The Trabecula cranii: development and homology of an enigmatic vertebrate head structure

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Abstract—The vertebrate cranium consists of three parts: neuro-, viscero- and dermatocranium, which differ in both developmental and phylogenetic origin. Traditionally, developmental origin has been used as a criterion for homology, but this becomes problematic when skull elements such as the parietal bone are now shown, by modern fate-mapping studies, to have different developmental origins in different groups of tetrapods. This indicates a flexibility of developmental programmes and regulatory pathways which has probably been very important in cranial evolution. The trabecula cranii is an intriguing cranial element in the anterior cranial base in vertebrates. It forms a viscerocranial part of the neurocranium and is believed to be neural crest-derived in gnathostomes, but a similarly named structure in lampreys has been shown to have a mesodermal origin. Topographically, this trabecula seems to be homologous to the gnathostome trabecula cranii, and might also have the same function: to form a border between adjacent morphogenetic domains, to constrain and redirect growth of both brain and stomodeum and thus to refine developmental schedules of both. We suggest that such a border zone can recruit cells from either the mesoderm (as in the lamprey) or from the neural crest (as in the gnathostomes investigated), and still retain its homology. In our view, the trabecula is an interface element that integrates the respective divergent morphogenetics programs of the preotic head into a balanced unit; we suggest that such a definition can be used to define "the sameness" of this element throughout vertebrates.

Keywords: head; neural crest; neurocranium; paraxial mesoderm; segmentation; trabecula cranii; viscerocranium.

THE CRANIUM AS A COMPOSITE STRUCTURE

Although merged into a harmonious unit, the vertebrate skull is a composite structure comprising three distinct parts with dissimilar phylogenetic origins (e.g., Kardong, 1995; Liem et al., 2001). The *viscerocranium* (or *splanchnocranium*) is

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the most ancient cranial part that, in the traditional view, evolved from pharyngeal arch elements supporting gill slits in a cephalochordate-like animal. Viscerocranial components support the gills and/or contribute to the jaw and hyoid apparatus in gnathostomes, but they probably form parts of the anteriormost neurocranium as well. The second part, the *neurocranium*, consists of endochondral bone or cartilage elements that surround and protect the brain and sensory organs. This part of the skull was originally supposed to have evolved from fused vertebrae. There is, however, no consensus as to the number of vertebrae which make up the neurocranium, or whether, in fact, it consists of vertebrae at all. Finally, the outer dermal skeleton or *dermatocranium* is composed exclusively of dermal (membrane) bones that, phylogenetically, arise from the bony armour of the integument of early fishes and is added onto the neuro- and splanchnocranium. The vertebrate skull elements can also be seen as belonging to either an *exoskeleton*, which is formed from the dermis within the integument (dermatocranium), or to an *endoskeleton*, which originates from mesoderm or from neural crest-derived mesenchyme deeper in the body (neuro- and viscerocranium).

Regarding the germ layer origin of cranial elements, the viscerocranium seems to be derived exclusively from neural crest cells (for a review and more species-specific quotations, see Hall and Hörstadius, 1988; Hall, 1999; Le Douarin and Kalcheim, 1999). Interestingly, the name 'viscerocranium' stems from the mistaken view that these cells originate from the same embryological source as the wall of the digestive tract, i.e., from the endoderm (Kardong, 1995). The neurocranium, according to a common opinion, evolved from fused vertebrae and these components are thus, *per definitionem*, of mesodermal (somitic) origin. Dermatocranial bones, on the other hand, develop as condensations in the dermis, which in the head originate either from the neural crest or from mesodermal cells, depending on the position alongside the antero-posterior axis (e.g., Larsen, 1993; Liem et al., 2001).

DOES THE EMBRYONIC ORIGIN OF CRANIAL ELEMENTS MATTER?

All these cranial components of diverse phylogenetic and developmental origin arise during ontogenetic development as a result of complicated cell movements and tissue interactions. Finally, however, they all become integrated into one structural and functional unit – the cranium. Knowledge about the embryonic origin of single skeletal elements might also shed light on their evolutionary origin, because similarity of development is one of the key tests used for deducing evolutionary homology (e.g., Matsuoka et al., 2005; also see Darwin, 1859: "Community of embryonic structure reveals community of descent").

It would, for example, be considered strange if a bone of mesodermal origin in one vertebrate is a direct homologue of a bone of neural crest origin in another species. But the parietal bone seems to illustrate just such a case. In the mouse it originates from cephalic paraxial mesoderm (Jiang et al., 2000; Morriss-Kay, 2001), whereas in chicken the parietal bone has been traced back to either mesodermal
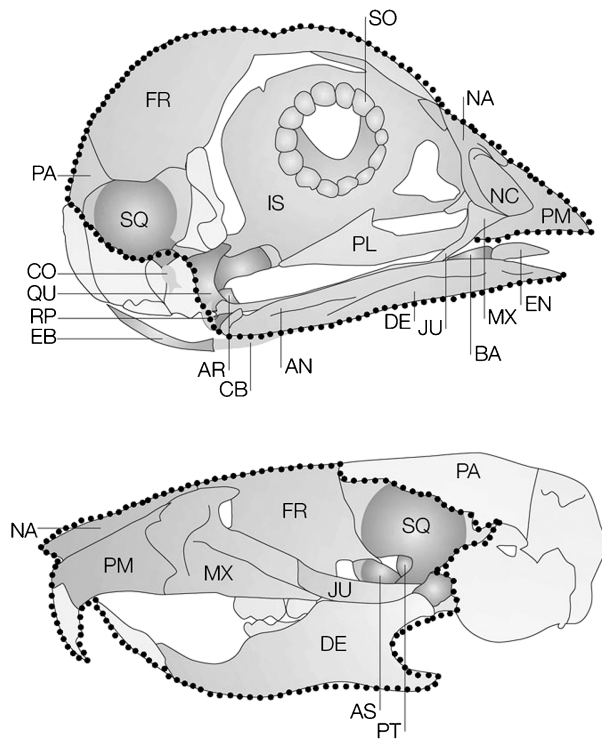
Figure 1. Contribution of neural crest cells (encircled by dark dotted lines) to the skull of chick (upper panel) and mouse (lower panel). Modified from Santagati and Rijli (2003); data according to Le Douarin and Kalcheim (1999); Morriss-Kay (2001). Notice that the parietal bone (PA) originates in chick from the neural crest, whereas in mouse from mesoderm. AN, angular bone; AR, articular bone; AS, alisphenoid; BA, basihyal; CB, ceratobranchial; CO, columella; DE, dentary bone; EB, epibranchial; EN, entoglossum; FR, frontal; IS, interorbital septum; JU, jugal bone; MX, maxillary bone; NA, nasal bone; NC, nasal capsule; PA, parietal; PL, palatine bone; PM, premaxillary bone; PT, pterygoid; QU, quadrate; RP, retroarticular process; SO, scleral ossicles; SQ, squamosal; ST, stapes; ZY, zygomatic bone.

(Noden, 1978) or, according to improved methodological approach, to neural crest cells (Couly et al., 1992, 1993) (fig. 1). In the clawed frog (*Xenopus laevis*), the only other animal for which the embryonic origin of a membrane bone is currently known (Gross and Hanken, 2005), a single fronto-parietal bone also originates from neural crest cells, although a contribution from cranial paraxial mesodermal cells cannot be excluded, as these have not yet been fate-mapped. These differences suggest that either the pattern of neural crest contribution to the vertebrate skull has changed significantly during evolution, or that widely accepted skull bone homologies across major clades may be incorrect (Hanken and Gross, 2005).

That the same final tissue, either cartilage or bone, can develop from independent embryonic cell lineages tells us something important about the flexibility of developmental programmes and regulatory pathways which underlies evolutionary changes. Head evolution seems to have been largely driven by functional co-option;

hence recruitment and elaboration of different embryonic cell lineages, by neural crest cells (e.g., Meulemans et al., 2003; Rudel and Sommer, 2003). Interestingly, cranial neural crest cells appear to be a highly plastic population (see Trainor and Krumlauf, 2000; Trainor et al., 2003; Santagati and Rijli, 2003, for recent reviews). For example, avian neural crest cells, when experimentally transplanted into the mesodermal mesenchyme, actually participate in the formation of bones and some neighbouring elements normally derived from mesoderm (Schneider, 1999). Neural crest cells are therefore able to respond to environmental cues that otherwise promote mesodermal skeletogenesis. This would have been seen as nearly unimaginable just a few years ago. There is now growing evidence for neural crest cell plasticity (see Trainor et al., 2003, for a review).

Admittedly, the embryonic origin of skull elements does matter; however, homology of these components does not equate simple constancy of embryonic origin of single elements, tissues or organs. The whole issue about defining 'sameness' in the vertebrate head is, therefore, much more complicated than previously thought, and we have to be able to determine the constancy of cell lineages, cell fates and gene regulation on many levels of biological organisation to solve this question (for reviews, see Raff, 1996; Hall, 1998a; Arthur, 2000; Hall, 2003; Wake, 2003).

NEURAL CREST CELLS AND VERTEBRATE HEAD MORPHOGENESIS

Clearly, in the head of all vertebrate species examined in some detail, neural crest cells create or at least contribute to most of the cranial cell types and tissues (Couly et al., 1992, 1993; Hall, 1999; Le Douarin and Kalcheim, 1999). Neural crest cells thus in fact 'build the vertebrate head' (Santagati and Rijli, 2003).

The neural crest is an embryonic cell population unique to vertebrates, and a key vertebrate character (Gans and Northcutt, 1983; Benton, 1998; Hall, 1998a, b; Ruffins et al., 1998; Selleck et al., 1998; Studer et al., 1998; Le Douarin and Kalcheim, 1999; Shimeld and Holland, 2000; Le Douarin and Dupin, 2003, but see Jeffery et al., 2004). It has been proposed that the evolution of vertebrates from a cephalochordate-like ancestor was driven largely by the origin and elaboration of the neural crest and of neurogenic ectodermal placodes (Gans and Northcutt, 1983; Northcutt and Gans, 1983). This assertion reflects the fact that neural crest and placodes give rise to adult structures that define the vertebrate clade (Shimeld and Holland, 2000), and thus the neural crest can be considered a fourth germ layer (Hall, 1998b, 2000).

Cranial neural crest cells differentiate into a wide variety of derivatives as different as myofibroblasts, fibroblasts, cartilage, bones, melanocytes, endocrine tissues and various types of neurons and glial cells in the peripheral nervous system (table 1) (for review see Couly et al., 1993; Etchevers et al., 2001; Le Douarin and Dupin, 2003; Le Douarin et al., 2004). Only the cranial part of the neural crest does normally give rise to connective and supportive tissues (Noden, 1991; Couly et al., 1993), although a hidden capacity of trunk neural crest to yield skeletal

Table 1.
Mesenchymal derivatives of the neural crest.

Cephalic neural crest
Dermatocranium
(bones derived from dermis):
Frontal, parietal, squamosal, sphenoid (basipre-), otic capsule (partly), nasal, vomer, maxilla, jugal, quadratojugal, palatine, pterygoid, dentary, opercular, angular, supraangular.

Chondrocranium
(cartilage elements, belonging either to viscero- or neurocranium):
Nasal capsule, interorbital septum, scleral ossicles, Meckel's cartilage, quadrate, articular, hyoid, columella, entoglossum.

Odontoblasts and tooth papillae

Other tissues
Dermis, smooth muscles, adipose tissue of the skin over the calvarium and in the face and ventral part of the neck; musculo-connective wall of the conotroncus and all arteries derived from aortic arches (except endothelial cells); pericytes and musculo-connective wall of the forebrain blood vessels and all of the face and ventral neck region; meninges of the forebrain; connective tissue component and tendons of ocular and masticatory muscles; connective tissue component of the pituitary, lacrymal, salivary, thyroid, parathyroid glands and thymus.

Trunk neural crest
Dorsal fins in lower vertebrates

Based mostly on data from avian embryos (Le Douarin et al., 2004).

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derivatives (Epperlein et al., 2000; McGonnell and Graham, 2002) can be revealed by appropriate environmental cues (Le Douarin et al., 2004).

PROBLEMATIC IDENTIFICATION OF NEURAL CREST CELLS

Neural crest cells are a predominant component of the vertebrate head, as seen in table 1. However, our knowledge of this issue is very limited and relies, in fact, on a few model animals. These results are then often generalised for all vertebrates. How is it possible that something as common as the embryological origin of main skeletal structures is not precisely known? The main problem is associated with the transient nature of the neural crest, the invasiveness of these cells and problems with their identification.

Neural crest cells were discovered by Wilhelm His (His, 1868) and named "Zwischenstrang" (intermediate cord). The participation of neural crest cells in the facial and visceral skeleton was first recognised at the end of the 19th century by the pioneering work of Kastschenko on shark embryos (Kastschenko, 1888) and of Julia Platt on the salamander *Necturus* (Platt, 1893, 1897). The importance and even existence of these cells had been controversial for a long time (see Hall, 1998b, 2000). As nicely pointed out by Langille and Hall (1993): "One hundred years ago, claiming that an ectodermal derivative such as the neural crest was in any

way involved with the formation of skeletal structures was the embryological and
evolutionary equivalent of nailing an additional thesis to the cathedral door. That
skeletal structures were mesodermal in origin was dogma, known and accepted by
all; an ectodermal origin was heresy". Establishing the neural crest as not only a
source of cranial and spinal ganglia, but actually as a major player in skeletogenesis
and in the evolution of the vertebrate head itself was a difficult process.

  After the recognition that neural crest cells do exist, the central issue in this
controversy was to distinguish cranial mesenchyme of neural crest origin (ec-
tomesenchyme or mes-ectoderm) from mesenchyme of mesodermal origin (mes-
endoderm). Classical studies attempted to analyse neural crest cell migration and
differentiation by means of simple histological techniques (Platt, 1897; Landacre,
1921; de Beer, 1947), extirpation and transplantation experiments (Stone, 1929;
Hörstadius and Sellman, 1946), chromatic dyes (Hirano and Shirai, 1984) or ra-
diographic labelling (Johnston, 1966; Chibon, 1967). However, the main problem
persisted: mesenchymal cells of very different embryonic origin do not contain any
specific morphological, histological, biochemical or molecular components which
can unequivocally be distinguished in adult tissue. Yet, despite the fact that, for
amphibians, the neural crest has long been attributed a direct role in cranial bone
development (Hörstadius and Sellman, 1946; de Beer, 1947), direct evidence for
neural crest contributions to the bony skull has remained elusive (but see Carl et al.,
2000; Gross and Hanken, 2004, 2005).

  The only way to detect neural crest cells reliably is to create lineage fate-maps
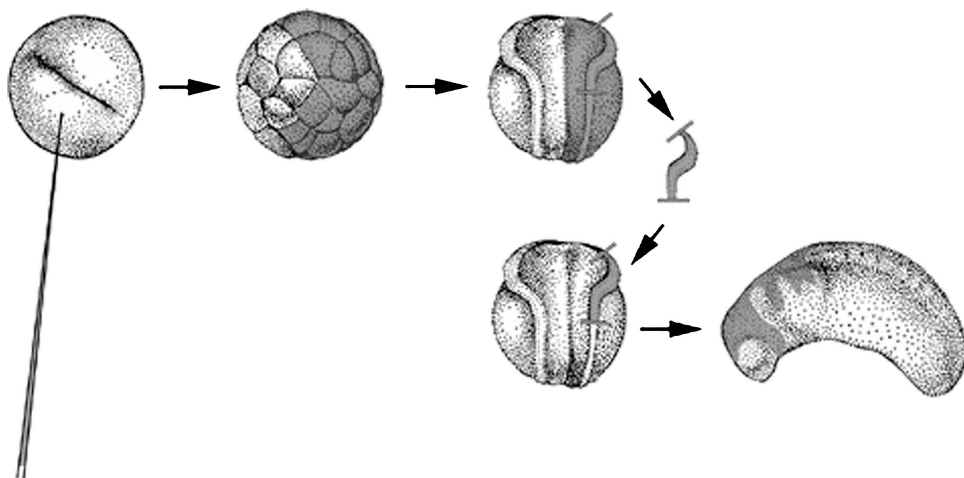(fig. 2), i.e., to trace single cells or small cell clusters from their origin to the

Figure 2. Lineage neural crest cell fate-mapping in axolotl. GFP mRNA is injected into one
blastomere where it incorporates into DNA; all descendant cells are GFP-positive (turn green after
illumination under UV light). When GFP-positive neural fold (a precursor population of neural crest)
is homotopically grafted into a host embryo, all neural crest cells emigrating from the graft are GFP-
positive and thus green. In this way, the population of neural crest cells can be traced with certainty
for a very long time.

final destination of their descendants in the larval or adult animal (Clarke and Tickle, 1999; Stern and Fraser, 2001; Cerny et al., 2004a, b; Ericsson et al., 2004). Such reliable cell markings have mostly been carried out using fluorescent dyes such as DiI and DiO (Lumsden et al., 1991; Serbedzija et al., 1992; Osumi-Yamashita et al., 1994; Epperlein et al., 2000; McCauley and Bronner-Fraser, 2003), or by injecting GFP mRNA or FITC-conjugated dextrans followed by experimental embryological or laser un-caging techniques (Carl et al., 2000; Sato and Yost, 2003). Recently, transgenic Cre/lox recombinant mice, where, for instance, the *Wnt-1* gene expression indelibly marks neural crest cells, have also been used (Chai et al., 2000; Jiang et al., 2000; Matsuoka et al., 2005). Another possibility is to create chimaeric animals, where neural crest cells from a donor species (like *Xenopus borealis*) are transplanted to a closely related host species (*X. laevis*) in which donor neural crest cells can be recognised by some specific feature (Sadaghiani and Thiebaud, 1987; Krotoski et al., 1988). Undoubtedly, the 'modern era' of neural crest research started after the introduction of quail chick chimaeras by N. Le Douarin in the late 1960s (see Le Douarin and Kalcheim, 1999, for a review). Therefore, our direct and reliable knowledge about cranial neural crest cell contributions to skeletal (and especially bony) tissues is based almost exclusively on research on avian embryos (Noden, 1983; Couly et al., 1992, 1993), with some recent contributions based on mice (Jiang et al., 2002; Matsuoka et al., 2005) and frogs (Gross and Hanken, 2004, 2005). Interestingly, data from these different tracing approaches tend to show that amphibians, birds and mammals possess unique patterns of neural crest contribution to the skull (Hanken and Gross, 2005). At least in some major groups of tetrapods the patterns of neural crest derivation of the skull seem to be evolutionarily labile and have not been rigidly conserved during vertebrate history.

THE VISCEROCRANIUM AND THE NATURE OF THE TRABECULA CRANII

Of all skeletal structures formed during embryogenesis, the viscerocranium, and especially the facial skeleton, is frequently considered to be the most intriguing (Richman and Lee, 2003; Clouthier and Schilling, 2004). The viscerocranium comprises the parts thought to be derived from pharyngeal arch elements; thus, interestingly, the only pragmatic definition of viscerocranial elements is their neural crest origin (Kardong, 1995; Le Douarin and Kalcheim, 1999). Consequently, the anterior internal cranium (roughly until the otic level; fig. 3), being neural crest-derived, is considered to belong to the viscerocranium, although it is topographically placed within the neurocranium.

A predominant component of this 'neurocranial' viscerocranium is a paired cartilage known as *trabecula cranii*. The trabeculae (or praechordalia) were discovered first by Rathke in the grass-snake (*Natrix natrix*) (de Beer, 1931) and later recognised as the essential elements of the cartilaginous anterior cranial base in all vertebrates, just as parachordal cartilages are the basis from which the posterior region of the skull arose (de Beer, 1931; Bertmar, 1959; Kuratani et al., 1997; Liem et al.,
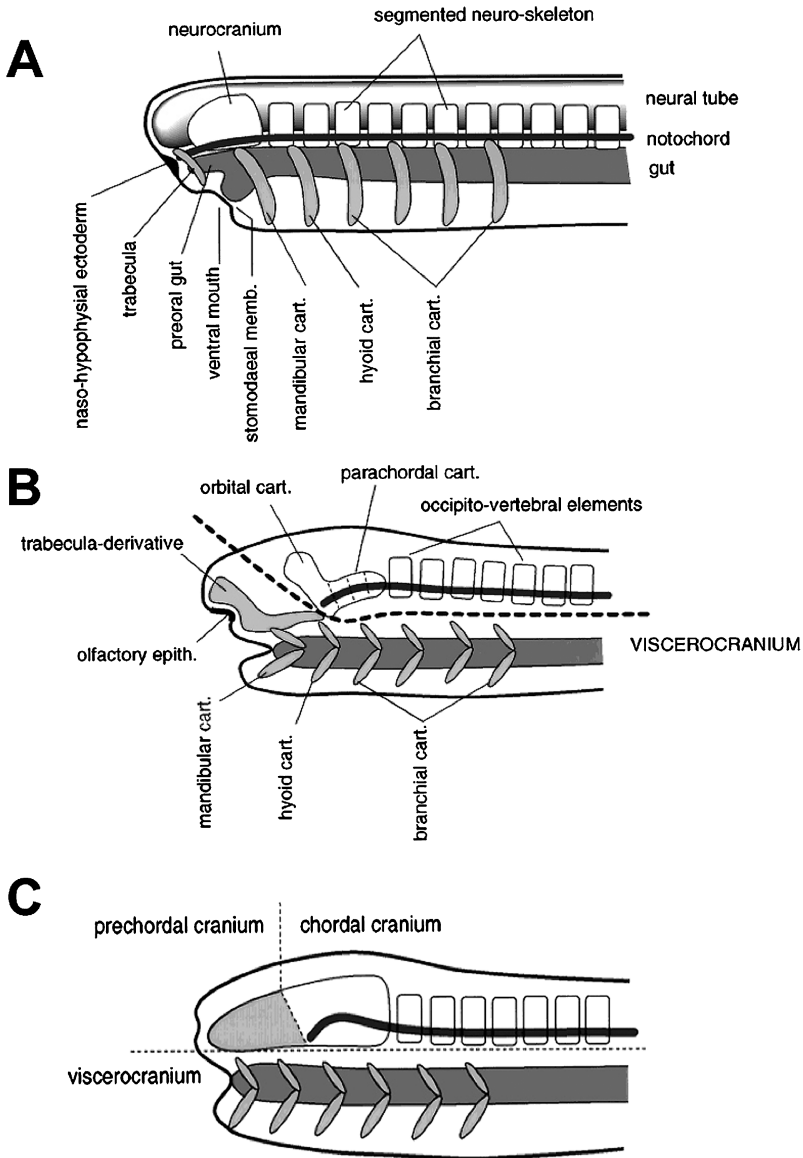
Figure 3. Evolutionary hypothesis explaining the trabecular cartilage as a viscerocranial element. A: An ancestral state: the trabecula comprises an anterior visceral arch structure being topographically in a pre-oral position. B: During the course of evolution, the trabecula enlarged to protect the anterior brain and subsequently become integrated into the neurocranium. The thick dotted line indicates the border between neural crest-derived viscerocranium (light gray) and mesoderm-derived chondrocranium (white). C: The resulting morphological plan of the vertebrate skull consists of dorsal neurocranium and ventral viscerocranium; however, the prechordal cranium is neural crest-derived whereas the chordal cranium is mesoderm-derived. Based on Kuratani et al. (1997).

2001). Whereas parachordal cartilages and thus elements of the posterior skull are mesoderm-derived, the opinion that trabeculae belong to visceral structures was validated only by their neural crest origin (de Beer, 1931, 1937; Kuratani et al., 2001). Therefore, according to an accepted evolutionary story (fig. 3), the trabecula cranii represents a viscerocranial component of the neurocranium, which is metamerically organised in register with cranial nerves and pharyngeal arches. From a radical segmentalist point of view (Bjerring, 1977), the trabecula might be considered as an original cartilaginous component of a hypothetical pre-oral arch (Allis, 1923; Jarvik, 1980), but this is very controversial (Janvier, 1996; Shigetani et al., 2005).

The radical segmentalism has it roots in idealistic morphology (Olsson et al., 2005) and it is quite difficult to find support for segmental theories in modern developmental biology. In the Mexican axolotl (*A. mexicanum*), for example, the trabecula originates among the neural crest cells on the dorsal side of the embryonic mandibular arch, in the maxillary portion (de Beer, 1931; Cerny et al., 2004a). A possible interpretation is that the maxillary part of the first arch represents the ancient pre-mandibular arch that has fused with the mandibular arch (de Beer, 1931). In this way, the trabecula could be regarded as a true element of the pre-mandibular arch (Allis, 1923, 1924). Alternatively the trabecula, with its origin in the maxillary portion, might be explained as the ancient dorsal element of the mandibular arch. This example shows that strict developmental support for segmental theories is lacking, and that this is a matter of interpretation. There is also no fossil evidence for either of these theories (for a recent review, see Janvier, 1996).

DO ALL VISCEROCRANIAL ELEMENTS INCLUDING THE TRABECULA NECESSARILY DEVELOP FROM NEURAL CREST CELLS?

In the context of the viscerocranium and its neural crest origin, it is interesting to remark that a condensation of the main anterior cranial component in the lamprey (which is called the trabecula as in the jawed vertebrates), could in fact be traced back to the first arch mesoderm rather than to the neural crest (Kuratani et al., 2004). The trabecula of the lamprey, i.e., the preotic basicranial element, is most likely an equivalent of the parachordal, a mesodermally derived element in gnathostomes, while a true homologue of the vertebrate trabecula in lamprey might be found in the ectomesenchyme of the upper lip (Kuratani et al., 2004).

The other interesting case, where truly viscerocranial elements do not seem to be derived from neural crest cells, is the basihyal and the second basibranchial cartilage in frogs. These elements are traditionally considered to arise from mesoderm (Hall, 1999). Moreover, many structures on the border between neural crest- and mesoderm-derived areas (e.g., the posterior trabeculae or *pila antotica*) seem to be of mixed origin (Olsson and Hanken, 1996).

We therefore ask whether elements traditionally regarded as being viscerocranial, such as the basibranchial and basihyal cartilages of frogs, as well as the trabecula of

lampreys, really belong to the viscerocranium, even if they do not seem to be derived from the neural crest? Clearly there is no answer yet, but any purely embryological definition of the viscerocranium is very problematic, because one has to take the surprising plasticity of neural crest cells (mentioned above) into account. Cranial neural crest cells are capable of condensing into cartilages in response to signals which normally initialise mesodermal skeletogenesis (Schneider, 1999). Therefore, in contrast to the classical theory, described above, that attempts to explain the trabecula as the ancient element of either the mandibular or the pre-mandibular arch which has been translocated during the course of evolution from a *viscerocranial* into a *neurocranial* position, it is conceivable that an anterior cranial element such as the trabecula could have developed *de novo* in order to support the enlarging anterior head. The only prerequisite needed would be an existing informative signal for condensation that meets pluripotent neural crest cells. Dermal bones, for example, might develop either from neural crest-derived dermis in the anterior head or from mesoderm-derived dermis, as is the case in more posterior head regions (for a review, see Le Douarin and Kalcheim, 1999), depending on the type of skeletogenic tissue available.

Because it seems that the actual diversity of the craniofacial skeleton strongly depends on an interplay between patterning and plasticity of cranial neural crest cells, the endless variation of vertebrate craniofacial features might be largely due to the creation of new structures according to actual functional needs, but also to functional modifications of anterior pharyngeal arch elements.

THE INTERFACING NATURE OF THE TRABECULA CRANII – A PROOF OF HOMOLOGY ACROSS THE VERTEBRATES?

Despite perpetual uncertainties concerning the evolutionary origin and developmental history of the trabecula cranii, its existence is beyond any doubt. Every craniate embryo possesses, at a certain developmental stage, a paired cartilage that topographically corresponds to what we normally call the trabecula cranii (de Beer, 1931, 1937). Despite the fact that different authors demonstrated different embryonic sources for this structure and proposed quite different models for its developmental meaning and homologies, no one doubts that such a structure actually exists and is identical in all craniates. In other words, the phenomenon of the trabecula cranii is not as problematic if it is restricted to the contextual and topological setting of the respective structure. This indicates that it is only the topology that is homologous in the case of the trabecula cranii.

Let us speculate on such a possibility:

A topological definition of the trabecula cranii

A paired cartilaginous element appearing in the preotic section of the head at the border between the brain and visceral structures.

Contextual meaning

The trabecula cranii is the first cartilaginous element appearing in the preotic section of the head. At that stage it may play the role of a rigid element that physically separates the domain of the anterior expansion of the anterior brain from the domain of posterior expansion of the stomodeum cavity, i.e., it forms a border between topologically divergent growth programmes.

In this respect the trabecula cranii represents, at least functionally, *both* the primordial base of the forebrain neurocranium and, at the same time, the most anterior element of the domain controlled by morphogenetic programmes for visceral development. Under such a setting, the trabecula can be viewed as an interface element that provides a topological blueprint for the preotic section of the head and integrates the respective divergent morphogenetic time schedules into a balanced morphogenetic programme for the entire head.

When attempting to understand the development and evolution of complicated structures such as the vertebrate head, one must always bear in mind that variations in the developmental programme can be adopted at various stages of development and thus can affect morphogenetic output at various levels of the developmental hierarchy. At the same time, any structure, whenever it appears, presents a contextual signal to growing tissues in its neighbourhood, at least in the form of a topological constraint on their expansion. Such spatial limitations or borders between adjacent morphogenetic domains represent true developmental constraints; they are real developmental causes of morphological homology (Wagner, 1994; Kuratani, 2005). Exactly this seems to be the case with the trabecula cranii: it constrains and redirects growth of both brain and stomodeum at a potential contact zone, and in that it contributes to refining the developmental time schedules of both. It is a constraint along which a developmental integration of both domains takes place.

At the same time, the trabecula can be viewed as a direct product of incompatibilities of the neighbouring developmental domains; an interface whose major role is to constrain them. In other words, the trabecula cranii should be looked upon as a border zone open to subsequent invasion of mesenchyme populations derived either from the cranial neural crest or from the cranial paraxial mesodem rather than as just a developmental product of either of these structures. It seems unproductive to search for a definite answer to the question about the developmental origin of the trabecula, as a postulate universally valid for all vertebrates. It cannot be excluded that the respective zone, i.e., the trabecula *sensu stricto*, is more tightly integrated into the neurocranial morphogenesis than into the morphogenesis of visceral arch structures in one group of craniates, but *vice versa* in another one, depending simply upon taxon-specific heterochronies in the development of these structures.

A substantial part of the morphogenesis of the vertebrate head is organised via intervention of multipotent cells of neural crest origin and via topological preformation of the adult structures with the aid of the mesenchymatic blastema they form. Such a 'diffusion dynamics' or 'nomadic morphogenesis' presents a new

dimension to morphogenetic interactions; something that dramatically changed both the playground and the rules of the triploblastic positional game.

The head, together with the neural crest, perhaps the most influential craniate apomorphy, is essentially generated in just that way. It develops beyond the constraints of the triploblastic morphogenetic game, segmentation control, etc., simply under its own rules, and not responding to the input developmental parameters. With respect to the clear morphogenetic rules of other triploblastic organisms, this is a development like in a looking-glass; something beyond the traditional interpretational paradigm of comparative morphology, i.e., the concepts established almost 100 years ago (Gegenbaur, 1870; Goodrich, 1930; de Beer, 1937). However, current models of neural crest development and further specificities of vertebrate morphogenesis related to it are much more recent (Hall, 1998a, 2000). It is no wonder that we still prefer to push our views on phenomena, such as the trabecula cranii, into the limits of traditional textbook alternatives. Should we not try – at least tentatively – to examine the morphogenetic playground at the looking-glass of head development with an optics more appropriate for this purpose? If so, among the first qualities which the neural crest cells brought to the developmental pathways is that they act as organisational agents which actively generate the interfaces between the resident tissues throughout the vertebrate body and integrate the specificities of their morphogenetic potential. Exactly this also seems to be valid for the structures to which they contribute; among others, the trabecula. Instead of attempting to define the trabecula as either an exclusively neurocranial or viscerocranial element, the trabecula might be seen as a true interfacing element amalgamating disparate developmental programmes together, and such a definition can be used to define 'the sameness' of this element throughout vertebrate groups. The case of the trabecula cranii also suggests that perhaps more items in head development should be looked upon as related to the entire context of head development and may originate in a way that reminds us of the recommendation for managing Looking-glass cakes: "You don't know how to manage Looking-glass cakes," the Unicorn remarked, "Hand it round first, and cut it afterwards" (from Lewis Carroll: Through the Looking Glass, Ch. VII: The Lion and the Unicorn).

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