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Short Contribution

Differential localization of Mox-1 and Mox-2 proteins indicates distinct roles during development

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ABSTRACT Transcript localizations for *Mox* genes have implicated this homeobox gene subfamily in the early steps of mesoderm formation. We have extended these studies by determining the protein expression profile of *Mox-1* and *Mox-2* during mouse development. The time of onset of Mox protein expression has been accurately obtained to provide clues as to their roles during gastrulation. Expression of Mox-1 protein is first detected in the newly formed mesoderm of primitive streak stage mouse embryos (7.5 days post-coitum, d.p.c.). In contrast, Mox-2 protein is first detected at 9.0 d.p.c. in the already formed somites. Additionally, immunostaining reveals new and distinct areas of *Mox* expression in the branchial arches and limbs that were not reported in our previous mRNA localization analysis. Mouse Mox-2 antibodies cross-react specifically in similar embryonic tissues in chick indicating the conservation of function of *Mox* genes in vertebrates. These expression data suggest that the *Mox* genes function transiently in the formation of mesodermal and mesenchymal derivatives, after their initial specification, but before their overt differentiation. Furthermore, while there appears to be some overlap in protein expression between Mox-1 and Mox-2 during somitogenesis, unique areas of expression indicate several distinct roles for the *Mox* genes during development.

KEY WORDS: *Mox, immunohistochemistry, mouse, mesoderm, differentiation*

We previously reported the isolation of two members of a novel subclass of the homeobox gene superfamily, the *Mox* genes (Candia *et al.*, 1992). *Mox* genes have subsequently been isolated from divergent vertebrate species including frogs, humans and zebrafish (Futreal *et al.*, 1994; Candia and Wright, 1995; B. Mankoo and V. Pachnis, personal communication). Expression studies in mice and frogs have led to the hypothesis that *Mox* genes are involved in the early steps of mesodermal differentiation prior to overt and terminal differentiation (Candia *et al.*, 1992; Candia and Wright, 1995). As homeodomain proteins, the gene products are probably an important part of a nuclear signaling cascade involving upstream immediate-early patterning genes such as *Brachyury*, *lim*, and *forkhead*, and downstream genes including the myogenic factors, *scleraxis*, *paraxis* and *pax*-related genes.

Despite the large number of homeobox genes isolated in vertebrates thus far, there is a paucity of reagents available to examine their respective protein products that ultimately carry out their function. To this end, and to extend our study of *Mox* genes during mouse development, Mox-1 and Mox-2 specific polyclonal antibodies were generated and used to analyze the spatial expression patterns of Mox proteins during embryogenesis. We define the onset of *Mox* protein expression, and demonstrate new areas of expression in the branchial arches and limb. In addition, we show that the Mox-2 antibodies display specific cross-reaction to chick tissue, which should allow the use of this reagent in other useful experimental systems.

Onset of expression

To determine the time at which Mox proteins appear, we immunostained several litters of mice spanning the onset of gene expression as determined by our previous *in situ* RNA analysis (Candia *et al.*, 1992).

Mox-1

Sections of a litter of gastrulation-stage mouse embryos were immunostained to pinpoint the start of Mox-1 protein expression. Figure 1A and B show Mox-1 immunostaining in parasagittal sections of two different mouse embryos at approximately 7.5 d.p.c., before the formation of definitive somites. In these embryos, nuclear Mox-1 signal marks the presumptive paraxial mesoderm, but not the anterior cardiac mesoderm, nor the most recently formed mesoderm towards the posterior of the embryo in the primitive streak. The closeness of age between the two embryos in Figure 1A and B suggests that detectable Mox-1 protein expression begins between 7.25 and 7.5 d.p.c.

Abbreviations used in this paper: *Mox*, mesoderm/mesenchyme specific homeobox gene; d.p.c., days post-coitum.

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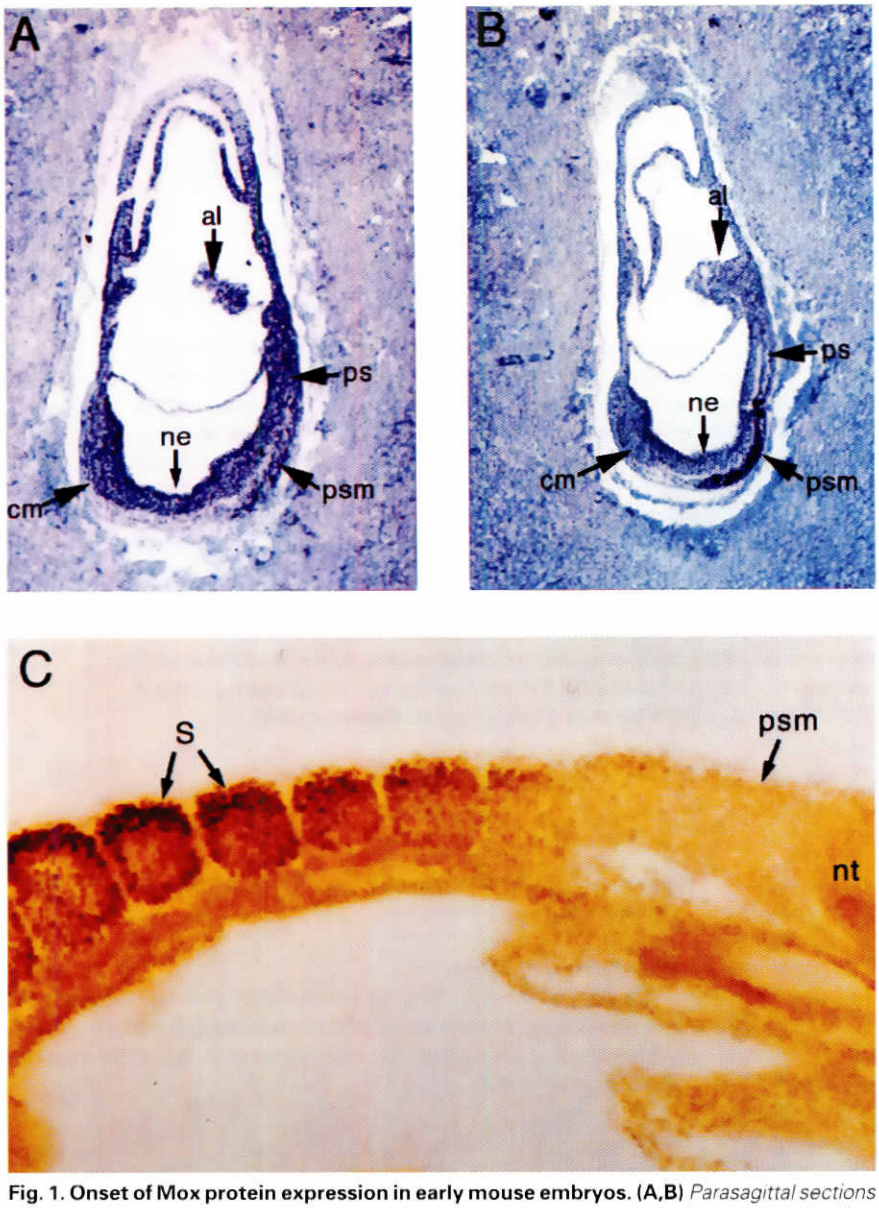

Fig. 1. Onset of Mox protein expression in early mouse embryos. (A,B) *Parasagittal sections of two different 7.5 d.p.c. embryos from the same litter immunostained with Mox-1 antibodies. Staining occurs only in the presomitic mesoderm (psm) and not the mesoderm of the primitive streak (ps) or cardiac mesoderm (cm). The lack of expression in* **A** *suggests that expression of Mox-1 protein begins between 7.25 and 7.75 d.p.c., but before the formation of somites.* **(C)** *Parasagittal section of a whole-mount Mox-2 immunostained 9.0 d.p.c. embryo. Expression of Mox-2 is detected in only the newly formed somites (s) and not the presomitic mesoderm. Anterior is to the left in all photographs. Other abbreviations: al, allantois; ne, neurectoderm.*

Mox-2

In contrast to Mox-1, Mox-2 protein is not detected by immunohistochemistry during gastrulation (data not shown, Fig 2B). Whole-mount immunostaining of a litter of mice at 9.0-9.5 d.p.c. revealed that specific Mox-2 immunostaining appears at approximately 9.0 d.p.c., at which stage it uniformly labels the epithelial somites (Fig. 1C).

The immunostaining pattern obtained for Mox-1 and Mox-2 during gastrulation stages is consistent with our former *in situ* RNA analysis showing expression over the majority of the mesoderm, but beginning at different stages of embryogenesis (Candia *et al.*, 1992).

Expression during somitogenesis

During somitogenesis (8.0 d.p.c.), Mox-1 protein is detected in all newly formed somites and in the presomitic mesoderm caudal to the somites over a length of approximately 2-3 somite-equivalents of presomitic mesoderm (Fig. 2A,B).

As mentioned above, Mox-2 protein is not detected until 9.0 d.p.c. (Figs. 1C and 2C). At this time, Mox-2 is expressed in all of the formed somites and, unlike Mox-1, cannot be detected in presomitic mesoderm. Neither Mox-1 nor Mox-2 are expressed in the most posterior, newly formed mesoderm of the primitive streak, nor in cardiac mesoderm (Fig. 2A,B,C). However, loosely packed Mox-1 positive cells are clearly detected anterior of the first somite (Fig. 2A), in a location indicating their derivation from the transiently formed cephalic somitomeres. We hypothesize that certain populations of these cells are in the process of migrating to craniofacial areas of Mox-1 expression that were detected previously by *in situ* RNA hybridization (Candia *et al.*, 1992).

At the beginning of somitic differentiation, the somites lose their epithelial character and become divided into the dermamyotome and sclerotome. At this stage, Mox protein expression is detected in all parts of the somite (10.5 d.p.c., Fig. 2D). However, at slightly later stages both Mox-1 and Mox-2 are no longer detected in the myotome (11.5 d.p.c., Fig. 2E and F). At this stage of development the myogenic factors (e.g. myf5, mrf4 and myoD) are known to be expressed at relatively high levels in the myotome (reviewed in Buckingham, 1992). This suggests that the myotome may begin to move towards a differentiated state before the dermatome and sclerotome, and that this is associated with the loss of Mox expression first in this compartment.

Mox protein expression domains appear to be spatially conserved among other vertebrates. During chick neurulation and somitic differentiation, the Mox-2 antibodies cross-react in chick embryonic tissue, marking the dermatome of the somite in a pattern very similar to that seen in mouse embryos (Fig. 2F). We have so far been unable to define conditions under which Mox-1 antibodies display specific cross-reaction with chick embryonic tissue.

Differential expression during organogenesis

The availability of these antibodies has allowed us to define areas of Mox expression that were not detected in our *in situ* hybridization study of *Mox* gene expression (Candia *et al.*, 1992). Moreover, given the great similarities in their expression pattern in

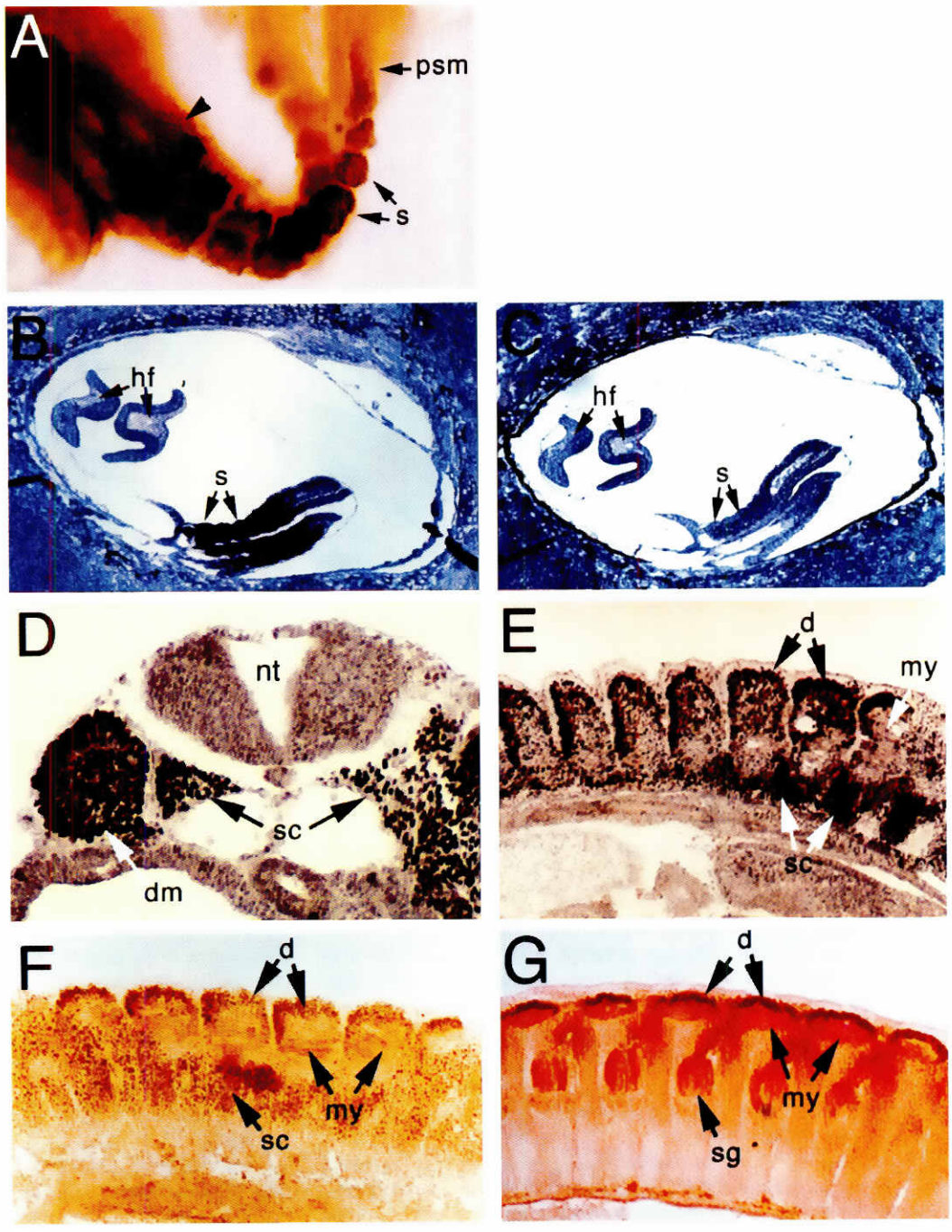

Fig. 2. Expression of *Mox-1* and Mox-2 proteins during somitogenesis. (A) *Whole-mount Mox-1 immunostained 8.5 d.p.c. embryo. Expression of Mox-1 is found in all of the formed somites (s) and approximately 2-3 somite equivalents of presomitic mesoderm (psm). Expression is also detected (arrowhead) in mesodermal cells anterior of somite one.* **(B,C)** *Immunostaining of two serial sections of an 8.5 d.p.c. embryo.* **(B)** *Mox-1 immunostaining is detected in similar tissues as described in* **A**, *and as indicated by the arrowhead, is absent from the mesenchyme of the head-fold (hf).* **(C)** *Mox-2 is not detected during gastrulation and somitogenesis.* **(D)** *Mox-1 expression during the separation of the sclerotome (sc) and dermamyotome (dm). Mox-1 expression continues in all parts of the somite of the 10.5 d.p.c. embryo.* **(E)** *Section immunostaining of an 11.5 d.p.c. mouse embryo with Mox-1 antibodies.* **(F)** *Parasagittal section of a whole-mount, 11.5 d.p.c. mouse embryo immunostained with Mox-2 antibodies. During somite differentiation, expression of both Mox-1 and Mox-2 continues in the dermatome (dm) and sclerotome (sc) but becomes extinguished in the myotome (my).* **(G)** *Mox-2 antibody cross-reacts in chick embryonic tissues. Whole-mount Mox-2 immunostaining of an approximate stage 24 (Hamburger and Hamilton, 1951) chick embryo detects a protein with a similar expression profile to mouse Mox-2 in the dermatome (dm) of the differentiating somite. As in mouse, expression is similarly absent from the chick myotome (my). Other abbreviations: nt, neural tube; sg, spinal ganglion*

the somite, we here define several important differences between the Mox-1 and Mox-2 expression patterns during the organogenesis phases of development.

Truncus arteriosus

The outflow tract from the vertebrate heart is initially comprised of a single tube that subsequently bifurcates into the aorta and pulmonary artery. The bifurcation process occurs by the epithelial-mesenchymal transformation of cells from the truncus endocardium (Markwald *et al.*, 1975, 1977), with the concomitant incorporation of newly-arrived neural crest-derived cells (Phillips *et al.*, 1987; Fukiishi and Morriss-Kay, 1992). At this time, Mox-1 protein is detected at high levels in the delaminated cells, but not in the epithelium (Fig. 3A), consistent with the general conclusion that expression of Mox-1 during embryogenesis is associated with an undifferentiated, proliferative status. Neither immunohistochemistry nor *in situ* RNA localization detect *Mox-2* expression in this region of the heart (Fig. 3B; Candia *et al.*, 1992).

Branchial arches

The branchial arches (BA) are outgrowths of neural crest-derived mesenchymal tissue that interact with the overlying epithe-

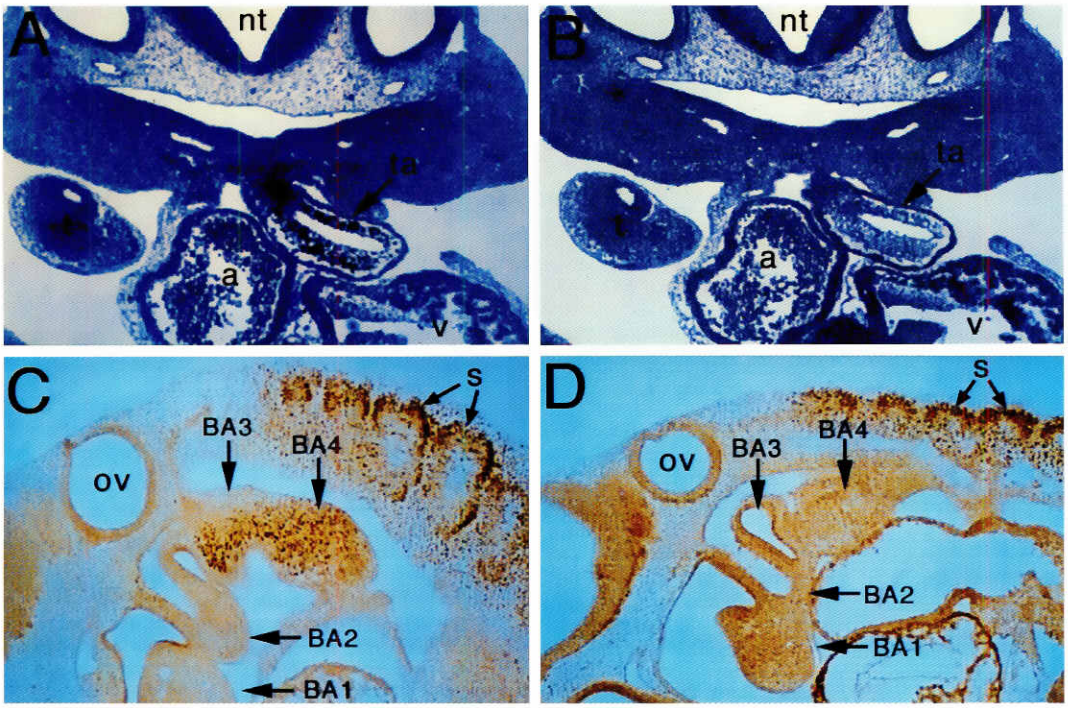

Fig. 3. Unique areas of Mox expression during organogenesis. *Two serial sections of an 11.5 d.p.c. mouse embryo showing expression of Mox-1* **(A)** *but not Mox-2* **(B)** *protein in the outflow tract. The dark blue nuclear staining indicates expression is detected in cells delaminating from the endocardium and is absent from the epithelium.* **(C,D)** *Mox expression in the branchial arches (BA).* **(C)** *Mox-1 antibodies detects Mox-1 protein in the mesenchymal component of BA 3 and 4.* **(D)** *In contrast, Mox-2 protein is not detected in the BA. Other abbreviations: a, atrium; nt, neural tube; ov, otic vesicle; s, somite; t, tail; v, ventricle.*

lium to form craniofacial structures, and contribute to the heart and vasculature of the thoracic region. While not reported in our original analyses of transcript localization, the immunostaining presented here shows that at least one *Mox* gene product is found in specific areas of the arches. In Figure 3C, Mox-1 is expressed in BA 3 and 4, but appears absent from BA 1 and 2. In these arches, Mox-1 is expressed in the mesenchymal component and not in the epithelium (Fig. 3C). In contrast, we do not detect Mox-2 protein in any of the BAs under these conditions (Fig. 3D). Whole-mount mRNA *in situ* analysis corroborates the expression of Mox-1 in the BA, although a lower level of Mox-2 RNA is detected in BA 1 and 2 (P. Sharpe, personal communication). The failure to detect Mox-2 protein in BA 1 and 2 could reflect a difference in the sensitivity of the two methods or the lack of translation of the *Mox-2* mRNA in these regions.

Mox-2, but not Mox-1, is expressed in presumptive muscle precursors of the limb

In addition to the areas mentioned above, a striking difference in expression of Mox-1 and Mox-2 exists in the developing limb. As shown in Figure 4B, Mox-2 is expressed at relatively high levels in mesenchymal masses in the dorsal and ventral regions of the limb bud, the site of presumptive limb muscle progenitors (Christ *et al.*, 1977; Ordahl and Le Douarin, 1992). We note that Mox-2 antibodies appear not to label the limb muscle progenitor cells during their migration from the ventrolateral margin of the somites, but transiently marks the cells that have already undergone the transition from a migratory state to the beginning of the muscle differentiation program.

Mox-2 immunostaining also labels a population of cells lying between the paraxial mesoderm and the limb buds (Fig. 4B) representing the sclerotomally-derived mesenchyme and precursor cells of the body wall (Christ *et al.*, 1977; Christ and Ordahl, 1995). Mox-1 antibodies detect no Mox-1 protein in the limb bud at these stages, although Mox-1 is also detected in sclerotomally-derived cells and muscle cells that form the body wall (Fig. 4A).

Analogous expression of Mox-2 is also observed in the dorsal and ventral mesenchyme in the limbs of chicken embryos, which further supports the specific cross-reaction of these Mox-2 antibodies with embryonic chick tissues, and the conservation of Mox gene expression patterns in vertebrate species (Fig. 4C).

Potential Mox-1 and Mox-2 functions

The data from this study supplement our initial findings on the expression of the *Mox* homeobox genes during embryogenesis. It is important to mention that we have carefully assessed the onset of Mox protein expression and have shown that Mox-1 protein is first detected during gastrulation (≈7.5 d.p.c.), while Mox-2 protein is first detected during neurulation (≈9.0 d.p.c.). Additionally, several areas of the embryo exhibit unique patterns of expression of Mox-1 or Mox-2 in the limb, truncus arteriosus and branchial arches.

The closeness of age between the two embryos (≈6 hours difference) taken from the same litter in Figure 1A and B suggests that the onset of Mox-1 protein translation occurs after the establishment of the primitive streak, but prior to the formation of somites. At this time *Mox-1* expression is restricted to the paraxial mesoderm. With Mox-2 antibodies, *Mox-2* protein expression is first detected when the somites have already formed at approximately 9.0 d.p.c. Interestingly, Mox-1 and Mox-2 have coincident expression over the entire somite at this time (Figs. 1C, 2D), which would be consistent with the proposal of functional redundancy between *Mox-1* and *Mox-2* at this time of development. Further, during somite differentiation, both proteins exhibit similar expression profiles (Fig. 2E and F). The question of functional redundancy is being addressed by assessing the phenotype of embryos homozygous for null mutations in *Mox-1* and *Mox-2* generated by gene targeting in embryonic stem cells.

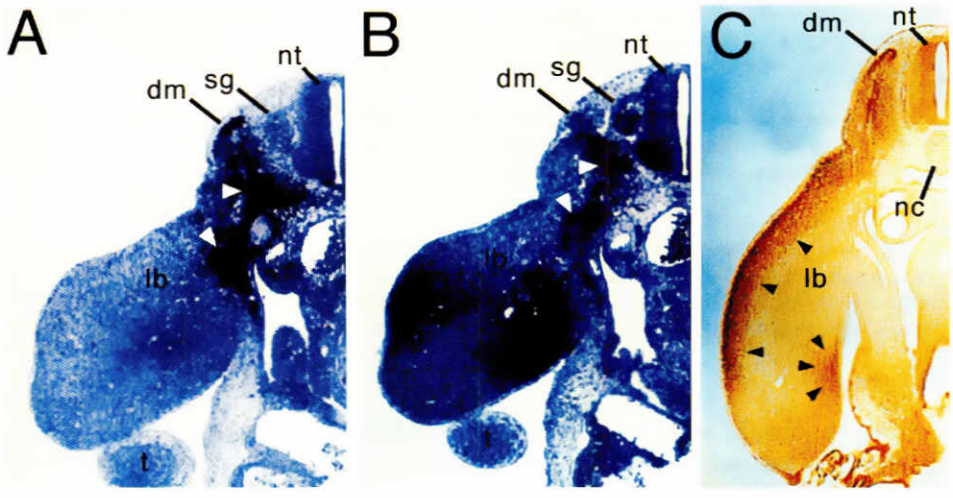

Fig. 4. Expression of Mox proteins in the limb bud. **(A,B)** *Two serial sections of an 11.5 d.p.c. embryo stained with Mox-1* **(A)** *or Mox-2* **(B)** *antibodies. Mox-2 protein is detected in ventral and dorsal mesenchymal patches of the developing limb in the area of the limb muscle precursors. Both Mox-1 and Mox-2 antibodies stain sclerotomally-derived cells and precursor body wall muscle cells (white arrowheads).* **(C)** *Transverse section of a whole-mount, Mox-2 immunostained chick embryo at Hamburger and Hamilton stage 22-24. Cross reaction of Mox-2 antibodies detects similar areas of expression in the chick limb bud (marked by arrowheads) in addition to maintained expression in the dermatome. The apparent signal in the motor neuron region of the neural tube in panel* **B** *represents a staining artefact since Mox-2 RNA is not detected in this region by in situ hybridization analysis (Candia et al., 1992; P. Sharpe, personal communication). Abbreviations: d, dermatome; nc, notochord; nt, neural tube; sg, spinal ganglion; t, tail.*

The highly differential expression of *Mox-1* and *Mox-2* in the limb, truncus and branchial arches indicates areas in older mouse embryos that may have unique requirements for *Mox* family gene function (another hypothesis to be tested by the gene inactivation studies). Nevertheless, a common link between the cells expressing *Mox* gene products in these tissues, as well as in all other tissues during embryogenesis, is that expression occurs within undifferentiated cells that are most likely in the process of determination, but yet to enter differentiation programs. Such an idea is consistent with the inability to detect *Mox* protein or mRNA in differentiating cells (Fig. 2E,F; data not shown; Candia *et al.*, 1992).

Since the *Mox* genes are nuclear factors likely to act as transcriptional regulators of other patterning genes, knowledge obtained by following the protein products as opposed to the transcripts of the *Mox* genes opens an additional level to our understanding of the potential regulation and roles of this gene family during development. These antibodies also add to the limited reagents that are available to study the early steps of mesoderm formation and gastrulation. Finally, their cross-reaction among vertebrate species indicates that they will be useful reagents for molecular studies in other embryonic systems.

Experimental Procedures

Mox-GST fusion proteins

Mox specific antigens were generated by creating fusion proteins with glutathione-S-transferase (Smith and Johnson, 1988). A 402 bp BamHI-Sau3A Mox-1 cDNA fragment encoding amino acids 2-136 was subcloned into pGEX-1. For Mox-2, a PCR generated fragment encoding the first 181 amino acids was subcloned into pGEX-KG (Guan and Dixon, 1991). Both of these regions were chosen to omit the sequences encoding the homeodomain. These constructs produced proteins of the appropriate molecular weight when translated *in vitro* (TNT kit, Promega) and in *E. coli* (data not shown).

GST-Mox constructs were transformed into JM109, fusion protein induced by the addition of IPTG, and harvested and purified from the bacteria according to Smith and Johnson (1988), with modifications as described by Wall *et al.* (1992) and Gamer and Wright (1995). Briefly, induced JM109 cells were lysed by sonication and cellular debris removed by centrifugation. The extracts were mixed with glutathione agarose beads to which the GST fusion proteins bound. The beads were collected by centrifugation and poured into a column which was washed with PBS/0.1% Triton X-100. Fusion protein was eluted from agarose beads with glutathione and frozen at -80°C until use.

Generation of polyclonal Mox antibodies

Polyclonal antisera directed against Mox fusion proteins were produced in female New Zealand White rabbits. The primary inoculant was a 1:1 emulsion of fusion protein and Freund's complete adjuvant. Subsequent boosts were carried out every 5 weeks with a 1:1 emulsion of fusion protein and Freund's incomplete adjuvant. Each injection contained 0.6-0.8 mg of fusion protein, equivalent to 0.2 mg of the Mox-specific antigen. Following the second and later boosts, 50 ml of blood was collected for purification of Mox-specific antibodies. Mox-specific antibodies were isolated by a two step purification method. To deplete GST specific antibodies, serum was mixed with a French-pressed, *E. coli*/GST extract coupled to Sepharose (CNBr activated sepharose, Pharmacia). Depleted serum was then incubated with a specific Mox-GST fusion protein similarly coupled to Sepharose. The slurry was then poured into a column and washed with TBS/0.05% Tween-20. Fractions of Mox-specific antibodies were eluted with 0.15 M glycine, pH 2.5, and neutralized with an equal volume of 2 M Tris, pH 8. Antibodies were tested by western blot analysis for reactivity on nitrocellulose strips containing purified bacterial GST-Mox fusion proteins. Fractions with the highest titer were pooled and split into aliquots that were stored at -80°C.

Specificity of the antibodies was demonstrated by two methods. Mox antibodies solely recognized their respective Mox fusion proteins and not cdx-4 (Gamer and Wright, 1993) or XlHBox8 fusion proteins (Wright *et al.*, 1988; Gamer and Wright, 1995) in western analysis. Additionally, Mox antibodies specifically immunoprecipitated their respective *in vitro* translated, full length proteins (data not shown).

Immunohistochemistry

Section immunostaining was carried out according to Wall *et al.* (1992) with the following modifications. Embryos were fixed in either Dent's fixative overnight or Bouin's fixative for 3-6 h. Antibodies were diluted 1:200 before use. Mox-2 antibodies were preabsorbed to embryonic acetone powder to reduce background.

Whole mount immunostaining was carried out according to Gamer and Wright (1993). Embryos were fixed in Dent's fixative. Antibodies were used at a dilution of 1:30-1:75. Following color development embryos were prepared for sectioning as follows: embryos were dehydrated through

methanols followed by two washes in xylene for at least 1 h. Embryos were passed through a 1:1 mixture of xylene and paraffin wax, followed by wax alone. Embryos were then embedded in paraffin wax and sectioned at 16 μm.

Except where noted in the text, the immunostaining patterns reported here are congruent with *in situ* hybridization expression profiles (Candia *et al.*, 1992; P. Sharpe, personal communication).

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References

BUCKINGHAM, M. (1992). Making muscle in mammals. *Trends Genet. 8:* 144-149.

CANDIA, A.F. and WRIGHT, C.V.E. (1995). The expression pattern of the *Xenopus* homologue of Mox-2 implicates a role in mesodermal differentiation. *Mech. Dev. 52:* 27-36.

CANDIA, A.F., HU, J.X., CROSBY, J., LALLEY, P.A., NADEAU, J.H. and WRIGHT, C.V.E. (1992). Mox-1 and Mox-2 define a novel homeobox gene subfamily and are differentially expressed during early mesodermal patterning in mouse embryos. *Development 116:* 1123-1136.

CHRIST, B. and ORDAHL, C.P. (1995). Early stages of chick somite development. *Anat. Embryol. 191:* 381-396.

CHRIST, B., JACOB, H.J. and JACOB, M. (1977). Experimental analysis of the origin of the wing musculature in avian embryos. *Anat. Embryol. 150:* 171-186.

FUKIISHI, Y. and MORRISS-KAY, G.M. (1992). Migration of cranial neural crest cells to the pharyngeal arches and heart in rat embryos. *Cell Tissue Res. 268:* 1-8.

FUTREAL, P.A., COCHRAN, C., ROSENTHAL, J., MIKI, Y., SWENSON, J., HOBBS, M., BENNETT, L.M. HAUGEN-STRANO, A., MARKS, J., BARRETT, J.C., TAVTIGIAN, S.V., SHATTUCK-EIDENS, D., KAMB, A., SKOLNICK, M. and WISEMAN, R.W. (1994). Isolation of a diverged homeobox gene, MOX1, from the BRCA1 region on 17q21 by solution hybrid capture. *Human Mol. Genet. 3:* 1359-1364.

GAMER, L.W. and WRIGHT, C.V.E. (1993). Murine cdx-4 bears striking similarities to the *Drosophila* caudal gene in its homeodomain sequence and early expression pattern. *Mech. Dev. 43:* 71-81.

GAMER, L.W. and WRIGHT, C.V.E. (1995). Autonomous endodermal determination in *Xenopus:* regulation of expression of the pancreatic gene XlHbox 8. *Dev. Biol. 171:* 240-251.

GUAN, K.L. and DIXON, J.E. (1991). Eukaryotic proteins expressed in *Escherichia coli:* an improved thrombin cleavage and purification procedure of fusion proteins with glutathione-S-transferase. *Anal. Biochem. 192:* 262-267.

HAMBURGER, H. and HAMILTON, H. (1951). A series of normal stages in the development of the chick embryo. *J. Morphol. 88:* 49-92.

MARKWALD, R.R., FITZHARRIS, T.P. and MANASEK, F.J. (1977). Structural development of endocardial cushions. *Am. J. Anat. 148:* 85-120.

MARKWALD, R.R., FITZHARRIS, T.P. and SMITH, W.N.A. (1975). Structural analysis of endocardial cytodifferentiation. *Dev. Biol. 42:* 160-180.

ORDAHL, C.P. and Le DOUARIN, N.M. (1992). Two myogenic lineages within the developing somite. *Development 114:* 339-353.

PHILLIPS, M.T., KIRBY, M.L. and FORBES, G. (1987). Analysis of cranial neural crest distribution in the developing heart using quail-chick chimeras. *Circ. Res. 60:* 27-30.

SMITH, D.B. and JOHNSON, K.S. (1988). Single step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione-S-transferase. *Gene 67:* 31-40.

WALL, N.A. JONES, C.M., HOGAN, B.L.M. and WRIGHT, C.V.E. (1992). Expression and modification of Hox 2.1 protein in mouse embryos. *Mech. Dev. 37:* 111-120.

WRIGHT, C.V.E., SCHNEGELSBERG, P., and DE ROBERTIS, E.M. (1988). XlHbox-8: a novel *Xenopus* homeo protein restricted to a narrow band of endoderm. *Development 104:* 787-794.

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