Patterning of connective tissues in the head: discussion report

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Summary

The three papers presented by Noden, Thorogood and Lumsden in this session encompassed the connective tissues as broadly defined, i.e. soft (fibrous) connective tissue, cartilage, bone, muscle and the dental tissues, enamel and dentine, and utilized a variety of experimental techniques on both avian and mammalian embryos to explore specificity and patterning of the vertebrate head. Whether similar developmental processes pattern homologous structures in different Vertebrate classes (Amphibia, Aves, Mammalia) was discussed with reference to patterning of the cranial musculature, chondrocranium and dental tissues. A number of challenging ideas emerged during this session. Does the premigratory neural crest consist of a homogenous population of totipotent cells or of subpopulations of bi- or tripotential cells? Is fundamental patterning of the head an early embryonic event, perhaps specified during primary embryonic induction or the consequence of neuroepithelial folding, brain growth, inductive interactions and/or spatially and temporally distributed extracellular matrix products? Can the fact that mesoderm and angioblasts do not display distinctive patterning that relates to their particular embryologic origins be extrapolated to patterning in general? How does the documentation of an ondontogenic trunk neural crest in mammals affect our theories of how patterning mechanisms arose or were modified during vertebrate evolution?

Specification of craniofacial mesoderm

Noden's paper provided an overview of the current evidence documenting the four sources of mesoderm in the head of the developing chick: prechordal plate, lateral plate and paraxial mesoderm, both somitomeres and occipital somites. Fibrous connective tissue, voluntary muscle, endothelial cells and laryngeal cartilages arise from these mesodermal elements. As beautifully illustrated in his paper, the angioblastic precursors of endothelial cells may differentiate in situ, or they may differentiate only after extensive migration; angioblasts are opportunistic, differentiating as and when required. Neural crest cells also differentiate into angioblasts (visualized using anti-quail antibodies after grafting quail neural crest cells into donor chick embryos) so that many of the craniofacial blood vessels are chimaeric, with mesodermally- and neural crest-derived endothelial cells participating in the formation of the same small segment of a blood vessel. The extensive migration of the angioblastic precursors coupled with chimaeric blood vessels clearly indicates that neither angioblasts nor their precursor cells carry any specificity with respect to patterning. Similarly, Noden demonstrated that individual muscles do not map to (or at least have not yet been mapped to) specific somitomeres, but rather map to regions of somitomeres. The patterns of the muscles are in fact determined by the connective tissue matrix in which they develop (Noden, 1982, 1983, 1984, 1986a,b).

Noden's paper also elegantly illustrated that a subpopulation of neural crest cells migrates into (invades?) the somitomeres that develop from the incompletely segmented paraxial mesoderm, segregating that mesoderm into units from which voluntary muscles of the visceral arches will subsequently develop. Given the recent demonstration that trunk neural crest cells invade the anterior halves of somites (Loring & Erickson, 1987; Lim, Lunn, Keynes & Stern, 1987; Stern & Keynes, 1987) Noden's observations on invasion of cranial neural crest cells into somitomeres highlights the similar functional environments provided by somites and somitomeres. Therefore, not only has there now been structural evidence documenting the existence of somitomeres provided by a variety of workers (the stereopair scanning electron microscopic images of somitomeres in representatives of all major vertebrate groups, see Bellairs, Ede & Lash, 1986 and Gilland & Alberch, 1988, for reviews) but for those who cannot 'see' somitomeres, there is now the evidence of functional equivalence between somites and somitomeres.

A point emphasized by Noden in his presentation, and obvious once our attention has been drawn to it, is that as a starting point for experimental transplantation and manipulation, the mesoderm and neural crest are not at the beginning of their development; they are cells with a history. It is becoming increasingly clear that we need to know much more about the early history of these cells, especially how the neural crest itself arises in the embryo, if we are to understand patterning of the connective tissues in the head. The localization of the neural crest at the junction of the neural and epidermal ectoderm both in the neural folds of the developing neurula (recognized by His (1868) in the initial description of the neural crest) and at earlier stages of development (Rosenquist, 1981) is suggestive of its possible origin via combined inductive interactions that occur at that site, and there is, at least for amphibians, experimental evidence to support such a notion (Rohrläuser-ter-Horst, 1980; discussed in Hall, 1987, 1988). The exciting recent finding by Couly & Le Douarin (1987) that much of the facial ectoderm arises from precisely localized areas of the prosencephalic neural folds, raises the distinct possibility that early inductive events may not only specify neural and neural crest cell populations, but that they may also specify the ectodermal populations with which they subsequently interact and which evoke their differentiative potential. As will be noted later in this report, the increasing likelihood that the neural crest consists of subpopulations of cells is going to require detailed knowledge of, what may turn out to be, very fine-scale specifications and/or interactions that pattern the neural crest.

Noden also emphasized the quite massive displacements that these early mesenchymal populations undergo during early embryonic growth, dramatically emphasized by his recounting that the furcula (wishbone) situated in the thorax, is derived from the same lateral mesoderm as is the larynx, extensive ventral displacement repositioning the lateral mesoderm into
the trunk during early embryonic development.

**Specification of skull form**

Noden's emphasis that unique tissue origins do not equate with distinctive patterning, fates or functions (mesodermal and neural crest-derived angioblasts forming chimaeric blood vessels) was echoed by Thorogood in his presentation on specification of skull form. Can homologous structures (e.g. trabeculae, Meckel's cartilages) found in different Classes of vertebrates develop by different developmental mechanisms or does constancy of phylogenetic pattern require constancy of developmental process from group to group? This important issue, highlighted by De Beer (1958) has resurfaced recently as an important interface between development and evolution (Horder, Witkowski & Wylie, 1986; Wagner, 1986; Roth, 1988).

Thorogood presented his data on how, in the embryonic bird, a subpopulation of migrating neural crest cells is trapped alongside the neuroepithelium at sites where subsequent epithelial–mesenchymal interactions will evoke chondrogenesis to form the primordium of the chondrocranial skeleton (trabeculae, parachordals, olfactory, optic and otic capsules). Type II collagen, localized both spatially and temporally along the neuroepithelium (perhaps synthesized and deposited by the neuroepithelium itself, although this point has yet to be verified) is the extracellular matrix component that traps migrating neural crest cells, or at least that traps those cells that express the anchor in CII cell surface properties, different final cell fates and perhaps even different states of determination. This possibility was emphasized by Weston in his remarks introducing the discussion that followed this session and has been elaborated in at least two recent reviews (Weston, 1986; Le Douarn, 1986). This emphasis on the morphogenetic role of epigenetic interactions involving components of the extracellular matrix subsequent to an initial patterning event, has surfaced in previous symposia and continually reappeared as a theme throughout the present conference. For example, Carlson (1981) in summarizing the proceedings of a symposium on 'Morphogenesis and Pattern Formation' held in Ann Arbor, Michigan in 1980, noted "The brain plays a prominent role in morphogenesis of the head. Both the brain and spinal cord, as well as the sense organs, induce protective coverings of hard tissue around themselves. Whereas the initial formation of the cranium depends upon inductive influences, the final shape of the skull is determined to a great extent upon forces generated by the brain and the pressure of the cerebrospinal fluid." (p 292). Similarly, in the Workshop ‘Strategies of Head Development’ (see Alberch & Kollar, this volume) an initial mosaic pattern of major head regions, inherent in the neural crest or in the neural–epidermal ectoderm, modulated by epigenetic interactions and responsible for establishing such parameters as relative growth and adaptation (as evidenced in both structural and functional coordination), was presented as a model for the development and evolution of the vertebrate head.

Thorogood in his paper makes a number of predictions based on his model of chondrocranial morphogenesis. One prediction is that the epithelia associated with type II collagen should be interchangeable in terms of their ability to evoke a chondrogenic response. A number of workers have examined this question of the exchangeability of epithelia with respect to their ability to evoke chondrogenesis from mesenchyme that they would not encounter in vivo (Holster, 1968; Hall, 1978, 1983a,b; Kratochwil, 1983; Nieuwkoop, Johnel & Albers, 1985). Apparently some specificity is evident. That notochord elicits chondrogenesis from somitic and head mesoderm but not from limb bud mesoderm, while otic vesicle elicits chondrogenesis from head mesoderm but not from somitic mesoderm, prompted Kratochwil to comment that such selectivity argues against the presence of a single 'cartilage-forming' signal to which any prechondrogenic mesenchyme can respond. However, this 'simple' story becomes more complex when embryos from various vertebrate Classes are compared, for while Benoit (1960) and O'Hare (1972) have shown that otic vesicle epithelium (a portion of the neuroepithelium with which type II collagen is associated) will not evoke chondrogenesis from chick somitic mesoderm (confirmed by B. K. Hall, unpublished observations) Grobstein & Holster (1955) and Holtfreter (1968) have shown that otic vesicle can evoke chondrogenesis from both mouse and amphibian somitic mesoderm. Notochord evokes cartilage from somitic mesoderm in all vertebrates tested (Hall, 1977) Notochord also evokes cartilage from mesodermally-derived cranial mesenchyme in amphibians (the parachordal cartilages and posterior portion of the trabeculae; Petricioni, 1964) and in the chick (otic capsule cartilage, bassiphenoid and occipital; Benoit, 1960; Schowing, 1974), from neural crest-derived cranial mesenchyme in amphibians (Hörstadius, 1950; Okada & Ichikawa, 1956; Holtfreter, 1968), as well as from sarcomas in both chicks and man (Mathis & Seiler-Aspang, 1962; Seiler-Aspang, Honus & Kratochwil, 1963). Do these experiments tell us that notochord has less specificity as an initiator of chondrogenesis than does otic vesicle epithelium (i.e. that they are not interchangeable) or does this apparent difference merely reflect the patchiness of the data in terms of species, tissues and ages tested? We will need a more
systematic survey of the specificity or lack of specificity of these interactions before we can tell.

**Specification of the mammalian tooth**

Until very recently, the mammalian neural crest has been refractory to experimental manipulation. The third presentation in this session was by Lumsden on tooth formation from the mammalian (mouse) neural crest.

A neural crest origin of the odontoblasts that deposit the dentine of the teeth has previously been based entirely on studies on amphibian embryos (Hörstadius, 1959). Lumsden utilized a tooth-shaping neural crest in combination with epithelial ectoderm, either from mandibular arches or from limb buds, to explore the differentiation and morphogenesis of the developing mouse tooth, and in so doing provided the first experimental evidence that mammalian odontoblasts are of neural crest origin. The teeth that formed in these tissue recombinations were fully-formed, complete with follicles, periodontal ligament, alveolar bone of attachment, and associated cartilage and bone, even to the point of showing signs of erupting in their ectopic sites as intraocular homographs.

Even though we might have expected that these pioneer experiments would produce surprises, Lumsden’s data challenges, in quite unexpected ways, produce surprises, Lumsden’s data bone, even to the point of showing signs of attachment, and associated cartilage and bone that these pioneer experiments would have contributed to the oral epithelium because of some component contributed to the epithelium by this underlying mesenchyme. Clearly, we eagerly await the future surprises concerning patterning of the connective tissues in the mammalian, and indeed in the vertebrate head, that will undoubtedly flow from future experimentation on the mammalian neural crest.

**References**


