Interactions and fates of avian craniofacial mesenchyme

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Summary

Craniofacial mesenchyme is composed of three mesodermal populations — prechordal plate, lateral mesoderm and paraxial mesoderm, which includes the segmented occipital somites and the incompletely segmented somitomeres — and the neural crest. This paper outlines the fates of each of these, as determined using quail–chick chimaeras, and presents similarities and differences between these cephalic populations and their counterparts in the trunk.

Prechordal and paraxial mesodermal populations are the sources of all voluntary muscles of the head. The latter also provides most of the connective precursors of the calvaria, occipital, otic–parietal and basi-sphenoid tissues. Lateral mesoderm is the source of peripharyngeal connective tissues; the most rostral skeletal tissues it forms are the laryngeal and tracheal cartilages.

When migrating neural crest cells encounter segmented paraxial mesoderm (occipital and trunk somites), most move into the region between the dermamyotome and sclerotome in the cranial half of each somite. In contrast, most cephalic crest cells migrate superficial to somitomeres. There is, however, a small subpopulation of the head crest that invades somitomeric mesoderm. These cells subsequently segregate presumptive myogenic precursors of visceral arch voluntary muscles from underlying mesenchyme.

Key words: mesoderm, craniofacial development, avian embryo, neural crest, muscle, blood vessels.

Definitions and problems

Head mesoderm is initially composed of three spatially segregated but contiguous populations. The mesoderm of the prechordal plate, which is the first formed, is a sparse, median condensation of mesenchyme extending rostrally from the tip of the notochord, beginning beneath the mid-mesencephalon (Adelmann, 1922, 1927; Meier, 1981). At the time of head folding, most prechordal mesodermal cells are displaced laterally; the population becomes integrated with and indistinguishable from paraxial mesoderm.

Flanking the brain, spinal cord and notochord is paraxial mesoderm (Fig. 1). Caudal to the level of the mid-myelencephalon paraxial mesoderm forms somites, which are transient, fully segmented, epithelial condensations (reviewed in Hay, 1968; Bellairs, 1985). In contrast, paraxial mesoderm from the otic level forward does not become epithelial, nor
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SEM examination of this population in all vertebrate classes has revealed the presence of a series of superficial transverse furrows (Meier, 1981; Meier & Tam, 1982; Meier & Packard, 1984; but see Wachtler & Jacob, 1986). The mesenchymal masses partially delineated by these furrows are called somitomeres; there are seven somitomeres in those birds and mammals that have been examined, fewer in anamniotes (see Holland, this volume). Caudal to the seventh somitomere is a complete cleft, behind which the first somite develops.

The paraxial mesoderm of the occipital region is fully segmented. The first somite becomes mesenchymal within a day of its appearance, and somites two and three follow this pattern shortly thereafter (Hinsch & Hamilton, 1956).

Within each somite three morphologically distinct regions develop: sclerotome, myotome and dermatome. Traditionally, each of these regions has been associated with a different set of tissue progenitors (reviewed in Ede et al. 1977). However, this scheme greatly under-represents the range of potentials present among cells in each region. Somitomeres do not
exhibit these morphologically distinct regions, yet they have the same range of potentials (Noden, 1983b, 1986a,b).

*Lateral mesoderm* in the developing avian head is even more regionally variable. Rostral to the third visceral arch lateral mesoderm does not separate into somatic and splanchnic layers, but appears as a sparse mesenchymal population between the wall of the pharynx and surface ectoderm (Fig. 1). No physical landmarks demarcate lateral from paraxial mesoderm. Beginning at the level of the third visceral arch, where the distal outflow tract of the heart initially forms, lateral mesoderm separates into splanchnic and somatic layers. The relation of these ventrally located mesodermal populations and adjacent endodermal and surface epithelial layers to axial and paraxial structures changes continuously and greatly during the closure of lateral body folds, the caudal shift of the heart and cervical flexure (see Searls, 1986).

It is into areas occupied by these mesodermal populations that the last-formed mesenchymal population, the *neural crest*, immigrates. Arising from elevating neural folds or the roof of the neural tube, neural crest cells inevitably contact paraxial mesoderm and, in regions where visceral arches will form, lateral mesoderm. The cephalic crest population is larger than that found at any trunk level, and it gives rise to a diverse array of connective tissues in addition to peripheral neuronal, glial and pigment cells (reviewed in Le Douarin, 1983; Noden, 1984a,b), and used with limited success in mammalian embryos (Jaenisch, 1985; Morris-Kay & Tan, 1987). Mouse chimaeras produced by mixing blastomeres from different species (see Rossant, 1984; Mullen, 1984; Herrup, 1986) or normal with transvected blastomeres (Price, 1987) have great potential in the study of mesenchymal lineages. For some populations, e.g. the neural crest and myotomal cells, the use of monoclonal antibodies that preferentially bind to these cells during their migratory phase has proved especially effective (Tucker *et al.*, 1984, 1986; Bronner-Fraser, 1986; Loring & Erickson, 1987). As each new method is developed and applied, our knowledge of the spatial relations between and among these mesenchymal populations during the critical periods of neurulation and early organogenesis is expanded. Undoubtedly, additional modifications will need be made as new methods of following cell lineages *in situ* are developed.

**Paraxial mesoderm**

Transplantation studies have confirmed earlier descriptive accounts defining those skeletal elements derived from cephalic paraxial mesoderm (e.g. Goodrich, 1930; de Beer, 1985; reviewed by Jarvik, 1980). Most of the calvaria, the lateral walls of the skull in the occipital and petrous regions, and the floor of the braincase from occipital through basisphenoid levels are all labelled following transplantation of occipital somites or somitomeres (Figs 2 and 3). Osteogenic precursors of the roofing bones of the skull arise within the sclerotomal region of each somite and the equivalent, though morphologically indistinguishable, deep region of somitomeres. These mesenchymal populations shift dorsally over the lateral walls and roof of the brain after neural crest cells
have emigrated. Deep paraxial mesodermal cells initially located beside the mid-myelencephalon shift laterally to circumscribe the otic vesicle (Noden, 1984a; Noden & Van De Water, 1986). Cells within this population also form all dermal and subcutaneous connective tissues of the dorsal region of the head, and the meninges caudal to the prosencephalon (Fig. 4).

**Lateral mesoderm**

Transplantation of lateral mesoderm at the level of the otic placode and first somite results in labelling of the laryngeal cartilages (arytenoid, cricoid; Fig. 5) and tracheal rings plus associated connective tissues (Noden, 1986b), but not of the syrinx. The median part of the clavicle and dermis of the ventrolateral and ventromedial regions of the neck are also derived from this tissue. Thus far, no connective tissue derivatives of more rostrally located lateral mesoderm have been identified, though these analyses are incomplete.

**Neural crest**

Many extirpation and transplantation experiments performed on amphibian and, more recently, fish and lamprey embryos have demonstrated that cells derived from the neural crest participate in the formation of midfacial and visceral arch skeletal structures (reviewed in Hörmstadus, 1950; Le

![Figure 3](image3.png)

*Fig. 3.* Schematic representation of cranial skeletal elements derived from neural crest (stipple), lateral mesoderm (lined), and paraxial mesoderm. This is based on extrapolation of avian and amphibian data to known homologues in the human. The ali- and orbito-sphenoid regions of these separate classes are difficult to homologize; thus, the precise contributions by crest and mesodermal populations in this area are uncertain.

![Figure 4](image4.png)

*Fig. 4.* This illustrates the distribution of connective-tissue-forming mesenchymal populations derived from neural crest, paraxial mesoderm and lateral mesoderm. Mesenchyme in each of the three areas is the exclusive source of local connective tissues.
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Fig. 5. The laryngotracheal diverticulum (left) and cricoid laryngeal cartilage (c) from a chick embryo into which quail subotic lateral mesoderm had been transplanted. The cartilage and local connective tissues are derived from the transplant.

Douarin, 1983; Maderson, 1987; Langille & Hall, 1988a, b). The quail–chick transplantation method has confirmed these studies in the avian embryo. Owing to the stability of the quail marker, the list of crest derivatives has been expanded considerably through the use of this method (Le Douarin, 1983; Noden, 1984a, b, 1987a).

As a result of their ventrad migrations, crest cells surround the prosencephalon and optic vesicle and occupy each of the visceral arches (Noden, 1975, 1978a, b, 1983a; Le Lièvre, 1978; Le Lièvre & Le Douarin, 1975). They constitute the only connective tissue-forming mesenchyme in these regions (Figs 4, 6). Thus, all cartilage, bone, tendon, dermal component, meningeal connective tissue, perivascular adventitial cell or glandular stroma that forms, for example, in the mandibular process (first visceral arch) will be derived from the neural crest; there are no other mesenchymal populations capable of forming connective tissues in this region.

Although the exact location of the crest–mesoderm interface shifts during the subsequent growth of the head and brain flexures, this demarcation segregating connective tissues according to embryonic origin remains. Those few situations in which a single skeletal element is formed by both crest and mesoderm (Noden, 1987a; Noden & Van De Water, 1986) represent secondary fusion of separate primordia (e.g. columella, frontal bone) or entrapment of a mesenchymal population by the growth of other tissues (e.g. otic capsule).

Fig. 6. This section is from the jaw region of a chick embryo into which quail midbrain neural crest had been transplanted. The grafted cells have formed the quadrate cartilage (q), the pterygoid bone (p), and all adjacent connective tissues. Myocytes (m) are derived from paraxial mesoderm, and thus are not labelled.

Significance

These results, and corroborating data on craniofacial development in other species (fish: Langille & Hall, 1988a, b; amphibians: Hörstadius, 1950; mammals: Morris-Kay & Tan, 1987), indicate that connective tissues in the vertebrate are derived from at least three sources. The possibility that the prechordal plate may contribute to median connective tissues below the midbrain has not been experimentally confirmed or refuted.

As a result of morphogenetic movements occurring during gastrulation and neurulation, paraxial mesoderm, lateral mesoderm and neural crest cells come to occupy different locations. Yet, each of these mesenchymes forms many connective tissues that are cytologically and biochemically identical. This suggests that despite the different histories and 'inductive' environments which these populations experience (see Thorogood, 1987 and this volume), many of the same genes ultimately become activated.
and functionally stabilized. This would be an example of *convergent ontogeny*.

The interface between mesenchymal tissues of different origin is not visible except in chimaeric embryos, and its significance, if any, to later morphogenetic events is unknown. In the formation of patterned fields in the integument, of aligned vascular channels, of oriented muscle fibres, and of specified routes of axonal outgrowth, the interface appears to be inconsequential.

**Origins and segregation of voluntary muscles**

Descriptive accounts of the contributions of paraxial mesoderm to craniofacial voluntary muscles have been conflicting, in part due to the difficulty of recognizing premyogenic populations (reviewed in Noden, 1983b, 1984a; Wachtler & Jacob, 1986). Condensations of the precursors of extrinsic ocular muscles are clearly visible within paraxial mesoderm (Neal, 1918; Adelmann, 1927; Edgeworth, 1935), and the hypoglossal cord, which contains precursors of tongue muscles, can be seen extending ventrally from the occipital somites (Hunter, 1935). However, these descriptive studies cannot establish the locations of myogenic precursors prior to their condensation.

Transplantation experiments have revealed that the myogenic precursors of all avian craniofacial voluntary muscles are in fact located within paraxial mesoderm (Fig. 7; Noden, 1983b). Moreover, these precursors are arranged in a rostral-to-caudal sequence that parallels their pattern of innervation, as summarized in Fig. 8. Wachtler *et al.* (1984; Wachtler & Jacob, 1986) have shown that the precursors of extrinsic ocular muscles arise initially within pericranial plate mesoderm, and secondarily come to reside in the more rostral somitomeres.

While the myogenic lineages of all voluntary muscles are traceable to a common source, paraxial mesoderm, the connective tissues associated with these muscles are of three different origins, as described in the previous section. Most cells committed to the myogenic pathway of cytodifferentiation in the head emigrate from paraxial mesoderm (occipital somites, cephalic somitomeres) and become associated with a different mesenchymal population, as illustrated in Fig. 7B. Those myoblasts that move into periocular, visceral arch or glossal locations become interspersed with neural-crest-derived mesenchymal...
cells, while myoblasts that migrate ventrally from the first somite integrate with lateral mesoderm adjacent to the laryngotracheal diverticulum. Some somitic, but no somitomeric, myoblasts remain associated with paraxial mesoderm and form, for example, all epaxial and most hypaxial muscles of the neck.

The formation of individual craniofacial muscles from these somitomeric populations follows several scenarios (McClearn & Noden, 1988). In the simplest case, exemplified by the dorsal oblique muscle, a premuscle condensation forms within one somitomere and shifts to its terminal site where a single muscle develops. In other cases, a common premuscle mass will give rise to several muscles. These may segregate from the parent condensation prior to the appearance of primary myotubes (e.g. separation of the protractor anlage from the mandibular muscle mass) or after elongated, aligned myotubes are present (e.g. individuation of adductor, pseudotemporalis and pterygoideus muscles from the mandibular muscle mass). Still another situation is represented by the intermandibularis and constrictor colli muscles, whose myoblasts condense and differentiate at their terminal location. Without the transplantation data it would be impossible to trace the precursors of these muscles to somitomeres and somites.

Neural crest migration and segmentation

The proposal that extant vertebrates are derived from an organism in which the head developed according to a segmental plan, i.e. was organized around a fundamental arrangement of serial segments, was enunciated by Balfour (1876) and elaborated by many early twentieth-century comparative morphologists, especially Goodrich (1930; see foreword by Hall & Hanken and review in de Beer, 1985) and, more recently, Jarvik (1980) and Bjerring (1977). Others, most notably Kingsbury (1926) and Romer (1972), rejected this proposition and suggested that the head is unique in its embryonic organization.

The discovery of somitomeres (Meier, 1981) and documentation of their presence in all vertebrate classes (see Holland, and Alberch & Kollar, this volume), renewed interest in the concept of head segmentation. At issue is whether a somite and a cephalic somitomere, though morphologically different, execute the same or different roles in development. Transplantation studies in avian embryos indicate that both structures have the same range of potentialities (see Noden, 1987a), but these results do not resolve the basic issue of functional identity.

Another approach to this problem has been to compare the patterns of neural crest movement...
Fig. 9. Migratory patterns of trunk neural crest cells (black stained cells) illustrated by applying anti-neural crest antibodies to sectioned embryos. A is a frontal section, with the cranial side at the top, showing that migrating crest cells are a continuous population when they contact somites, but become segmentally segregated within the cranial half of each somite as they move ventrally. B is a transverse section at the level of somite no. 3. This shows that crest cells move into and through the somite near the interface between sclerotome and dermamyotome. At this level, many crest cells become associated with the roof of the caudal pharynx and subsequently contribute to enteric ganglia.

around or through trunk and head paraxial mesoderm. Migratory pathways in the trunk have recently been well defined by the application of antibodies specific for migrating crest cells (Tucker et al. 1986; Bronner-Fraser, 1986; reviewed by Erickson, this volume). As shown in Fig. 9, most neural crest cells emigrating from occipital and trunk levels move ventrally between the dermamyotome and the dispersing sclerotome. Once contact is made with a somite, the crest population becomes segmented and moves exclusively through the rostral half of each somite or the intersomitic cleft (Keynes & Stern, 1984; Teillet et al. 1987; Loring & Erickson, 1987).

Application of anti-crest antibodies to sections of the avian head at the late neurula stages largely confirms the results of earlier transplantation studies (Johnston, 1966; Noden, 1975). Most cephalic crest cells migrate ventrally as a continuous sheet beneath surface ectoderm and superficial to the optic vesicle, rostrally, and somitomeric paraxial mesoderm elsewhere in the head (Fig. 10).

However, beside the midbrain and preotic hindbrain a small population of crest cells breaks away from this sheet and invades paraxial mesoderm. This population subsequently forms the medial boundary of developing visceral arch muscle masses, which suggests that these crest cells may be involved in segregating the superficial myogenic population from other components within each somitomere (Noden, 1987a, b). Similar deep pathways of cephalic crest migration have been described in mammalian embryos (Jaenisch, 1985; Tan & Morris-Kay, 1985; Chan & Tam, 1988).

Frontal sections reveal that crest cells that penetrate somitomeres do not remain as a continuous longitudinal population. Rather they appear as individual cells or small clusters (Fig. 10D). Whether the locations of these clusters corresponds to the rostral part of each somitomere has not been determined, because the only known demarcations between adjacent somitomeres are transverse clefts present on their dorsal surface.

These results are surprising in that none of the morphological features believed to be essential in guiding migrating crest cells into and through somites are evident within cephalic somitomeres. It will be interesting to learn whether there are deposits of fibronectin, cytotactin and tenascin (see Erickson, 1986 and this volume; Tan et al. 1987; Mackie et al.
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Fig. 10. The migratory patterns of cephalic neural crest cells, revealed using an anti-neural crest antibody. (A,B) Transverse sections through the prosencephalic and caudal mesencephalic levels of a stage-10/11 chick embryo. Crest cells are the sole mesenchymal population atop the forebrain and optic evaginations, although paraxial mesoderm will later move into these regions. Note in B that three divisions of the crest population are visible: a, precursors of the trigeminal ganglion; b, a population that is penetrating somitomeric mesoderm, and c, the major population that will move ventrally between surface ectoderm and underlying mesoderm to form the mandibular process. (C,D) Transverse and frontal sections of stage-11/12 embryos showing the superficial and smaller deep (arrows) migrating crest populations. Note the large supra-ocular/future maxillary population of labelled crest cells in D. (E) Transverse section at the level of the first visceral arch, stage-13 chick embryo. The deep and superficial crest populations have both moved into the developing first visceral arch and are surrounding the first aortic arch.
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1988) within somitomeres along pathways used by crest cells.

Origins of vascular endothelial cells

The anatomy of aortic arches and cardinal veins, and their transformation to permanent arteries and veins of the head, is described similarly in all introductory vertebrate embryology texts (including Noden & de Lahunta, 1985). If truth were based on the number of such repetitions, the story would be beyond reproach. Unfortunately, such is not the case.

Many carefully executed descriptive studies of craniofacial vasculogenesis in birds (e.g. Sabin, 1917; Hughes, 1934) and mammals (e.g. Congdon, 1922; Padget, 1948, 1957) have documented, for example, the presence of two, sometimes three, separate cranial cardinal veins that develop anastomoses and form the definitive internal jugular and its primary branches. Similarly, there are at least two separate first aortic arches; the one that is present immediately caudal to the optic vesicle in the well-studied 33 h chick is not the same as the one that is present penetrating the first visceral arch in the 48 h embryo.

It has been well documented that the muscular tunics and connective tissues surrounding craniofacial blood vessels are derived from local mesenchyme, either the neural crest (Le Lièvre & Le Douarin, 1975; Noden, 1978b; Kirby et al. 1983) or mesoderm, depending upon the location. However, the embryonic ancestry of endothelial cells has received less attention.

His (1868) reasoned that because patent blood vessels were grossly visible first in the extraembryonic yolk sac, this was the site at which endothelial cells arose. These progenitors then invaded embryonic tissues. Based on an extensive series of india-ink-injection studies on avian embryos, Sabin (1917) supported the concept of ingrowth from the periphery, but believed that intra-embryonic lateral plate mesoderm was also a source of endothelial cells. In contrast, McClure (1921) and most other investigators since then (Lanot, 1980; reviewed by Wagner, 1980; Noden & Reiss, 1988) have suggested that the ability to form endothelial vessels is more widespread throughout embryonic mesoderm, particularly in the

![Fig. 11. Transverse sections of chick embryos into which a somite-size piece of quail lateral mesoderm (A) and paraxial mesoderm (B) had been implanted at the level of the otic placode. Sections have been treated with anti-quail endothelium antibodies. Note the widespread distribution of graft-derived (black) endothelial cells, and their participation in the formation of aortic arches (2, 3), the dorsal aorta (da), the cardinal veins (cv), and numerous minor vessels. In B endothelial cells derived from the graft are forming the periotic vascular and meningeal plexuses. Note that some have begun invading the neural epithelium (arrows, B).](image-url)
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Head region (Rosenquist, 1970).

The recent application of monoclonal antibodies that specifically recognize quail endothelial cells has confirmed the latter views (Peault et al. 1983; Pardanaud et al. 1987; Coffin & Poole, 1988; Poole & Coffin, 1988). Endothelium-lined vesicles and cords appear beside and within many mesodermal populations shortly after the formation of the first few somites. Often these labelled populations are separate and isolated from earlier-formed vessels, suggesting that they arose independently.

However, such studies do not exclude the possibility that the angiogenic progenitors of these vesicles may have arisen elsewhere and migrated into the sites where vasculogenesis is initially detected. To test this, it is necessary to transplant suspected angiogenic precursors from quail into chick embryos and examine host embryos using anti-quail endothelial antibodies.

Following transplantation of either paraxial or lateral mesoderm at the level of the otic placode in embryos of 2 to 7 somites, labelled endothelial cells contribute to the formation of aortic arches and their tributaries, and all components of the cranial cardinal venous plexus (Fig. 11; Noden, 1987c). Transplantation of comparable trunk tissues give similar results. Neither prechordal plate mesoderm nor the neural crest contains angioblasts.

Unlike most grafted muscle- and connective tissue-forming cells that remain near the site of implantation, labelled endothelial cells are found great distances (several hundred micrometers in a 4- to 5-day embryo) and in all directions from the graft site. Typically, several aortic arches and patches of the cardinal vein from the midbrain to the cervical level will have quail endothelial cells. In most locations, areas of donor- and host-derived endothelium are interspersed, indicating that an extensive intermingling of angioblasts occurred before definitive vessels formed. The polyclonal antibodies used in this study (generated using the protocol of Lance-Jones & Lagenaur, 1987) bound to many isolated single cells and solid clusters as well as to isolated vesicles and parts of definitive vessels (Fig. 12).

These results indicate that all intra-embryonic mesoderm except the prechordal plate contains angioblasts. Some of these develop in situ, by clonal proliferation or aggregation of individual cells to form a solid cluster that subsequently becomes patent and fuses with other endothelial channels. Others move in all directions, invading distant mesenchymal and epithelial tissues. This 'metastatic' capability of
angiogenic precursors is unmatched by any other mesenchymal cell type in the avian embryo!
In all cases, both arterial and venous channels, and in older embryos the lymphatics, were labelled. However, since the transplant contained several hundred cells, it is not known whether a common stem cell or several different committed lineages are present in the embryo.

Origins of peripheral neurones and glia

Cells of the peripheral nervous system arise from mesenchymal precursors. In the head region, there are two sources of peripheral neurones, the neural crest and neurogenic placodes (Narayanan & Narayan, 1980; Noden, 1978c; D'Amico-Martel & Noden, 1983; reviewed by Le Douarin, 1983, 1984), and also two proven sources of glia and Schwann cells, the neural crest and the olfactory placode (Noden, 1978c; Couly & Le Douarin, 1987).

Crest- and placode-derived sensory neurones occupy different ganglia or separate parts of ganglia, as summarized in Fig. 13. Except for the vestibulocochlear ganglia, placode-derived neurones develop earlier (D’Amico-Martel & Noden, 1980) and are initially much larger (12–20 μm versus 8–15 μm) and more argentophilic than neurones from the neural crest. Later in development these cytological distinctions are lost (Gaik & Farbman, 1973). All peripheral autonomic neurones of the head are derived from the neural crest.

The significance of this dual origin of sensory neurones is not known. It has been shown for the trigeminal ganglion that placodal cells are preferentially responsive to nerve growth factor (Ebenlal & Hedlund, 1975), and only the crest-derived neurones contain substance P, which suggests these serve as the primary conveyors of nociceptive extracellular stimuli (Le Douarin, 1983). However, anatomic mapping studies in birds (Noden, 1980b,c; Covell & Noden, 1988) have shown that neurones derived from both embryonic sources have similar peripheral and central projections.

Spatial organization and integration

How do each of these mesenchyme-derived tissues develop in the proper position within the head, and with the appropriate shapes, alignment and attachments, channels, or projections? These are problems in spatial patterning, which remain the most difficult ones in which to define the links among gene function, cell and tissue interactions, and phenotypic expression.

Certainly, the spatial and temporal depositions of extracellular matrix materials that are contacted by each of these shifting mesenchymal populations are an important component; their possible roles are discussed elsewhere (Thorogood, 1987; see articles by Thorogood, Lumsden and Davies elsewhere in this volume). That these differentiation-promoting influences are necessary is not in dispute; but whether they, acting alone or in concert, are the essential, information-bearing cues for establishing spatial patterning within mesenchymal (and epithelial) populations is controversial.

Orientation and sculpturing of voluntary muscles

Morphological patterning of striated muscles involves three sets of events: the segregation of individual muscles or their precursors from common myogenic mesenchymal masses, the alignment of primary then secondary myotubes, and the establishment of definitive attachments. Studies of limb muscle formation involving transplantation of myotomes (Adelmann, 1938; Christ et al. 1977; Chevallier et al. 1977; Chevallier, 1979) or of lateral plate somatic mesoderm (Jacob & Christ, 1980; Chevallier & Kiemy, 1982) clearly indicate that it is the connective tissue forming mesenchyme, not the myogenic precursors, which carry the patterning information for each of the above processes (reviewed in Jacob et al. 1983).

The same is true for craniofacial muscles. When trunk somites are transplanted in the place of cephalic somitomeres, transplanted myogenic cells invade local connective tissue-forming mesenchyme, which is derived largely from the neural crest. Subsequently, they form extrinsic ocular and visceral arch muscles (Fig. 14) with normal fibre alignment and attachments (Noden, 1986a). These data confirm the essential role of connective tissue-forming mesenchyme in muscle patterning. Additionally, they suggest that interactions between progenitors of muscle and connective tissue that result in fibre alignment and attachments are common throughout the body, irrespective of the source of the connective tissue mesenchyme.

Patterns of blood vessel formation

Given the chaotic migratory behaviours of angiogenic precursors described earlier, it is remarkable that so constant a vascular morphology arises in vertebrate embryos. To assess the relative roles of endothelium-producing cells and surrounding mesenchymal and epithelial tissues in the patterning of blood vessel formation, quail paraxial or lateral mesoderm from the trunk has been implanted beneath the otic placode of a chick host at the 5- to 10-somite stage.

Transplanted angioblasts move extensively away from the implant site and contribute to the formation
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Fig. 14. This illustrates that trunk paraxial mesoderm will form normal craniofacial muscles if transplanted in the place of cephalic somitomeres. B and C are enlargements of areas indicated in A, which is a section through the mandibular abductor (ma), protractor pterygoideus (pp) and pseudotemporalis (ps) muscles and the quadrate (q) cartilage. sc, scleral cartilage, nV, mandibular nerve. Note quail cells in the sectioned muscles in B and C (from Noden, 1986a).

Fig. 15. Transverse section through a stage-21 chick embryo into which quail trunk somitic mesoderm had been implanted at the level of the otic placode. Transplant-derived endothelial cells are labelled black. This demonstrates that somitic mesoderm contains angioblasts and that these are capable of forming normal craniofacial vessels (compare with Fig. 11).

of aortic arches, dorsal and ventral aortae, endocardium, and various venous channels that are normal and appropriate to the developing head region (Fig. 15; Noden, 1987c; Noden & Shimamura, 1988). The timing and sites of vascular invasion of the hindbrain, which are quite distinct from those of the spinal cord, are also normal. Thus, as with the myoblasts described above, the patterns of angioblast movement, condensation, vesiculation and channel elongation are determined by the mesenchyme surrounding these cells.

Role of the neural crest in pattern formation

Patterning of visceral arches

The preceding experiments implicate connective tissue-forming mesenchyme in the establishment of three-dimensional organization within many regions of the developing head. Experiments on the neural crest support this.

Andres (1946, 1949) and Wagner (1949, 1959) transplanted cephalic neural crest primordia from salamander into frog embryos. In the resulting chim-
Fig. 16. Cross-species neural crest transplantations in amphibians. The normal visceral skeletal structures in each species is indicated in A. Note specially that the palatoquadrate (Pq) of *Bombinator* has a large rostral process, and that the mandible (Md) of *Triturus* bears teeth. In B the results of replacing *Triturus* crest with that of *Bombinator* are shown on the left, the reciprocal on the right. In both cases the grafted crest formed donor-type skeletal tissues in the host. Redrawn after Wagner (1949).

Fig. 17. This illustrates the results of replacing the precursors of the second visceral arch neural crest population with an identical (control) population or else crest precursors that normally would invade the first visceral arch (experimental). The coarse stippled structures are those derived from the experimental transplant. Note that they are located in the second visceral arch, but that most of these have the morphology and spatial organization of first visceral arch skeletal structures (from Noden, 19846).

Heterotopic transplantations similar to these have been performed in the avian embryo, in which the presence of a stable (quail cell) marker permit the origin of each cell to be ascertained (Noden, 1983a). Presumptive first (mandibular) arch neural crest primordia grafted in the place of presumptive second (hyoid) or third (branchial) arch crest primordia migrate normally into the closest arch but therein form a jaw skeleton, complete with squamosal, pterygoid and angular bones, and quadrate and mandibular (Meckel’s) cartilages (Fig. 17).

Not only are crest-derived structures morphologically inappropriate for their new location, but some muscles in the second arch location form alignments and attachments typical of first arch muscles. Also, the development of the integument overlying transplant-derived crest populations is altered, forming first arch-specific specializations such as an external auditory meatus and ectopic beak (Fig. 18).

These studies indicate that neural crest precursors, while they are still part of the immature neural
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epithelium, acquire spatial programming. Those cells destined to populate the first arch are different from those that would normally seed the second or third arches. The differences are not of commitment to particular cell phenotypes, for all these cephalic crest populations normally develop the same range of tissues. Rather, the differences involve the patterns in which mesenchymal populations derived from these separate axial levels will become organized within each visceral arch, and how they will form subpopulations that are selectively responsive to various inductive stimuli. Invading myogenic and angiogenic populations, as well as ingrowing peripheral neurites and overlying surface ectoderm, receive spatial cues from this connective tissue-forming population.

These results are identical to those obtained in analyses of limb development. Substituting leg mesenchyme for that of the wing bud results in the development of a leg from the thoracic region (Zwilling, 1955; Saunders et al. 1959; reviewed by Gumpel-Pinot (1984). The spatial organization of both the muscles, internally, and the integument are changed.

Although these two systems, the limb and the head, are phenomenologically similar, there is one major difference. Namely, the location in which neural crest primordia acquire spatial programming is known; they do so while part of the neural plate (or possibly sooner). It is not known when or where limb-forming somatic mesoderm acquires its pattern-generating capabilities.

This role for cephalic neural crest mesenchyme has important implications in understanding vertebrate craniofacial evolution (see Northcutt & Gans, 1983.) It implies that changes in the formation or delineation of rostrocaudal compartments within the vertebrate neural plate will likely have profound sequelae both in the central nervous system and in the musculoskeletal assembly of visceral arches.

Patterning of the frontonasal process

Shortly after their formation, all neural crest cells that will populate visceral arches move away from the neural epithelium. In contrast, crest cells destined to form the maxillary and frontonasal processes remain in close proximity to the prosencephalon or optic vesicles (Johnston, 1966; Noden, 1975). Many descriptive and experimental embryological analyses have indicated that disruption of bilateralization or normal closure of the rostral neural plate inevitably results in dysmorphologies of frontonasal structures (Adelmann, 1936; Evans et al. 1966; Couly, 1981; Kokich et al. 1982; see Sulik, this volume). Similarly, clinicians have documented the positive correlation
between forebrain lesions and frontonasal reductions in various forms of the holoprosencephalic syndrome (Lemire et al. 1981; Siebert et al. 1981; Cohen, 1982).

In the avian system, transplantation of neural crest cells that normally would form frontonasal or maxillary structures in the place of second arch crest precursors gave unexpected results. The grafted cells moved normally into the second arch, but therein formed a mandibular skeletal array (Noden, 1983a).

Explanations of this apparent paradox are conjectural. One possibility is that rostral-to-caudal gradients (see Saxén & Toivonen, 1962; Slack, 1983) persist and continue to affect crest progenitor populations. Surgically moving crest precursors slightly along this axis, as described in the preceding section, is insufficient to alter their intrinsic programming. Following greater shifts, however, intrinsic positional values within the crest precursor population become altered by influences emanating from surrounding axial, or possibly pharyngeal, tissues.

An alternative explanation is based on the observation that in most vertebrates there exists a small region of the developing brain near the junction of the metencephalon and myelencephalon in which no neural crest cells are formed (Noden, 1980a). This corresponds to the demarcation between first and second arch crest precursors. Perhaps all of the crest populations located rostral to this null zone have an identical spatial programming (i.e. to form first arch (mandibular) structures) that is expressed whenever they migrate into a visceral arch. This spatial programming is altered by prolonged contact with proencephalic neural epithelium, which elicits the formation of maxillary or frontonasal structures.

**Perspectives**

From the phenomenological and often paradoxical results presented in this chapter it can be seen that our foremost task is to document the complexity and many regional differences in the patterns of morphogenesis and spatial programming of mesenchymal populations evident within the developing vertebrate head. Although our understanding of the cellular events underlying these processes is incomplete, and knowledge of the genetic basis for initiating and implementing 'spatial programming' in vertebrate systems is poor, there is reason for enthusiastic optimism.

The tools to establish a complete descriptive craniofacial embryology in all accessible species are available. As indicated throughout this review, even in the supposedly exhaustively examined chick embryo there remain many craniofacial cell populations and tissues that have not been critically and thoroughly unexamined structurally or biochemically.

Molecular biologists are applying probes to identify functional genes in vertebrates that share sequence similarity with homeobox genes in *Drosophila*, and finding many situations in which these genes function in specific rostrocaudal and dorsoventral regions of the embryo (e.g. Gaunt, 1987; Toth et al. 1987). Holland & Hogan (1988) have described one mouse gene, *Hox 2.1*, which is active in cells of the central nervous system beginning at the level of postotic brainstem, and also in cells of peripheral sensory ganglia beginning with the distal Xth (nodose). Is it a coincidence that neural crest and placode cells that form this ganglion are derived from the postotic brainstem and adjacent surface ectoderm?

Neither the products nor the functions of homeobox genes have yet been characterized in vertebrate systems. However, it is exciting to speculate that similar, soon to be identified, gene sequences are acting to establish spatial programming within craniofacial mesenchymal and epithelial populations. When probes for these are available, their application can be combined with the types of transplantations discussed in this review. Perhaps then a discussion based on understanding rather than phenomenology and conjecture can be written.

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**References**


Noden, D. M. (1983a). The role of the neural crest in...


