by studying the expression profile of Hox 1.1 (m6) during differentiation of F9 cells in parietal endoderm (Colberg-Poley *et al.*, 1985b). Since F9 cells are nullipotent and thus restricted in their differentiation pathway (Strickland and Mahdavi, 1978; Hogan *et al.*, 1983), mouse embryos were investigated at various stages of development. Specifically, using Northern analyses transient expression of Hox 1.1, Hox 1.2 (Colberg-Poley *et al.*, 1985a,b), Hox 1.3 (M.Fibi, B.Zink, M.Kessel, A.M.Colberg-Poley and P.Gruss, in preparation), Hox 1.4 (Wolgemuth *et al.*, 1986; Rubin *et al.*, 1986; Duboule *et al.*, 1986), Hox 1.5 (Ruddle *et al.*, 1985), Hox 2.1, Hox 2.2 (Hart *et al.*, 1985; Jackson *et al.*, 1985; Hauser *et al.*, 1985; Krumlauf *et al.*, 1987), and Hox 3.1 (Breier *et al.*, 1986; Awgulewitsch *et al.*, 1986) was reported. Temporal and spatial restriction of the transcripts was found for Hox 1.1, 1.3 and Hox 2.1 which peak at 11–12 days post coitum (p.c.), whereby Hox 3.1 is enriched in spinal cord (Colberg-Poley *et al.*, 1985a; Hart *et al.*, 1985; Awgulewitsch *et al.*, 1986; Jackson *et al.*, 1985; Krumlauf *et al.*,1987). Recent work with Hox 1.5 also showed expression at day 7.5 and later in spinal cord and mesonephric kidney (Gaunt *et al.*, 1986), as well as in spermatocytes and spermatids of adult male animals (Wolgemuth *et al.*, 1986; Rubin *et al.*, 1986). Human homeo box studies confirm the spatial and temporal expression profile of mammalian homeo box-containing genes (Mavilio *et al.*, 1986).

Although these data point to marked similarity between mammalian homeo box genes and the respective *Drosophila* genes, they are far from being conclusive. Thus, more information is required concerning the temporal and spatial expression of murine homeo box genes. Since only little material can be extracted from early stage murine embryos, *in situ* hybridization techniques are a means of overcoming this experimental obstacle. More importantly, this technique can yield information about the gene activity down to the single cell level. Here we report an *in situ* analysis of the spatial and temporal expression profile of Hox 1.3. We identified the transcripts in embryonic structures of segmental origin, such as somitomeres, somites, sclerotome, vertebrae and ribs. Additionally, embryonic structures induced to develop by mesenchymal tissue such as mesonephric kidney, lung and intestinal tissue actively express Hox 1.3. In this expression pattern Hox 1.3 is distinctly different from Hox 1.1 (K.Mahon personal communication), suggesting a possible role in the control of the formation of these structures.

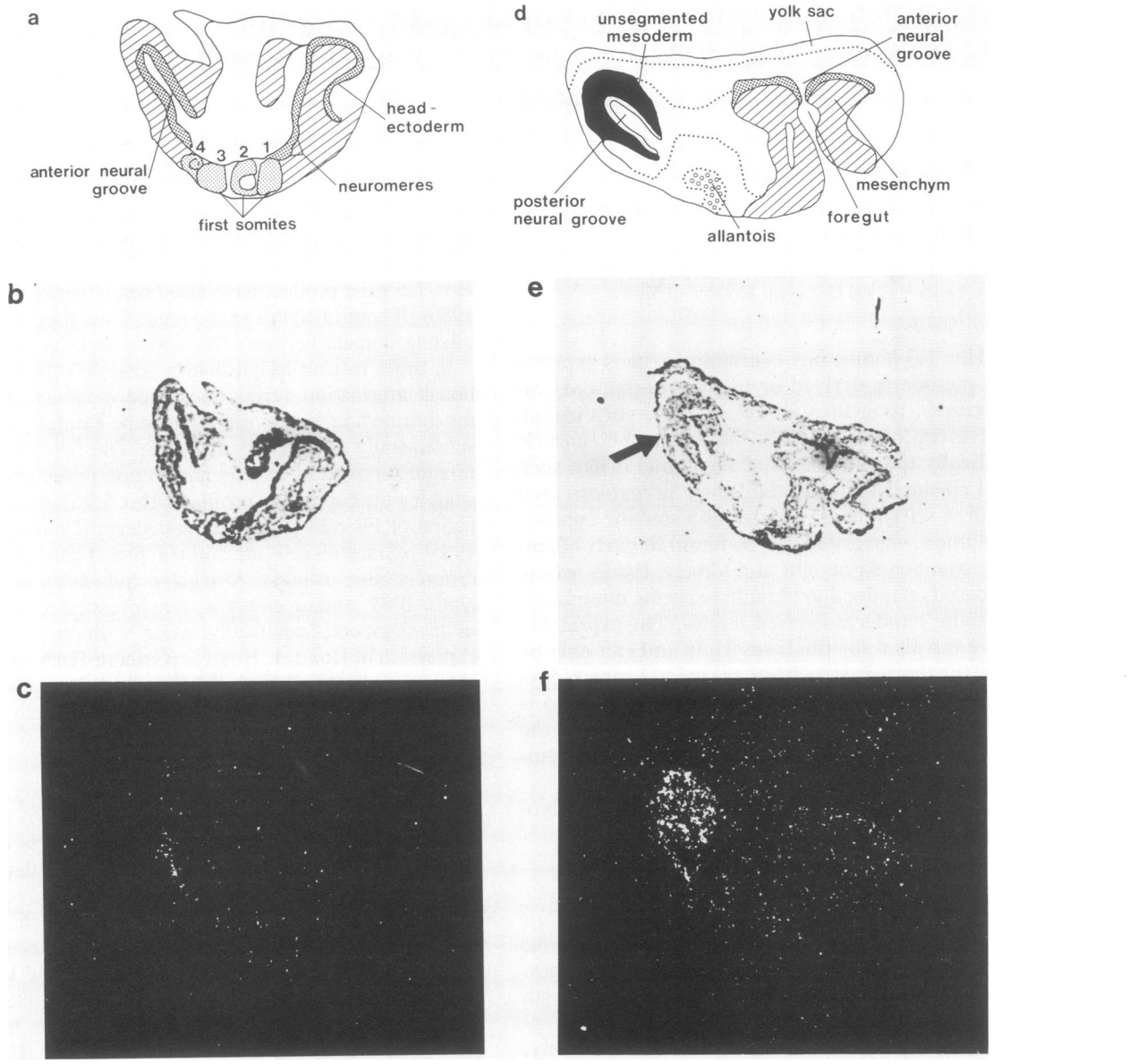

Fig. 1. Expression of Hox 1.3-specific RNA hybridising to an anti-sense transcript from a 470 bp Hox 1.3 fragment (*Sac*I, *Bgl*II) in 8-day-old mouse embryos. (**a**) Schematical representation, (**b**) bright field and (**c**) dark field view of a section from the anterior region of the embryo containing the head, the first four somites (indicated in **a**), and a portion of the anterior neural groove. (**d,e,f**) *In situ* analysis of the same embryo representing the head region and the posterior unsegmented mesoderm, which is marked black.

Results

Expression of Hox 1.3 in mesodermal structures of segmented origin

The Hox 1.3 (m2) gene is a member of a homeo box gene cluster located on chromosome 6 (McGinnis *et al.*, 1984a; Bucan *et al.*, 1986; Colberg-Poley *et al.*, 1985a; Duboule *et al.*, 1986) and constitutes the third gene in tandem. Our previous studies (M.Fibi, B.Zink, M.Kessel, A.M.Colberg-Poley and P. Gruss, in preparation) using Northern analysis indicated the expression of Hox 1.3 (m2) RNA (1.9 kb) during prenatal murine development. To analyze whether the signal obtained can be attributed to a spatially restricted expression of Hox 1.3 transcripts, we performed *in situ* hybridization to sections of frozen embryos at different stages of embryogenesis.

In 8-day-old embryos two important developmental processes are taking place. One is the formation of the neural folds and the other is the condensation of the mesoderm cells on either side of the notochord into pairs of morphologically distinct somites (Theiler, 1972; Hogan *et al.*, 1985). Drawn in Figure 1 (a,d) are serial parasagittal sections of an 8-day-old mouse embryo, demonstrating the localization of the head ectoderm, the first four somites and the adjacent unsegmented mesoderm of the anterior (Figure 1a) and posterior (Figure 1d) neural groove. With the intention of analyzing the expression pattern of the murine homeo box-containing gene Hox 1.3, comparable sections (Figure 1b,e) were used for *in situ* hybrization. As demonstrated by the dark field photographs in Figure 1 (c,f), no signal could be detected in the head region or in the anterior body part up to the fourth somite. Also, the directly adjacent presomitic mesoderm turned out to be negative (Figure 1a,b,c,). In contrast, a clear signal was found in an area containing the unsegmented mesoderm which eventually generates the thoracic region later in development (Figure 1d,e,f). Control by hybridization using either sense

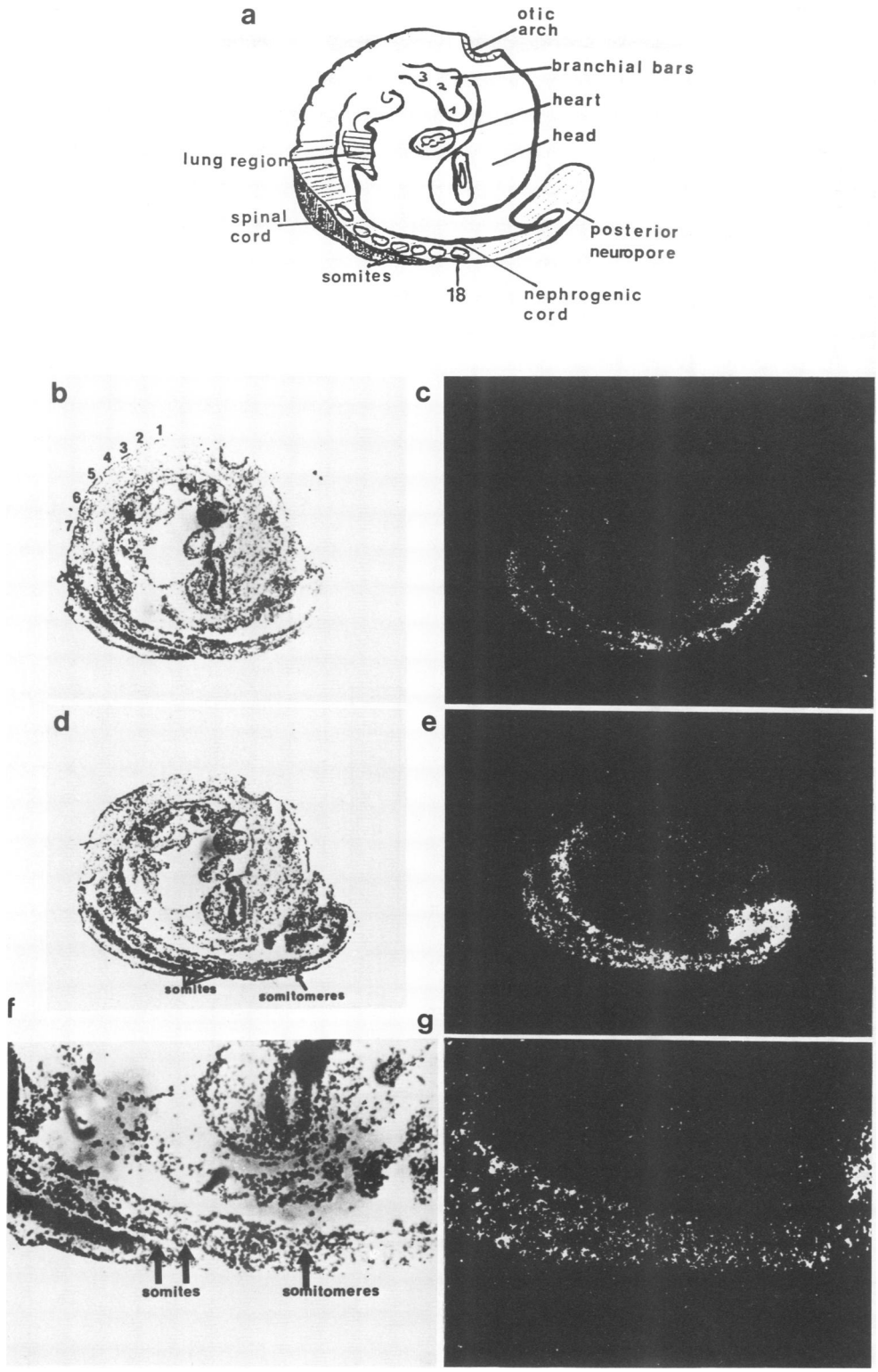

Fig. 2. Localization of Hox 1.3 RNA by *in situ* hybridization on parasagittal sections of 9-day-old mouse embryos. (**a**) Schematical drawing of the original section. Localization of somite number 18 is indicated. (**b,c**) Bright and dark field views of the frozen sections from 9-day-old embryos after hybridization to a Hox 1.3 RNA probe (470 bp *Sac*I, *Bgl*II). Localization of the first seven somites is indicated by numbers. (**d,e**) *In situ* hybridization to another serial section of the same embryo with the same probe. The region containing somitomeres is shown by the arrow. (**f,g**) Higher magnification of Figure 3d and representing the posterior position of the embryo containing somites and adjacent somitomeres (see arrows).

orientation transcripts or a heterologous probe (c-fos) was completely negative (data not shown). In order to discern whether the Hox 1.3-expressing unsegmented mesoderm will generate somites which maintain this expression, we performed *in situ* hybridization to parasagittal sections of 9-day-old embryos which have already formed the first 13–20 somites (Theiler, 1972).

As pointed out in Figure 2b,c, again no Hox 1.3-specific RNA was discovered in the anterior region of the embryo. Instead,

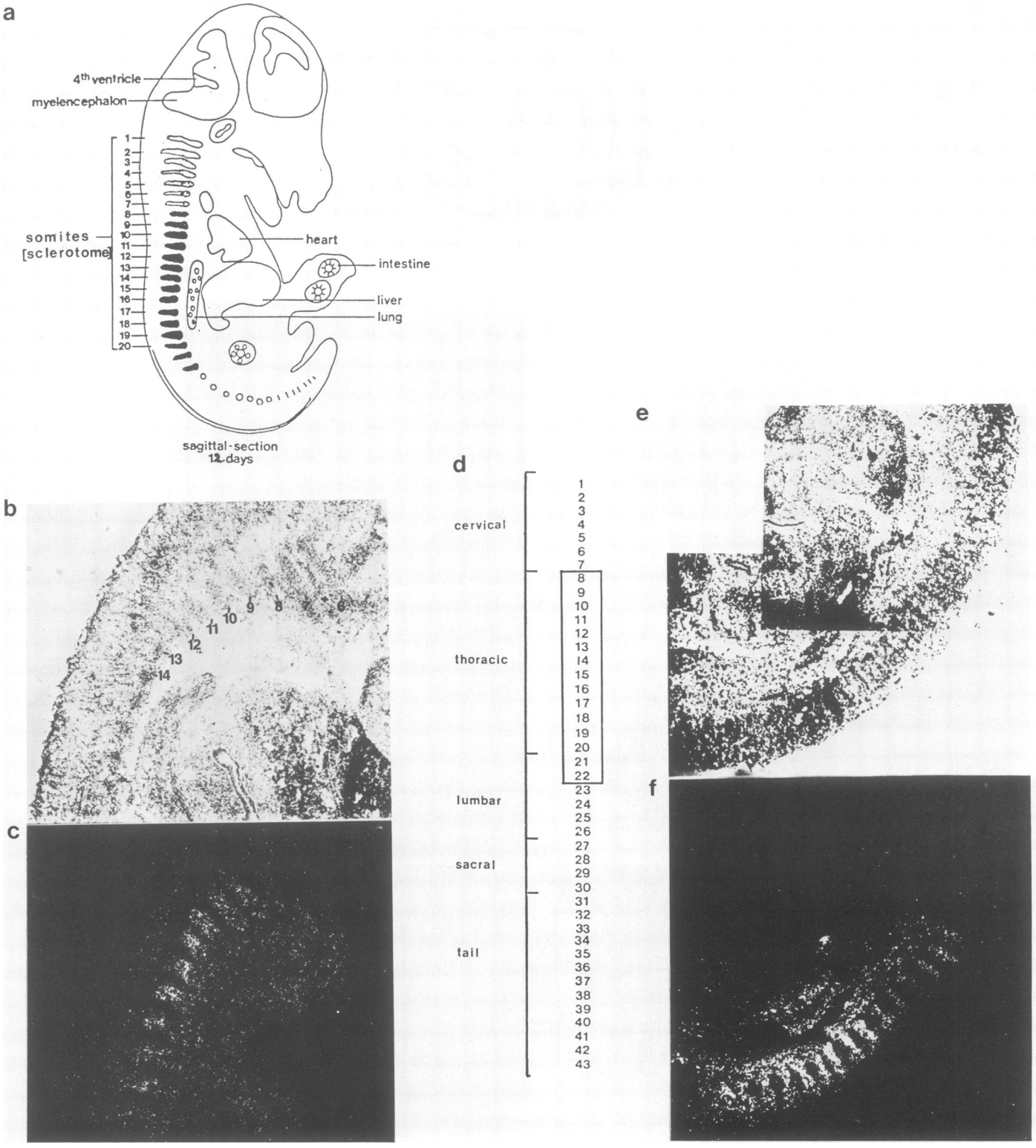

Fig. 3. Expression of the homeo box-containing gene Hox 1.3 in a parasagittal section of 11- and 12-day mouse embryos. (**a**) Schematical representation, (**b**) anterior thoracic region of the section under bright field illumination (12-day embryo). Sclerotomes along the anterior-posterior axis are indicated by numbers. (**c**) Dark field view of the same section after hybridization to ^{35}S-labeled RNA probe of Hox 1.3 [470 bp Hox 1.3 fragment (*Sac*I, *Bgl*II)]. (**d**) Schematical demonstration of the localization of Hox 1.3 expressing sclerotomes in 12-day-old mouse embryos. Hox 1.3 positive sclerotomes are indicated by the black bar. (**e**) Dark field and bright field view of the whole Hox 1.3 expressing sclerotome region in a parasagittal section of 11-day mouse embryo. After control hybridization using a c-fos antisense RNA and also a Hox 1.3 sense probe no spurious hybridization could be detected (data not shown).

the Hox 1.3 transcript is present in the somites, in the presumed lung tissue, and in the spinal cord (see below) of the successive thoracic body region (Figure 2a–e). Additionally, in all sections a particularly strong signal is seen in the area where new somites and somitomeres develop (indicated by arrows, Figure 2d) and in the tail bud region harboring the posterior neuropore (Lipton

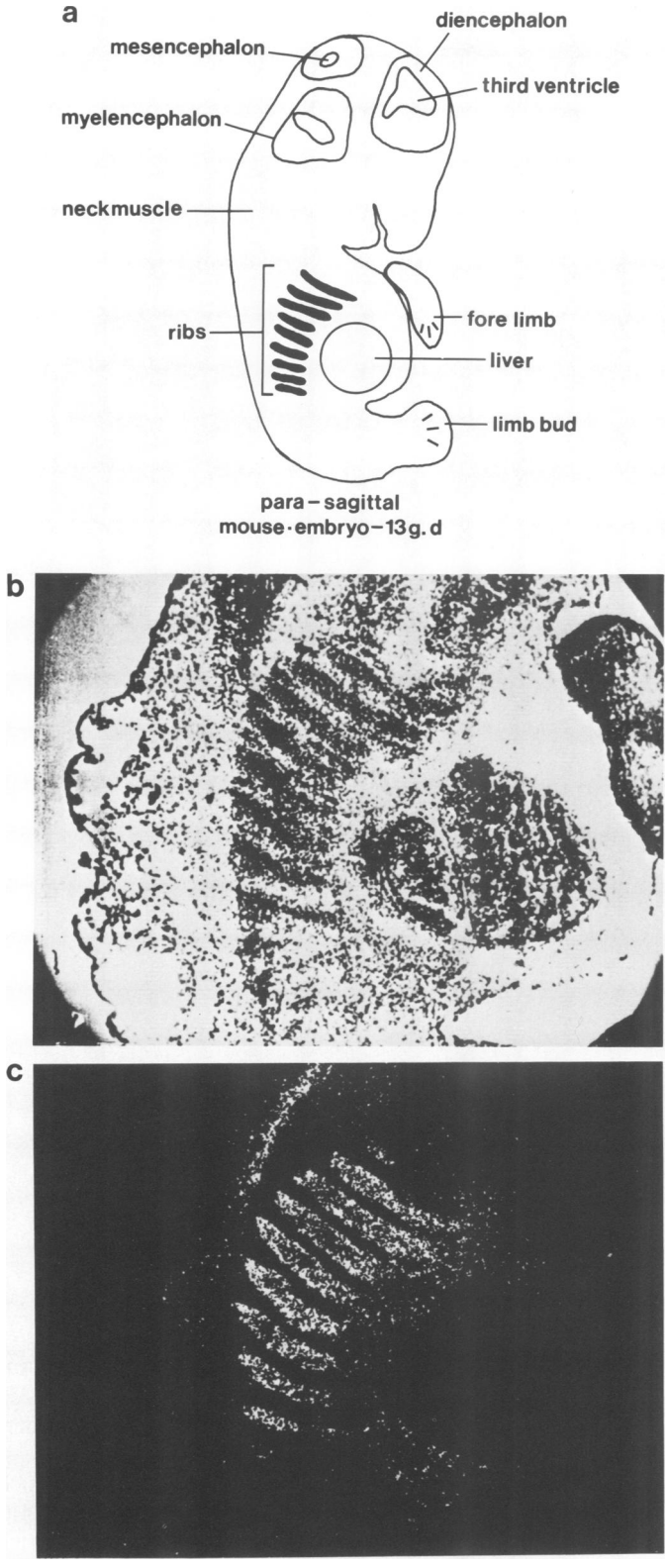

Fig. 4. Localization of Hox 1.3 RNA by *in situ* hybridization on a parasagittal section of a 13-day-old mouse embryo. (**a**) Schematical drawing of the section, (**b**) thoracic region of the section under bright field illumination and (**c**) distribution of silver grains on the same section viewed under dark field illumination. For probe the 470 bp Hox 1.3 fragment (*Sac*I, *Bgl*II) was used as a template.

and Jacobson, 1974; Tam *et al.*, 1982; Tam and Meier, 1982). A higher magnification of the region containing somitomeres is shown in Figure 2f,g.

The observation that Hox 1.3 RNA specifically characterizes the segmented mesodermal structures of the thoracic body region was strengthened and extended by examining 11 and 12-day-old embryos. Drawn in Figure 3a is a parasagittal section of a 12-day-old mouse embryo, demonstrating the location of different organs like brain, liver, heart and lung. Additionally, along the craniocaudal axis of the embryo, a seris of mesodermal condensations, which represent the sclerotomic part of the somites, is indicated (numbers 1–20). These structures, which proliferate from the segmented axial mesoderm (somites), contribute to the formation of the axial skeleton in later stages of murine development (Langman, 1969; Gilbert, 1985). The data from *in situ* analysis of comparable sections are presented in Figure 3. The anterior thoracic part of the original section (numbers of sclerotomes are indicated) is shown by the bright field photograph in Figure 3b. The dark field photograph (3c) of the same section after hybridization reveals a striking signal in sclerotomes numbers 8–17 at the dorsal site of the embryo. Interestingly, in the sclerotomes indicated by numbers 6 and 7 no signal of Hox 1.3 (m2)-specific RNA was detectable. These results and further analyses of successive sections containing sclerotomic structures of other body regions reveal that, in 12-day mouse embryos, only those sclerotomes which are located in the thoracic body part are positive for the expression of Hox 1.3 (m2)-specific RNA (Figure 3d). These data are in good agreement with the results we obtained from 8- and 9-day-old embryos, indicating that Hox 1.3 expression is characteristic of a specific region of the embryo and is not due to the maturation state of the somites. Thus, the expression pattern of Hox 1.3-specific RNA in mesodermal, segmented structures follows a developmental lineage (unsegmented mesoderm, somitomeres, somites, sclerotomes) which is restricted to components eventually leading to formation of the thoracic body region.

Expression of Hox 1.3 in somite-derived structures

Mesodermal condensations, the somites, represent the original segmentation of the early mouse embryo and are known to be the source of a number of important segmentally arranged mesodermal derivatives, such as the vertebrae and the ribs in older embryos (for references see Gilbert, 1985; Hogan *et al.*, 1985; Langman, 1969). Moreover, parts of the somites differentiate into muscle tissue and connective tissue layers of the skin (Chevallier *et al.*, 1977). Therefore, we were interested to know whether these somite-derived tissues also express Hox 1.3 RNA. Figure 5 demonstrates a parasagittal section of the presumptive rib region of a 13-day-old mouse embryo. The rib centers of the anterior thoracic region appear early, by 12 days, and develop ahead of the more posterior regions (anterior–posterior gradients; Theiler, 1972). At day 13 of development the presumed rib tissue consists of mesodermal cells of a cartilaginous stage (Carlson, 1981). Ossification centers are formed later during embryogenesis, beginning at day 14 p.c. (Theiler, 1972).

The *in situ* analyses of this section using Hox 1.3 probes are shown in Figure 4b,c. The bright field microscopic image of the section with embryonal liver, which at that stage of embryogenesis is well developed and contains scattered blood-forming foci (Theiler, 1972), is presented in Figure 4b. Also, presumed rib tissue is evident with a forelimb at the right of the section. The *in situ* analysis reveals a striking signal in embryonic rib structures and the Hox 1.3 RNA in this tissue is evenly distributed as indicated by the homogeneous signal. Other structures like the liver or the limbs clearly do not express Hox 1.3. Interestingly an additional signal can be seen most likely represen-

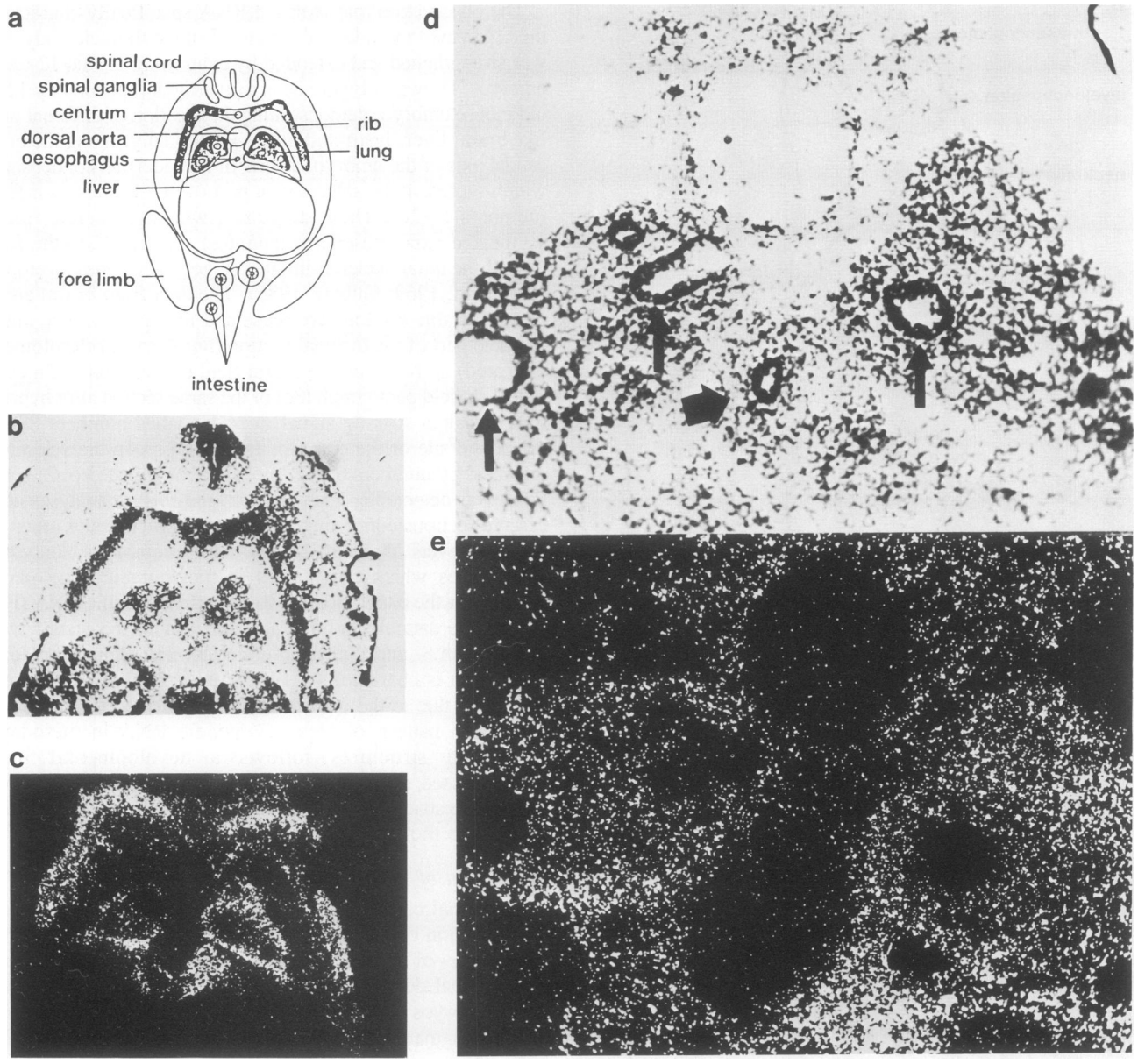

Fig. 5. *In situ* hybridization to a transverse section from the lung region of a 12-day mouse embryo. (**a**) Schematical representation, (**b**) bright field and (**c**) dark field photograph of the transverse section after hybridization with a Hox 1.3 probe (*Eco*RV, *Eco*RI). (**d,e**) Higher magnification of the lung region shown in (**b**). The esophagus is indicated by the large arrow, the endodermal portions are shown by the small arrows.

ting embryonic trunk muscles. Significantly other somite-derived tissues were found to be negative for Hox 1.3 RNA.

In order to analyze whether the demonstrated Hox 1.3 expression pattern also remains constant in later stages, we performed *in situ* hybridizations to sections of 18-day embryos. At this time, nearly all tissues and organs have reached their final stage of differentiation. Interestingly, no signal of Hox 1.3 RNA could be detected in differentiated ribs and trunk muscle (data not shown).

Expression of Hox 1.3 in parenchymal tissues

Further sections of 12-day embryos revealed that several additional organs can be identified expressing Hox 1.3 RNA. These tissues represent mesodermal portions of embryonic lung, stomach and midgut. In Figure 5 we show *in situ* analyses using a transverse section through the lung region of a 12-day-old embryo. As indicated schematically in Figure 5 and documented in Figure 5b,c, again sclerotome/rib structures are positive. Additionally, embryonal lung tissue, which at this stage is subdivided into lobes and in which segmental bronchi are branching (Theiler, 1972), reveals a strong Hox 1.3-specific signal.

Figure 5(d,e) shows a higher magnification of the lung which reveals that only some regions of this tissue express Hox 1.3 RNA. Specifically, the endoderm-derived epithelial cells, which will form the bronchi of the lung (for references see Carlson, 1981), are completely negative, but the surrounding lung mesenchyme reveals a strong signal for Hox 1.3. Esophagus (indicated by an arrow) is also shown to be negative. These results demonstrate the strong spatial restriction of Hox 1.3 RNA to a few distinct tissues.

In analogy to the results we obtained from the mesodermal and endodermal portions of the lung, the dark field photograph in Figure 6b reveals that, besides the Hox 1.3 expression of the ribs, a strong signal can also be found in the stomach mesen-

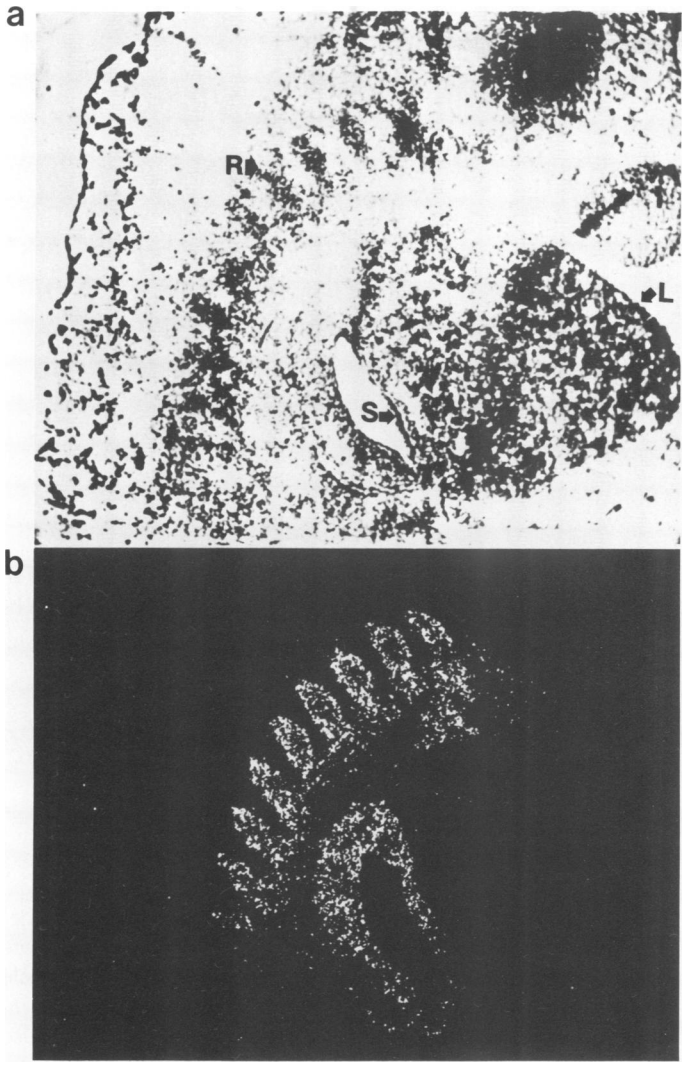

Fig. 6.(a) Bright field and (**b**) dark field view of a parasagittal section from a 12-day-old embryo hybridized with ^{35}S-labeled RNA complementary to the anticoding strand of Hox 1.3 (*Sac*I, *Bgl*II). The bright field picture shows several portions of the ribs (R), the stomach (S) and a part of the liver (L). Silver grains in Figure 6b are restricted to the ribs and the stomach region.

chyme of the 12-day-old embryo. Similar results were obtained from the mesodermal and endodermal part of the midgut (data not shown). However, analyses of the Hox 1.3 transcripts in these organs in 18-day-old embryos reveal that terminally differentiated tissues of the lung and the stomach as well as ribs have ceased Hox 1.3 expression (data not shown).

In contrast to rib, lung and stomach tissues the midgut tissue of 18-day-old embryos still expresses Hox 1.3-specific RNA, though the extent of the expression is more restricted than in 12-day-old embryos. As demonstrated in Figure 7, only one distinct layer of the gut wall retains the expression of Hox 1.3 RNA. The higher magnification in Figure 7e reveals that the label is distributed as a regular broken line, which presumably traces the course of the longitudinal muscle layer of the gut wall. The dotted appearance of the signal can be attributed to transverse sectioning of the longitudinal muscle fibers. Further sections reveal that the expression of Hox 1.3 RNA in the gut is restricted to the midgut region of the animal (data not shown). The last tissue that was found to be positive and which is also known to be formed after mesodermal inductions represents embryonal kidney (data not shown).

Expression of Hox 1.3 in the central nervous system

As already demonstrated in Figure 2, the spinal cord of 9-day-old embryos shows expression of Hox 1.3-specific RNA. All other structures of the central nervous system, including the ganglia and the brain, are negative at this stage of development. In more mature embryos (day 12), besides the spinal cord (date not shown) one other distinct region of the brain becomes positive for Hox 1.3 RNA. As pointed out in Figure 8a,b the posterior region adjacent to the myelencephalon is strongly positive for the expression of Hox 1.3 RNA. The homologous region was also found to be positive in 18-day-old embryos (Figure 8c,d). In contrast, the spinal cord was found to be negative at this stage of development. No expression of Hox 1.3 RNA was found in any other tissues of the central nervous system, including the brain, at any stage examined.

Discussion

Homeo box-containing genes of *Drosophila* constitute an integral part of the developmental program of the fruit fly (see Gehring, 1985, for review). As for homeo box-containing genes of the vertebrates, most of the efforts to establish cognate relationships have gone into analyses of *Xenopus* (Carrasco *et al.*, 1984; Harvey *et al.*, 1986), mouse (see Colberg-Poley *et al.*, 1987, for review), and human genes (Hauser *et al.*, 1985; Boncinelli *et al.*, 1985; Mavilio *et al.*, 1986). As in *Drosophila*, some homeo box genes of the mouse are present in clusters and are differentially expressed in teratocarcinoma cells and during mouse embryogenesis (see Colberg-Poley *et al.*, 1987, for review). However, no detailed information is available concerning the differential expression of mouse homeo box-containing genes at different stages during embryogenesis. If homeo box-containing genes play a regulatory role during mouse embryogenesis, a spatially and temporally restricted expression profile of these genes can be predicted. Here we have concentrated on the expression profile of Hox 1.3, the third gene in tandem located on mouse chromosome 6 between T-cell receptor β and Immunoglobulin Kappa genes (Bucan *et al.*, 1986). For our *in situ* experiments SP6-generated ^{35}S-labeled RNA probes were utilized. The region transcribed excluded the homeo box or contained only parts of the least homologous 5′ region (54 bp) in order to obtain Hox 1.3-specific signals and to avoid possible cross hybridizations with other highly homologous homeo box-containing transcripts. Also, the sense transcript of the same fragment and a c-*fos* transcript were used as controls in order to document the specificity of the signals. In no case with these controls and under the conditions used could we detect spurious hybridizations.

Thus, having confirmed the specificity of the signal, we examined in our experiments the expression of the Hox 1.3 gene at various stages (days 8−18) of embryogenesis. At day 8 of gestation, two major activities are progressing in the embryo. One is the formation of the neural folds in the ectoderm in response to inductive signals from the underlying mesoderm (neural induction). The other is the development of somites and neuromeres, which are formed in a cranio−caudal sequence (Theiler, 1972). Therefore, at this stage of development when the first somites are condensing, new mesoderm cells are still being generated at the posterior end of the primitive streak which represents presomitic mesoderm (Flint *et al.*, 1978; Tam, 1981; Woo Youn *et al.*, 1980). Later these cells will give rise to

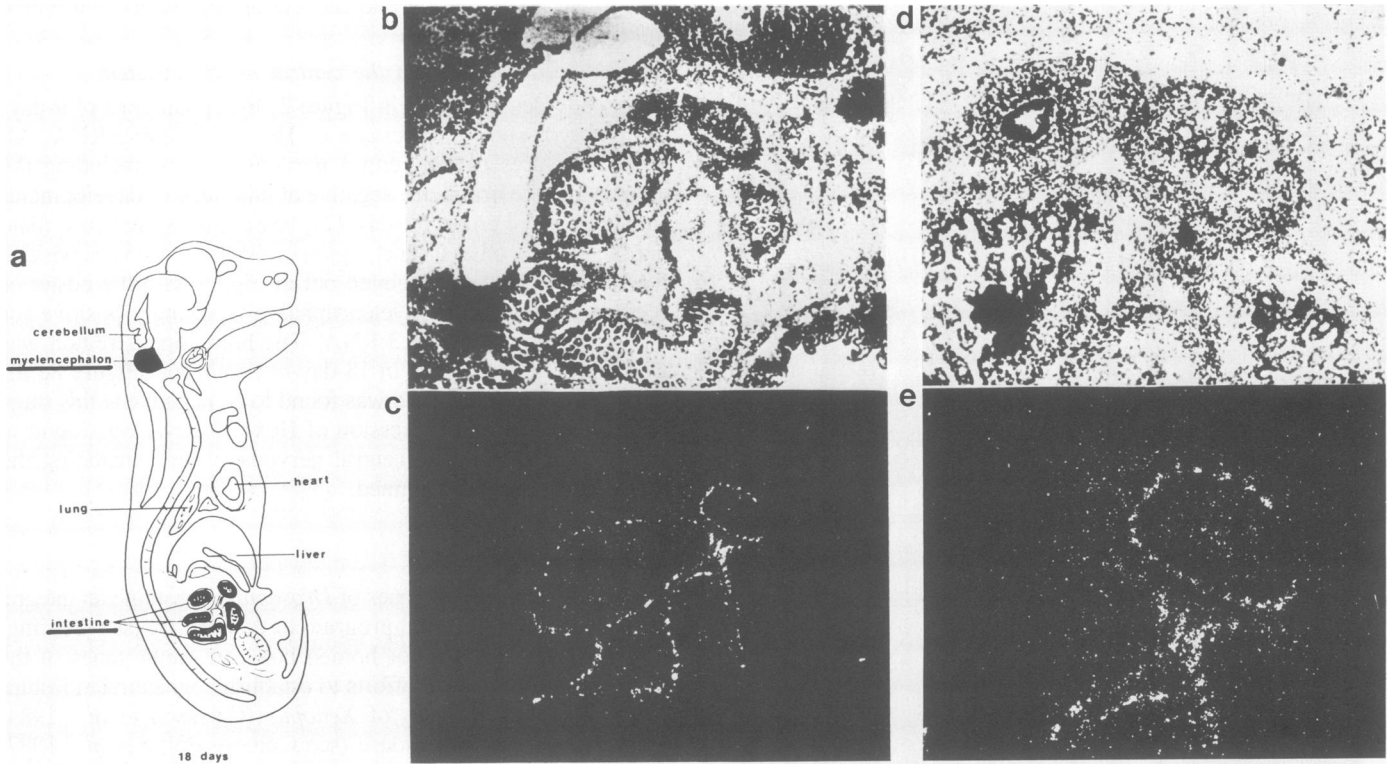

Fig. 7. *In situ* hybridization to detect Hox 1.3 transcripts (*Sac*I, *Bgl*II) in 18-day-old mouse embryos. (**a**) Schematical drawing indicates the localization of the gut region which was found to express Hox 1.3 specific RNA. Gut region of the section under (**b**) bright field and (**c**) dark field illumination. (**d,e**) Bright field and dark field photograph of the higher magnification of Figure 7b and c.

somitomeres which subsequently generate somites of the corresponding region (Tam *et al.*, 1982; Tam and Meier, 1982). *In situ* hybridization to serial sections of 8-day-old embryos using a Hox 1.3 probe results in a clear signal in the posterior part of the embryo containing the region of presomitic mesoderm, which eventually generates the thoracic region later in development. Notably, the head region, the first four somites visible at this stage (Theiler, 1972) and the directly adjacent somitomeres do not exhibit any specific signal. These somites are assumed to develop into cranial vertebrae and into the extrinsic eye musculature (Meier and Tam, 1982; Balinksy, 1975; Noden, 1983).

The generation of mesoderm cells at the end of the primitive streak and the formation of newly condensed somites progresses over several days, resulting in an anterior–posterior gradient in the maturation of the somites (Pearson and Elsdale, 1979). Considering this maturation and differentiation pathway of the somites, it was of interest to examine the subsequent stages of development for their expression of Hox 1.3 RNA.

In the 9-day embryo, organogenesis is greatly accelerated and the contour of the embryo is simultaneously altered. At this stage of development a mouse embryo has ~ 13–20 somites (Theiler, 1972). *In situ* analysis of the expression pattern of Hox 1.3 RNA reveals that, again, the head and the anterior body region (up to about somite number 7) do not show any expression of this RNA, but a strong signal was found in the adjacent posterior body part from somite numbers 8–18 and also in the following somitomeres and in the tail bud region containing presomitic mesodermal cells. Moreover, Hox 1.3 RNA could be detected in the posterior spinal cord and in the presumed lung region.

Subsequently, the original segmentation of the embryo becomes more and more obscure because the cells derived from different subregions of the somites (sclerotome, myotome, dermatome) proliferate from the segmented axial mesoderm and participate in formation of different tissues such as vertebrae, ribs, muscle and dermis (see Hogan *et al.*, 1985, for review). Using *in situ* hybridization, we demonstrate that in 11- and 12-day-old mouse embryos the somitic sclerotomes which are located in the thoracic body region (somites 8–22) intensify the expression of Hox 1.3 RNA, whereas the sclerotomes of the adjacent anterior or posterior region remain negative. Most interestingly, this expression pattern was maintained by the corresponding somite-derived tissues like the ribs and the trunk muscle of the thoracic region in 13-day-old mouse embryos, whereas both organs in their terminally differentiated state are negative. Expression of Hox 1.3 thus seems to be spatially restricted to presumed segmented structures which are involved in the formation of the thoracic body region.

Our results concerning the Hox 1.3 expression pattern obtained from 8-, 9- and 12-day-old embryos also indicate that the expression of Hox 1.3-specific RNA is not due to a particular maturation stage of the somites of the region, but rather Hox 1.3 is expressed characteristically in a distinct body part very early in murine development. Therefore, a lineage restricted to the thoracic region beginning with presomitic mesoderm yielding somites yielding sclerotomes yielding ribs was found to express Hox 1.3 transcripts.

Further hybridizations to different sections of 12-day-old embryos reveal that, besides the somite-derived structures, distinct tissues of the lung, the stomach and the midgut exhibit a strong expression of Hox 1.3-specific RNA. Significantly, all three organs are located in a body part which was originally characterized by Hox 1.3 expression, the thoracic region (Figure 9). The histogenesis of these organs is based on processes called

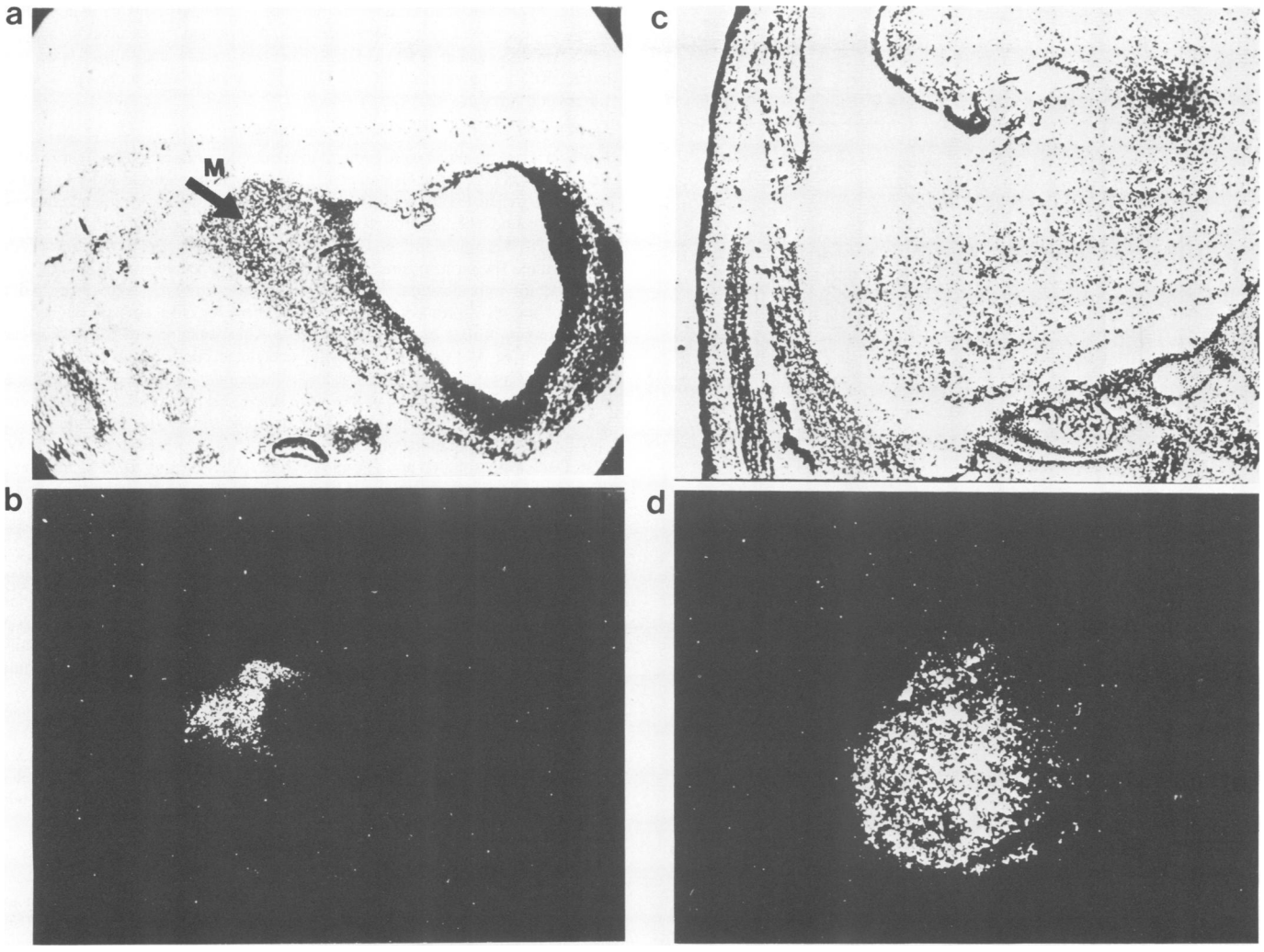

Fig. 8. Expression of Hox 1.3-specific RNA in the brain of 12- and 18-day-old mouse embryos. (**a**) Bright field and (**b**) dark field view of the myelencephalon (M) (parasagittal) of a 12-day-old embryo after hybridization. (**c,d**) Homologous region of an 18-day-old embryo after hybridization.

epithelio/mesenchymal interactions (Rudnick, 1952; Wessels, 1970, 1977; Deuchar, 1975; Spooner and Wessells, 1970; O'Rahilly, 1978; Holtzer, 1968).

The epithelial portions of the lung, stomach and midgut are derived from endodermal cells of the primitive gut, whereas the mesenchyme is derived from region-specific embryonic mesoderm. In early embryos, the epithelial structures of the endodermal tube are not strictly limited in their differentiation capacity. They can develop differently at different locations depending on the mesenchyme (Rudnick, 1952). This regional specificity of the mesenchyme can be seen dramatically in the formation of the respiratory system. Depending on the origin of the mesenchyme, the lung bud epithelium is able to differentiate either into bronchial buds or gastric glands or intestinal villi (Deuchar, 1975). Interestingly, our hybridizations have shown that the expression of Hox 1.3 RNA occurs only in the mesenchymal part of these organs, whereas the endoderm-derived epithelia are negative. Moreover, we found that expression of Hox 1.3 RNA is restricted to the developing stage of the lung and the stomach. These results indicate a possible role of the Hox 1.3 gene product in the inductive processes which are responsible for the formation of these organs. Only in the terminally differentiated gut tissue were stable transcripts of Hox 1.3 RNA retained. In these 18-day-old embryos the expression of Hox 1.3 was found to be restricted to one smooth muscle layer of the gut wall. As described recently, another murine homeo box gene, Hox 2.1, also reveals an analogous cell-type specific expression in embryonic lung tissue. However, in contrast to Hox 1.3, there was essentially no change in RNA levels between fetal and adult stages (Krumlauf *et al.*, 1987).

In conclusion we were able to demonstrate that Hox 1.3-expressing mesodermal structures are derived from somites or from region-specific mesoderm (Carlson, 1981; Holtzer, 1968; Deuchar, 1975) of the thoracic body part, yielding for the first time clear evidence that a murine homeo box gene is expressed following a 'segmented pattern'. In addition to these results we also found a Hox 1.3-specific signal in the central nervous system of the mouse embryo. Whereas the spinal cord was labelled only during the differentiating stage, one distinct region of the brain, namely the myelencephalon, was marked by a strong expression of Hox 1.3 RNA throughout development. Recently, a regionally localized transcription pattern in the central nervous system was also reported for several other murine homeo box genes (Hox 1.5, 2.1, and 3.1). In analogy to our results, significantly, each of these genes displayed a unique anterior boundary of expression within the CNS (Utset *et al.*, 1987; Krumlauf *et al.*, 1987; Gaunt *et al.*, 1986). Although in constrast to *Drosophila* the spinal cord and the brain are not characterized by an obvious segmented

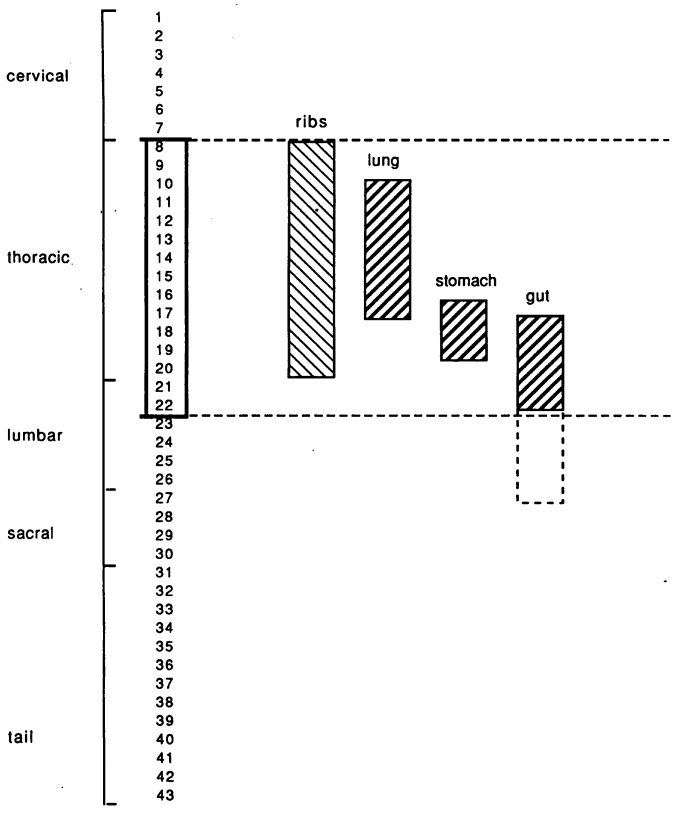

Fig. 9. Localization of Hox 1.3 expressing tissues in 12-day mouse embryos. Somites (sclerotomes) are indicated by numbers. Localization of rib tissue (directly somite derived), lung, stomach and gut (formation of these tissues is based on mesodermal induction) region expressing Hox 1.3 is shown by the hatched bars.

arrangement, the formation of these organs is based on region-specific inductive processes from the underlying mesoderm (for review, see Hogan *et al.*, 1985). Thus, the expression of murine homeo box genes in the CNS could serve as a positional cue during the development of the anterior–posterior body plan in embryogenesis.

Materials and methods

Embedding, sectioning and fixation of mouse embryos

After dissection, embryos (8,9,12 and 18 days) were rinsed in phosphate-buffered saline (PBS) and frozen immediately in OCT embedding medium (Miles Laboratory) using a cold chamber placed on dry ice. Embryo blocks can be stored at −70°C for several weeks. For sectioning, blocks were mounted on a cryostat specimen holder at −20°C using OCT and were trimmed to the right size. Sections of ~5 μm were prepared at −18°C to −20°C and picked up on acid-cleaned, prewarmed slides, rubbed according to Gall and Pardue (1971). Several serial sections were collected on one slide in order to perform parallel hybridization using Hox 1.3 antisense and control probes (Hox 1.3 sense and c-*fos* sense) on the same slide. Sections were dried at 55°C on a hot plate for ~15 min and afterwards they were taken straight into post-fixation according to Hafen *et al.* (1983). After dehydration and air-drying they can be stored in a dry chamber at −20°C for several weeks.

Preparation of SP6-labeled RNA probes

Several unique fragments of the murine homeo box-containing gene Hox 1.3 (fragments:470 bp Hox 1.3 genomic DNA insert without box sequences (*Sac*I, *Bgl*II), 1.7 kb Hox 1.3 cDNA (*Eco*RV, *Eco*RI) (containing 34 bp of box sequences)) and one fragment of the c-*fos* cDNA (1 kb *pst*I–*pst*I) (Curran *et al.*, 1982) were cloned into the polylinker regions of PSPT18 vectors (Promega Biotec) by standard methods. High specific activity RNA (~1 × 10^8 c.p.m. μg^{-1}) was prepared from the coding and the anticoding strand of the fragments using 100 μCi alpha [^{35}S]UTP (400 Ci mol^{-1}, Amersham). After separating the unincorporated labeled nucleotides, probes for *in situ* hybridization were subjected to limited alkaline hydrolysis generating a size range of ~ 50–100 bp (Cox *et al.*, 1984). Size was determined by gel electrophoresis. After ethanol precipitation the RNA pellet was dissolved in ~100 μl DEPC (diethylpyrocarbonate)-treated water containing 50% formamide and 10 mM DTT. In this solution the probe can be stored for several days at −20°C.

Section pretreatment, hybridization and washing

For hybridization slides were brought to room temperature and prehybridization treatment was performed (Hafen *et al.*, 1983) with variations in order to remove proteins associated with the mRNA that might inhibit hybridization and increase the background labeling. For protein removal slides were incubated in 2 × SSC (30 min, 70°C), rinsed in 1 × PBS, and treated with pronase for 10 min at room temperature. The pronase concentration was varied depending on the kind of tissue we used for hybridization. 8- and 9-day mouse embryos were incubated in 0.5–0.1 mg ml^{-1} pronase (Boehringer), whereas for older embryos higher concentrations up to 0.2 mg ml^{-1} were used. After washing in PBS, slides were fixed again in 4% paraformaldehyde, acetylated, dehydrated and air dried. Hybridization was carried out according to Ingham *et al.* (1985) with variations. Just prior to hybridization the probes were suspended in hybridization buffer containing 50% deionized formamide, 10% dextrane sulfate, 10 mM dithiothreitol (DTT), 0.3 M NaCl, 10 mM Tris, 0.1 M $NaPO_4$pH 6.8, 2 mM EDTA, 1 × Denhardt's solution and 100 mM S-ATP. After heating to 95°C for 2 min, 10–20 μl of the hybridization solution was applied to each section, which was then covered with a siliconized coverslip.

Hybridization was performed in a humid chamber at 50°C overnight. For washing the coverslips were allowed to float off in a solution of 2 × SSC (0.03 M NaCl, 0.03 M Na_3 citrate), 50% formamide, 10 mM DTT. Washing was continued for 4 h in the same solution with several changes and afterwards an RNase digestion (20 μg ml^{-1} RNase A) (Ingham *et al.*, 1985) was performed in 0.5 M NaCl in TE [10 mM Tris (pH 7.6), 1 mM EDTA] at 37°C for 15 min. Finally, the washing was continued overnight at 50°C in 2 × SSC, 50% formamide, 10 mM DTT, then slides were dehydrated and air dried.

For autoradiography the slides were immersed in Kodak NTB2 emulsion (diluted 1:1) with water. After air drying autoradiography was allowed to proceed for ~7 days at 4°C in a dry, light-tight box. Developing was performed at 15°C in Kodak D19 developer for 3 min, then slides were rinsed briefly in a water-stop bath and fixed for 10 min in Kodak Unifix. Finally, slides were stained with Giemsa for light microscopy.

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