Cranial paraxial mesoderm: regionalisation of cell fate and impact on craniofacial development in mouse embryos

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SUMMARY

A combination of micromanipulative cell grafting and fluorescent cell labelling techniques were used to examine the developmental fate of the cranial paraxial mesoderm of the 8.5-day early-somite-stage mouse embryo. Mesodermal cells isolated from seven regions of the cranial mesoderm, identified on the basis of their topographical association with specific brain segments were assessed for their contribution to craniofacial morphogenesis during 48 hours of in vitro development. The results demonstrate extensive cell mixing between adjacent but not alternate groups of mesodermal cells and a strict cranial-to-caudal distribution of the paraxial mesoderm to craniofacial structures. A two-segment periodicity similar to the origins of the branchial motor neurons and the distribution of the rhombencephalic neural crest cells was observed as the paraxial mesoderm migrates during formation of the first three branchial arches. The paraxial mesoderm colonises the mesenchymal core of the branchial arches, consistent with the location of the muscle plates. A dorsoventral regionalisation of cell fate similar to that of the somitic mesoderm is also found. This suggests evolution has conserved the fate of the murine cranial paraxial mesoderm as a multiprogenitor population which displays a predominantly myogenic fate. Heterotopic transplantation of cells to different regions of the cranial mesoderm revealed no discernible restriction in cell potency in the cranio-caudal axis, reflecting considerable plasticity in the developmental fate of the cranial mesoderm at least at the time of experimentation. The distribution of the different groups of cranial mesoderm matches closely with that of the cranial neural crest cells suggesting the two cell populations may share a common segmental origin and similar destination.

Key words: cell fate, somitomere, craniofacial development, branchial arches, mouse embryo

INTRODUCTION

Segmentation is a feature characteristic of the axial skeleton, musculature and nervous system of vertebrates (Keynes and Stern, 1984; Lumsden and Keynes, 1989; Puelles and Rubenstein, 1993). Craniofacial structures such as the branchial arches, pharyngeal pouches and cranial nerve ganglia are typically arranged into serially repeated patterns in mammalian embryos (Hunt and Krumlauf, 1991; Hunt et al., 1991a; Thorogood, 1993). The craniofacial tissues are composed principally of neuroectodermally derived neural crest cells and the cranial paraxial mesoderm, both of which are segmentally organised during early embryogenesis (Couly et al., 1993; Noden, 1986, 1988). Recent studies on the ontogeny of cranial neural crest cells have revealed that the neuramic organisation of the neural tube has a significant impact on neural crest differentiation (Wilkinson et al., 1989; Figdor and Stern, 1993; Fraser, 1993; Puelles and Rubenstein, 1993). In essence, neural crest cells destined for different branchial arches or cranial nerve ganglia always arise from specific neuromeres and their migration pattern faithfully follows their original metameric order in the neural tube (Tan and Morriss-Kay 1986; Chan and Tam, 1988; Lumsden, 1990; Lumsden et al., 1991; Serbedzija et al., 1992; Wilkinson and Krumlauf, 1990; Hunt et al., 1991a,b,c). Furthermore, neural crest cells that originate from specific segments of the hindbrain (e.g. rhombomeres r2, r4 and r6) express a similar set of Hoxb genes characteristic of their rhombomeric origins (Hunt et al.,1991a). The emulation of rhombomeric Hox gene expression by neural crest cells suggests that the early segmental organisation of the hindbrain may have long-term effects on cell differentiation and patterning of the craniofacial structures.

Presently it is not known whether the cranial paraxial mesoderm also exerts a similar segmental influence on craniofacial morphogenesis. A meristic pattern of somitomeres (Meier, 1979) has been described in head mesoderm of eight vertebrate embryos, including the mouse (Tam and Trainor, 1994). Somitomeres are clusters of loosely packed mesenchymal cells that form in the paraxial mesoderm along the length of the embryo (Meier and Tam, 1982). The somitomeres in the segmental plate or presomitic mesoderm have been regarded as the primordial structures for somites (reviewed by Tam and
Trainor, 1994). In the mouse, the cranial mesoderm consists of seven pairs of contiguous somitomeres, which maintain a consistent topographical relationship with specific regions of the neural tube (Tam and Meier, 1982; Meier and Tam, 1982; Jacobson and Meier, 1986). Somitomere I (Sm-I) underlies the prosencephalon. Somitomeres II and III (Sm-II and Sm-III) reside lateral to the mesencephalon. Somitomere IV (Sm-IV) is associated with the metencephalon. Somitomeres V, VI and VII (Sm-V, Sm-VI and Sm-VII) underlie the myelencephalon (Fig. 1). However, to this date, lineage restriction and molecular heterogeneity of cells in different somitomeres comparable to those displayed by the neuromeres (Fraser et al., 1990; Puelles and Rubenstein, 1993) have not been found.

In the chick, different groups of voluntary cranial muscles and some components of the chondrocranium are found to be derived from different portions of the cranial paraxial mesoderm (Noden, 1983b, 1988; Couly et al., 1992). Such regionalisation of cell fate has been attributed to the partitioning of the different myogenic and skeletogenic precursors to the cranial somitomeres. The chimaeric avian embryos analysed in these studies had developed beyond the stages of branchial arch and facial primordia formation such that fusion and growth of these structures had already occurred. The early pattern of distribution and the fate of individual cranial somitomeres were not examined, leaving unresolved the question of whether the difference in the myogenic fates between somitomeres is a reflection of the regionalisation of cell fate or a restriction in cell potency. What is also lacking in previous studies of cephalic mesodermal organisation is a clear appreciation of the precise somitomeric origins of the branchial arch mesenchyme. It remains to be seen if, like the neural crest cells, there is a segmental allocation of paraxial mesoderm to craniofacial structures. In the present study, we have taken the first step to analyse the role of somitomeres in craniofacial morphogenesis by testing if there is any regionalisation of cell fate along the craniocaudal axis of the cranial paraxial mesoderm regarding its contribution to serially repeated craniofacial structures of the mouse embryo. Specifically, we aimed (i) to trace the distribution of cells in different segments of cranial mesoderm as defined by the somitomeres and (ii) to determine if the craniocaudal order of cells in the cranial mesoderm is transposed into patterning of craniofacial structures such as the branchial arches. We have also examined the cranial mesoderm for evidence of restriction of cell fate by heterotopic transplantation of lacZ-tagged cells and by differential labelling of different parts of the paraxial mesoderm.

**MATERIALS AND METHODS**

**Experimental strategy**

For tracking the fate of the cranial mesoderm, small groups of mesodermal cells were isolated from specific regions of the paraxial mesoderm (Figs 1, 2A–C), and were then transplanted to an equivalent site in isochronic 8.5-day host embryos. The transplanted cells were marked by the β-galactosidase activity encoded by a lacZ transgene, which is ubiquitously expressed during development (Tam and Tan, 1992). Alternatively, the mesodermal cells were labelled in situ with fluorescent carbocyanine dyes. Heterotopic transplantations of mesoderm to different sites in host embryos were also performed to assess the prospective potency and state of commitment of the cells. In the absence of regional specification, the transplanted cells would develop according to their site of transplantation rather than their origin. Transplantations and labelling of cells were performed by micromanipulating embryos that were explanted from the uterus. Experimental embryos were cultured in vitro for the assessing the fate of the cells.

**Recovery and in vitro culture of host embryos**

Early-somite-stage embryos were obtained from pregnant female mice of ARCS strain at 8.5 days post coitum (p.c.). Whole conceptuses were dissected out from the uterus. The parietal yolk sac was removed leaving the embryo proper with an intact visceral yolk sac, amnion and ectoplacental cone. Previous studies on the migration of cranial neural crest cells in the mouse embryo have established that the first population of neural crest cells to leave the neural tube are those of the mesencephalon and it happens at about 5- to 6-somite stage (Jacobson and Tam, 1982; Chan and Tam, 1986, 1988; Serbedzija et al., 1992). To ensure that only the paraxial mesoderm and no neural crest cells were labelled or grafted, we applied a stringent criterion that only embryos having ≤5 pairs of somites were used for the experiments. After cell grafting or labelling, embryos were cultured in vitro for 48 hours (Sturm and Tam, 1993).

**Isolating donor transgenic tissue and grafting**

8.5-day embryos were obtained from matings of transgenic H253 mice, which express a bacterial lacZ transgene under the control of the 3-hydroxy-3-methylglutaryl co-enzyme A (HMG-CoA) reductase promoter (Tam and Tan, 1992). Donor H253 embryos were first bisected along their midline (Fig. 2A). Wedge-shaped fragments containing the paraxial mesoderm and the associated neuroectoderm and surface ectoderm (Fig. 2B) were isolated by cutting the embryo transversely at various neuromeric junctions (Fig. 2A).

![Fig. 1. The subdivision of the cranial paraxial mesoderm into seven (I to VII) somitomeres (Meier and Tam, 1982) in accordance to their topographical relationship to major segments of the prosencephalon (Pro), mesencephalon (Mes), Metencephalon (Met) and Myelencephalon (Mye). The short dashes demarcate the boundaries between brain segments. The first 2 somites are numbered and HT denotes the heart.](attachment:fig1.png)
The embryonic fragments were then incubated in a solution of 0.5% trypsin, 0.25% pancreatin, 0.2% glucose and 0.1% polyvinylpyrrolidone in PBS for 20 minutes at 37°C to loosen the mesoderm and ectoderm layers. Finely polished alloy and glass needles were used to separate the ectoderm from the underlying mesoderm (Fig. 2C). The mesoderm was dissected into fragments containing approximately 30 cells which were then grafted into the cranial mesoderm of the host embryo (Fig. 2D). For the purpose of this study, the mesoderm isolated from different cranial fragments and the sites to which they were transferred are referred to by their somitomeric designation, i.e. Sm I-VII (Fig. 1).

**Transplantation experiments**

1. **Orthotopic transplantations of cranial mesoderm to isochronic hosts**

Donor mesoderm derived from Sm I-VII was transplanted to the equivalent somitomere in host embryos (Fig. 2E). Seven types of transplantation were performed, designated I→I, II→II, III→III, IV→IV, V→V, VI→VI or VII→VII, to denote the somitic origin of donor cells and the site of transplantation, respectively.

2. **Heterotopic transplantations of somitomeric mesoderm to isochronic hosts**

In the first set of experiments, donor mesoderm from somitomeres I, II, III, IV, V and VI was transplanted to different somitomeres in host embryos. In the second set, somitomeres I, II, IV, V and VI were chosen to demonstrate how tissues both rostral and caudal to somitomere III develop when ectopically grafted to somitomere III. Nine experiments designated III→I, III→II, III→IV, III→V, I→III, II→III, IV→III, V→III and VI→III referring to the source and destination of the donor mesoderm were performed. Somitomere III was chosen respectively as a source of donor mesoderm and a site for transplantation because it is centrally located within the head. This allowed us to assess its development in ectopic positions in the head both rostral and caudal to its normal axial position.

**Analysis of the results of transplantation experiments**

Host embryos were harvested after 48 hours of in vitro culture and grossly abnormal embryos were discarded. Embryos were fixed in 4% paraformaldehyde for 30 minutes and stained by X-gal histochemistry to reveal the β-galactosidase activity (Tam and Tan, 1992). Embryos containing blue X-gal-stained cells were processed for wax histology and counterstained with nuclear fast red. The location of labelled cells in structures such as the branchial arches and craniofacial mesenchyme was determined on serial sections of the embryo.

**Preparation of fluorescent dyes for injection**

0.05% solutions (w/v) of DiI (1,1-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate) and DiO (3,3′-dioctadecyloxacarbocyanine perchlorate, Molecular Probes) were made from a 0.5% stock ethanolic solutions. The stock was diluted 1:7.5 in 0.3 M sucrose immediately before injection. Micropipettes were backfilled with dye solution and injection was done under positive pressure generated by a deFonbrune oil pump (Alcatel).

**Dye labelling of individual somitomeres**

The same topographical landmarks employed for locating somitomeres in the transplantation experiments were also used for targeting the dye injection. Following insertion of the micropipettes into the

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**Table 1. Summary of the patterns of tissue colonisation in transplantation and labelling experiments**

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**Abbreviations.** FNM, frontonasal mass; POM, periocular mesenchyme; MXP, maxillary component of first arch; MDP, mandibular component of the first arch; FM, facial mesenchyme; SA, second arch; CV, cervical mesenchyme; PTM, periotic mesenchyme; TA, third arch; FA, fourth arch.
mesoderm, a small amount of DiI or DiO solution was expelled and the labelled sites were visible through the dissecting scope as red (DiI) or orange (DiO) patches of labelled mesoderm (Fig. 2F). For testing dorsoventral regionalisation, different parts of somitomeres III and IV were labelled with different fluorescent dyes (Fig. 2G). The ventral region of the somitomere was labelled first, which provided the reference point for a dorsal label. The two injection sites were always separated by a zone of unlabelled cells.

**Analysis of the results of dye labelling experiments**

Embryos cultured in vitro for 48 hours were viewed without any fixation as whole mount under a standard fluorescence microscope.

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**Fig. 2.** (A) The bisected halves of a 8.5-day (5-somite) mouse embryo showing the somitomeric boundaries (arrows). Transverse cuts made at these specific neuromic landmarks resulted in isolation of single somitomeres. (B) Embryonic fragment containing the Sm-III and its associated surface ectoderm and neuroectoderm obtained by cutting the half-head at mid-mesencephalon and at the mesencephalon-metencephalon boundary. (C) The wedge-shaped somitomeric mesoderm (arrowhead) dissected free of the surface ectoderm and neural plate. (D) Grafting somitomeric cells to a 8.5-day early-somite-stage embryo by inserting the injection micropipette through the extraembryonic membranes into Sm-III. (E) A 8.5-day embryo examined 2 hours after transplantation to reveal the location of the tissue grafts (X-gal-stained blue cells), which were grafted into the dorsal region of Sm-I and the caudal-ventral region of Sm-IV (arrowheads). (F) A 8.5-day embryo examined immediately after DiI injection into Sm-I, Sm-III, Sm-IV and Sm-VI (arrows). (G) A 8.5-day embryo with the ventral and dorsal regions of Sm-III labelled by injection of DiO (arrow) and DiI, respectively. Abbreviation: hb, hindbrain; ht, heart; mb, midbrain. Bar, 100 mm.
Fig. 3. For legend see p. 2402
RESULTS

Cranial somitomeric mesoderm contributes to branchial arch formation

Table 1 summarises the pattern of distribution of transplanted cells and DiI/DiO-labelled cells to various craniofacial structures in the mouse embryo after 48 hours of culture. Embryos to be analysed were selected on the basis that (i) they are morphologically similar to the 30- to 36-somite-stage embryo in vivo, (ii) the branchial arches were well formed and (iii) both the forelimb and hindlimb buds had developed.

Results of the transplantation and labelling experiments showed that the cranial mesoderm contributes cells to the first four branchial arches in a manner strictly in accordance with their craniocaudal order in the axis (Table 1a,b). Each of the first three branchial arches, were colonised by cells from neighbouring regions of the paraxial mesoderm that are defined by two consecutive somitomeres: namely Sm-II and III for first arch, Sm-IV and V for the second arch and Sm-VI and VII for the third arch (Table 1a,b; Fig. 5). In contrast, only one somitomere participated in fourth arch development and the fifth and sixth pairs of branchial arches did not receive any contribution from the cranial mesoderm.

Both II→II grafts and Sm-II dye injection experiments revealed that mesoderm derived from Sm-II extensively colonised the proximal maxillary prominence of the first branchial arch (Fig. 3A). In 8% (2/25) of cases where Sm-II was labelled with either DiO or DiI and in 10% (1/10) of II→II grafts, a small number (5-10) of cells were displaced to the distal region of the first arch which gives rise to the mandibular prominence (Fig. 3B). Mesoderm derived from Sm-III colonised both the mandibular and the maxillary prominences of the first branchial arch (Fig. 3C). There was a widespread distribution of grafted cells in the facial mesenchyme surrounding the proximal end of the first arch. Cells derived from Sm-III were also found in the maxillary component of the arch, overlapping with that of Sm-II (Fig. 3D). Histology of the first arch revealed that the graft-derived cells were localised in the central to medial core mesenchyme (Fig. 3E). Sm-II and Sm-III did not participate in morphogenesis of more caudal branchial arches. The distribution pattern of Sm-III when labelled with DiI resembled closely that observed for grafted mesoderm (Fig. 3F). The fluorescent mesoderm again colonised the central regions of the maxillary and mandibular prominences. The distribution of fluorescent cells, however, seemed broader than that observed for the grafted cells. The quantitative difference is due to the fact that more cells are labelled through dye injection than are transplanted. There were no instances in which the mandibular epiblast or the surface ectoderm were colonised with mesoderm derived from Sm-II and Sm-III.

The second branchial (hyoid) arch received contribution from the preotic mesoderm associated with the upper hindbrain (Sm-IV and Sm-V). Mesoderm derived from Sm-IV, which underlies the metencephalon, contributed primarily to the central region of the hyoid arch (Fig. 3G). The distribution of IV→IV grafted cells was widespread in the rhombencephalic mesenchyme adjacent to the second arch but, within the arch, the mesoderm colonised a central core region (Fig. 3H). Marking somitomere IV with DiI produced similar results to IV→IV grafting experiments, with the labelled cells populating the hyoid arch (Fig. 3I). Sm-V, which underlies the rostral myelencephalon, also contributed to the hyoid arch. There was a clear segregation of donor mesoderm derived from the dorsal and ventral regions of Sm-V respectively such that the dorsal mesoderm was found in a more proximal position in the second arch than the ventral mesoderm (Fig. 3J). The distribution of Sm-V-derived cells overlapped significantly with that of Sm-IV. There was no evidence of Sm-IV and Sm-V contributing to formation of either the first or third branchial arches. In one instance only, the investing mesenchyme and epithelium of the first pharyngeal pouch were populated with Sm-V-derived mesoderm (data not shown).

Similar to the first two arches, the third arch received a dual contribution from two adjacent somitomeres, Sm-VI and Sm-VII of the caudal myelencephalon. VI→VI grafts showed that the mesodermal cells were mostly confined to the central region of the third arch (Fig. 3G). Sm-VI did not contribute to formation of first, second or fourth branchial arches. Sm-VII, which is the causal most cranial somitomere, participates primarily in formation of the third arch. The distribution of VII→VII grafted cells in the third arch overlapped with cells derived from Sm-VI. VII→VII grafts also reveal that Sm-VII cells were found in the central core region of the fourth branchial arch.

Prosencephalic and mesencephalic mesoderm contribute to the periorcular, frontoasal and facial mesenchyme

The two most rostral somitomeres (Sm-I and Sm-II), con-
tributed extensively to the periocular mesenchyme. I→I and
II→II grafts showed that the rostral and ventral portions of the
periocular mesenchyme were derived from Sm-I, whereas the
dorsal and caudal periocular mesenchyme came primarily from
Sm-II (Fig. 4A,B). There was, however, some overlap in the
distribution of Sm-I and Sm-II cells. In 9% (1/11) of I→I trans-
plantations, the dorsal and caudal periocular mesenchyme were
colonised by graft-derived cells. In 10% (1/10) of II→II grafts,
Sm-II-derived cells contributed to the rostral periocular mes-
encephyme and also the ventral periocular mesenchyme in the
vicinity of the primitive oral cavity. Similar but broader dis-
tribution patterns were observed for Sm-I and Sm-II, respec-
tively, when they were directly injected with DiI (Fig. 4C). In
addition to the periotic mesenchyme, Sm-I and Sm-II con-
tributed to the endothelium of blood vessels surrounding the
optic epithelium (data not shown). These two somitomeres also
contributed extensively to the facial mesenchyme lateral to the
telencephalon and diencephalon. The frontal area of the
embryo associated with the lateral and medial nasal processes
was colonised by Sm-I only (Fig. 4A).

Sm-III colonised the facial mesenchyme and some vascular
and endothelial tissue lateral to the caudal midbrain and meten-
cephalon (Fig. 4E). The distribution of Sm-III cells overlapped
with Sm-II cells in the facial mesenchyme lateral to the
midbrain and with Sm-IV cells in the region lateral to the
rostral hindbrain, adjacent to the mandibular and hyoid arches.
III→III grafts showed that Sm-III colonised the branchial arch
tissue, the connective tissue surrounding the truncus arteriosus
and the endothelium of the primary head vein (anterior cardinal
vein).

Rhombencephalic mesoderm contributes to the
periotic and cervical mesenchyme

The otic placode develops superficially to Sm-VI and VII. The
periotic mesenchyme was derived from Sm-IV, V, VI and VII.
IV→IV and V→V grafts revealed that Sm-IV and Sm-V con-
tributed significantly to both the rostral and rostroventral
periotic mesenchyme, respectively, and to the facial mes-
encephyme associated with the rostral hindbrain (Figs 3J, 4F).
VI→VI and VII→VII grafts showed that the ventral and the
caudal periotic mesenchyme were colonised by mesoderm
derived from Sm-VI and Sm-VII, respectively (Figs 3G, 4G).
Sm-VI and Sm-VII also contributed to the cervical mesenchyme
associated with caudal segments of the hindbrain and to the
dorsal periotic mesenchyme, which is bounded by the
otic placode and neuroectoderm. In one instance, Sm-VI-
derived mesoderm transplanted into Sm-VI (VI→VI graft)
colonised the otic epithelium. This was probably the result of
grafting cells too superficially to the surface ectoderm. A
summary of the distribution of somitomeric mesoderm to cran-
iofacial structures is presented in Table 2 and Fig. 5.

Evidence for dorsoventral constraints

The relative positioning of cells within cranial somitomeres
was maintained during their deployment to craniofacial struc-
tures, such that the dorsal and ventral regions of a somitomere
have different fates with respect to craniofacial morphogene-
sis. Table 1d summarises the results of experiments in which
the ventral and dorsal mesoderm of Sm-III and Sm-IV were
labelled with DiO and Dii, respectively. There was a strong
tendency for the ventral mesoderm of Sm-III to colonise the
distal mandibular and maxillary regions of the first branchial
arch. Progressively more dorsal mesoderm derived from Sm-
III contributed to the maxillary and more proximal regions of
the first arch, and to the facial mesenchyme (Fig. 4H). Similarly,
the ventral Sm-IV mesoderm was confined ultimately to the
hyoid arch proper and the dorsal mesoderm con-
tributed to the cervical mesenchyme and to the proximal region
of the hyoid arch (data not shown). Although some cell mixing
occurred at the interface between the DiO- and Dii-labelled
populations, mesoderm derived from the dorsal region of the
somitomere seldom colonised the distal region of the branchial
arch. Conversely ventrally derived mesoderm never con-
tributed to the facial mesenchyme adjacent to the neural tube.
These results indicate that regionalisation of cell fate may exist
in the cranial mesoderm. The dorsoventral partition within
somitomeres is transposed into a proximal-distal disposition of
cells in the branchial arches and adjacent paraxial mes-
enchyme.

The fate of the cranial paraxial mesoderm is not
positionally restricted

Heterotopic transplantations showed that mesoderm derived
from any axial level, when grafted to a heterotopic site would
develop according to its new position and not its site of origin.
Table 1c summarises the results of several types of heterotopic
transplantation. Sm-III due to its central location in the head,
was chosen as the source of donor cells for most of the grafts
(45%; 24/53). Sm-III mesoderm was grafted into somitomeres
both rostral and caudal to its origins (III→I, III→II, III→IV,
III→V). The final distribution of graft-derived cells was
always characteristic of the site of transplantation. For example,
in III→V transplantation experiments, the mesoderm
cells contributed to the hyoid arch mesenchyme and to the
rostral periotic mesenchyme (Fig. 4I). This was almost
identical to the distribution pattern observed from V→V grafts
(Fig. 3J). Similarly in III→II transplantations, the pattern of
distribution resembled very closely II→II grafts: the mesoderm
colonised to the caudal and ventral periocular mesenchyme,
to the mesenchyme lateral to the diencephalon and upper
midbrain, and also to the maxillary component of the first
branchial arch (Table 1c). As expected there was no colonisa-
tion of the mandibular component of the first arch, previously
shown to be derived from Sm-III (Fig. 3C and 3D). Hetero-
topic transplantations were also performed for Sm-I. The
distribution of cells observed after I→III grafts showed that the
ectopic mesoderm cells colonised the maxillary prominence
and facial mesenchyme (Fig. 4J). This pattern of colonisation
was characteristic of III→III grafts. The grafted mesoderm did
not contribute to the mesenchyme of the frontal area, nor to the
rostral and ventral periocular mesenchyme, which were deriva-
tives of I→I grafts (Fig. 4A). Mesoderm cells derived from
Sm-IV (IV→III), V (V→III) and VI (VI→III) were also het-
erotopically grafted into Sm-III. The grafted mesoderm exten-
sively colonised the facial mesenchyme, the maxillary and
mandibular prominences and the perivascular tissues of the
truncus arteriosus (Fig. 4K,L). In each case where cranial
mesoderm was derived from different cranio-caudal levels and
grafted into Sm-III, it always developed in a manner charac-
teristic of III→III grafts. These results suggest that the normal
fate of the cranial paraxial mesoderm is not positionally or
somitomERICally restricted at least at 8.5 days p.c. when the het-

**DISCUSSION**

The impact of regionalisation of cell fate in the cranial paraxial mesoderm on craniofacial patterning

Results of cell transplantation and fluorescent dye marking experiments reveal that the cranial paraxial mesoderm participates extensively in craniofacial morphogenesis and in particular impacts upon the development of the branchial arches (Table 2). There is an orderly cranio-caudal distribution of cells from different regions of the cranial mesoderm to the craniofacial tissues in the mouse embryo (Table 1a,b). A previous morphological study has revealed that the murine cranial mesoderm is segmented into clusters of cells known as somitomeres (Meier and Tam, 1982). Each of the seven somitomeres are related topographically to a particular segment of the brain (Jacobson, 1993; Tam and Trainor, 1994). Our discovery of a regionalisation in cell fate may argue for the presence of a segmental prepattern in the cranial mesoderm although the findings neither require the existence of somitomeres nor give support for their existence. For clarity of discussion, however, we shall refer to the different fragments of cranial mesoderm by their somitomeric address.

By following the distribution of cells from individual somitomeres...
omeres to the branchial arches, it was found quite unexpectedly that each branchial arch is colonised by cells from a set of two consecutive somitomeres. The mandibular arch is derived form somitomeres II and III. Somitomeres IV and V contribute to hyoid arch development and somitomeres VI and VII form part of the visceral arch mesenchyme. The fourth arch receives a cranial mesoderm contribution from somitomeres VII only. However, an additional contribution by the most rostral cervical somites cannot be ruled out. The fifth and sixth pairs of branchial arches were not colonised by any somitomeric mesoderm cells. It is interesting to note that this pairwise restriction of cell distribution only occurs in the branchial arches. Outside the arches, cells from adjacent somitomeres mixed extensively in the pericranial, facial and cervical mesenchyme. Cell mixing, however, is minimal between alternate somitomeres.

The contribution by dual somitomeres to each branchial arch is reminiscent of the so-called ‘two segment periodicity’ associated with the derivation of the branchial arch motor neurons from pairs of rhombomeres (Lumsden and Keynes, 1989). The motor nerves innervating the three arches (trigeminal, facial and glossopharyngeal) develop from nuclei found in consecutive pairs of rhombomeres (r2 and r3 for trigeminal; r4 and r5 for facial; r6 and r7 for glossopharyngeal; Lumsden, 1990; Lumsden et al., 1991; Hunt et al., 1991a). The segmentally repeating patterns of rhombomeres and motor neurons are associated with the differential expression of genes such as Krox-20, Hoxb-1 and Sek (Wilkinson et al., 1989; Nieto et al., 1991). Differential expression of a similar set of genes is also found in the migrating neural crest derived from specific rhombomeres (Hunt et al., 1991a). All the known genes for embryonic mesoderm however, such as Mox1, Mox2 (Candia et al., 1992), M-twist (Wolf et al., 1991), Sek (Nieto et al., 1992) and S8 (de Jong and Meijlink, 1993) do not display any spatially restricted patterns of expression that may reflect the initial somitomeric organisation or the distribution of the mesenchymal derivatives from these somitomeres (Tam and Trainor, 1994).

**Cranial mesoderm does not display any positional identity in developmental fate**

The orderly and predictable distribution of cells from specific somitomeres to different craniofacial structures may imply that a stringent specification of developmental fate has been endowed on individual somitomeres. A comparison of the disposition of cells from within the dorsal and ventral components of the paraxial mesoderm to different parts of the branchial arches further reinforces the view that some form of regional restriction in potency may be present. Cells in the ventral region of the cranial mesoderm contribute to the distal mesenchyme of the mandibular and hyoid arches, respectively. Progressively more dorsal regions contribute to tissues in the proximal regions of the arches and preferentially to facial mesenchyme. Whether the maintenance of the dorsoventral segregation of these two cell populations is the result of diminished cell mixing or an intrinsic regional difference in tissue’s fate has not been tested. It has been shown in the chick, however, that regionalisation of cell fate does exist between the medial and lateral cell populations in the cranial mesoderm (Couly et al., 1992).

Cell fate analysis following heterotopic transplantation revealed that cells taken from any axial level of the cranial mesoderm can contribute to formation of the maxillary and mandibular prominences of the first arch in a manner typical of the caudal mesencephalic mesoderm to which they are transplanted. Similarly mesoderm ectopically grafted into Sm-II or Sm-IV will migrate in a manner characteristic of tissues at the native site. These results indicate that in the 8.5-day early somite-stage mouse embryo, the differentiative pathway that could be taken by cranial mesoderm is not positionally restricted. Our results agree with previous studies in the chick, which demonstrate remarkable morphogenetic plasticity of the paraxial mesoderm (McLachlan and Wolpert, 1980; Noden 1982; Cauwenbergs et al., 1986; Butler et al., 1988).

**Co-distribution of cranial mesoderm and the neural crest cells**

The distribution of cranial mesoderm to the frontonasal and branchial regions (this study) is generally similar to that of the neural crest cells originating from the same axial level (Lumsden and Keynes, 1989; Serbedzija et al., 1992). Mesoderm from the first two somitomeres and the forebrain and upper mesencephalic neural crest cells contribute primarily to the periorcular region in a similar pattern to the somitomeric mesoderm. The maxillary region of the first arch is colonised by mesenchyme derived from Sm-II and also the mesencephalic crest (Lumsden et al., 1991; Serbedzija et al., 1992; Osumi-Yamashita et al., 1994). Neural crest cells from the caudal mesencephalon and the mesoderm of Sm-III both migrate into the distal region of the mandibular arch. The preotic or metencephalic neural crest cells and mesoderm derived from Sm-IV and Sm-V both contribute to hyoid arch morphogenesis. A similar concordance of cell contribution is also found for the postotic or myelencephalic neural crest cells and Sm-VI and SM-VII, which collectively colonise the third branchial arch. Therefore cranial neural crest cells and the somitomeric mesoderm generally share a common segmental
origin and final destination. This raises the question of how the two tissue populations are spatially distributed at their final destinations. The Dlx-2 gene, which is a vertebrate homologue of the *Drosophila Distal-less* gene, is uniformly expressed throughout the arches excluding a core of mesenchymal cells (Bulfone et al., 1993). These mesenchymal arch cores, which are devoid of Dlx-2 gene expression, are consistent with the location of the mesodermally derived muscle plates. Migrating neural crest cells also express Dlx-2. This suggests that, in the branchial arches, the muscle plates, which are mesodermally derived become enveloped by neural crest cells. This could be important as it has been shown that, in the limb and the face of the chick embryo, the region-specific organisation of muscle groups depends critically upon the connective tissue pattern set up locally by the neural crest cells (Chevallier, 1979; Chevallier and Kieny, 1982; Noden, 1982, 1983a, 1986). The co-distribution of the craniofacial progenitors could therefore be important for morphogenetic interactions that lead to the establishment of tissue patterns during craniofacial development. The somitomeric organisation of the paraxial mesoderm, if it does exist, may be a part of the metameric patterning process that operates during the establishment of the neuromeres and the cranial neural crest cells.

### A presumptive myogenic fate of cranial mesoderm in the branchial arches

Cells derived from the cranial somitomeres colonise the mesenchymal core of the branchial arches. Interestingly, mesenchyme at the core of newly formed branchial arches has been found to express muscle-specific genes such as *Mnf5* (Ott et al., 1991) and *Htx* (R. Harvey, per comm) at 9.25 to 10.0 days. This mesenchyme later condenses into a myogenic mass known as the muscle plate of the branchial arch (Bulfone et al., 1993). In the chick, the cranial mesoderm displays a predominantly myogenic fate (Noden, 1983b). More significantly, the precursors of different groups of craniofacial muscles and skeletogenic cells are regionalised craniocaudally in specific sets of somitomeres (Table 2). For example, the myoblasts of the extrinsic ocular muscles in the chick were derived from Sm-I and Sm-II and the jaw opening and hyobranchial muscles came from Sm-VI and Sm-VII. Although the mouse culture system only permits in vitro development up to about 10.5 d.p.c. and therefore does not allow a definitive elucidation of the final histogenetic fate of the cranial somitomeres, parallel findings in the chick and the initial localisation of somitomeric cells to the myogenic regions of the branchial arch are strongly suggestive of a myogenic fate for the cranial somitomeres in the mouse.

However, other genes not specific for the myogenic lineage, such as the type II collagen gene (*Col2a-I*, Cheah et al., 1991; Ng et al., 1993) and the murine homologue of the human *Hox11* gene (*Tlx-I*, Raju et al., 1993) are also expressed in the so-called muscle plate. It has been shown recently in the avian embryo that cranial somitomeres also participate, in addition to the differentiation of craniofacial muscle, in the formation of skull bones such as the orbitosphenoid, sphenoidalis and otic capsule (Coulby et al., 1992). The expression of *Col2a-I* gene in the mesenchyme colonised by the somitomeric cells therefore suggests that cranial somitomeres may also contribute to the formation of at least part of the facial skeleton which previously was thought to be entirely derived from neural crest cells (Le Lievre, 1978; Noden, 1978; Le Douarin, 1982). Finally, the murine cranial mesoderm also contains vascular or endothelial tissue precursors just as in the avian embryos (Peault et al., 1983; Pardanaud et al., 1987; Coffin and Poole, 1988; Noden, 1988; Poole and Coffin, 1988). Somitomeres differentiate to form supporting tissues of the primitive cephalic vein and also contribute frequently to the endothelium of branchial arch arteries. The cranial paraxial mesoderm therefore consists of a multiprogenitor mesodermal cell population.

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### REFERENCES


Fraser, S. E., Keynes, R. J. and Lumsden, A. (1990). Segmentation in the chick embryo hindbrain is defined by cell lineage restrictions. *Development** 102, 453-455.


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