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The neural crest as a fourth germ layer and vertebrates as quadroblastic not triploblastic

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Next to cells, germ layers—the fundamental embryonic cell layers from which tissues and organs form—are the most long-standing units of structural organization of the embryos of multicellular animals (metazoans). First identified in chicken embryos by Pander in 1817, the history of germ layers through the nineteenth century was one of increasing appreciation of their generality and importance:

- layers equivalent to those in the chick were found by Rathke (1825) in a decapod crustacean;
- von Baer (1828) discovered germ layers in the embryos of vertebrates other than the chicken;
- Huxley (1849) showed that the outer and inner layers of vertebrate embryos, which George Allman in 1853 named "ectoderm" and "endoderm," were homologous with the two germ layers seen in adult coelenterates.

Thus, Huxley, who coined the term "mesoderm" for the middle layer, extended the germ layer concept from embryos to adults and from embryology to evolution, although it took Huxley some time to appreciate the significance of his discovery. For decades, germ layers were the backdrop against which research programs were initiated and the context into which new findings were inserted; see Hall (1998a, 1998b) for overviews.

E. R. Lankester gave germ layer theory an even more substantial role when in 1877 he divided the animal kingdom into three grades:

- the Homoblastica (protozoa) had a uniform single "layer";
- coelenterates with two germ layers (ecto- and endoderm) comprised the Diploblastica;
- all other animals possessed three germ layers (ecto-, endo-, and mesoderm) and comprised the Triploblastica.

Lankester's scheme remains to this day: metazoans are regarded as either diploblastic or triploblastic. My aim is to demonstrate that the neural crest, a layer of cells associated with the developing neural tube and discovered in chick embryos in 1868 but present in all vertebrate embryos (Hall 1999), is a fourth germ layer, and that vertebrates (or more strictly craniates (vertebrates + hagfishes) are not triploblastic but quadroblastic. What is the basis for such an apparent radical change to a scheme that has stood for almost 125 years?

A germ layer is a fundamental embryonic layer from which tissues and organs arise. Ectoderm and endoderm are primary germ layers. They are present from the outset of development, having been specified maternally during development of the egg (Gilbert 1997; Hall 1998b). Mesoderm is a secondary germ layer arising after fertilization, often only after inductive interactions between future ectoderm and endoderm (Hall 1998a, 1998b, 1999). The neural crest, which is the dorsalmost portion of the neural folds in all vertebrate embryos, also arises secondarily, following inductive interactions between two types of ectoderm—neural and epidermal (Fig. 1). Both mesoderm and neural crest break up into populations of cells that migrate away from the midline to form a diversity of cell types (Hall 1999).

Mesoderm is recognized as a germ layer because of the tremendous diversity of cell and tissue types that originate from it; mesoderm breaks up into recognized populations of cells (sclerotome, dermamyotome, lateral plate, somatic, and splanchnic mesoderm) and forms the same tissues and structures across the animal kingdom. Neural crest breaks up into populations of cells that are conserved in all vertebrates and form the same tissues and organs across the vertebrates (Hall 1999). Indeed, neural crest produces an even greater array of cells and tissues than does mesoderm, including neural, pigment, skeletal, connective tissue, cardiac, dental, and endocrine cells. Mesoderm and neural crest both give rise to embryonic mesenchyme. On all points, if mesoderm qualifies as a secondary germ layer, so does neural crest (Hall 1998a).

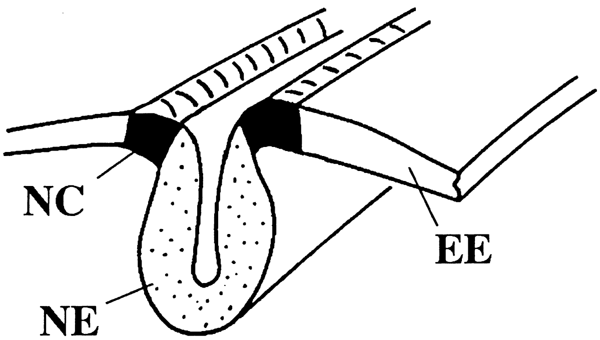

Fig. 1. A diagrammatic view of the developing neural tube of a typical vertebrate embryo at the stage when neural ectoderm (NE, stippled) has folded to form open neural folds. Brain and spinal cord develop from the neural tube. Neural crest cells (NC, black) are found at the dorsal edge of the neural folds between the NE and the epidermal ectoderm (EE), from which the skin arises.

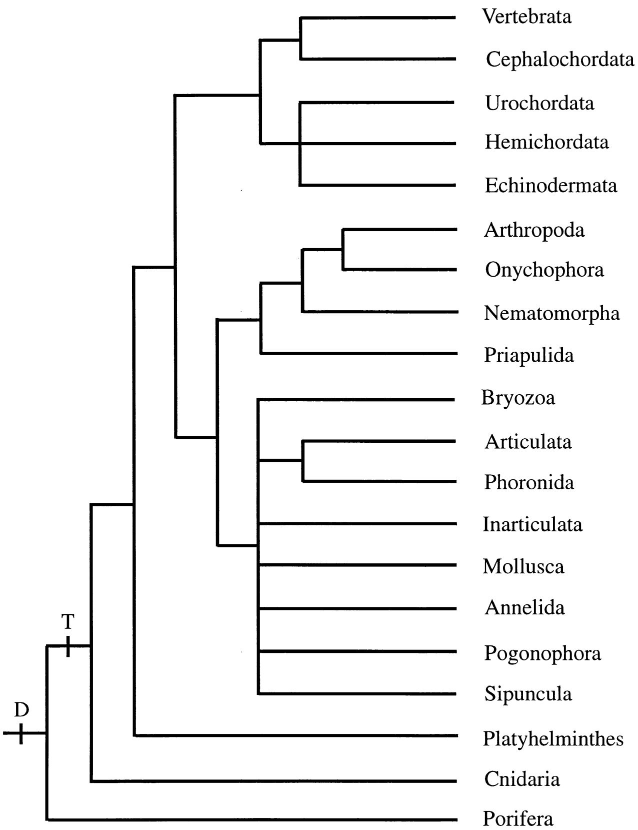

Fig. 2. A phylogeny of 20 major phyla to show the origins of the germ layers. D shows the evolution of the diploblastic level of organization, characterized by the origin of the ectodermal and endodermal germ layers. T refers to the origin of the triploblastic level of organization and the evolution of mesoderm as a germ layer. Phylogeny adapted from data in Erwin et al. (1997).

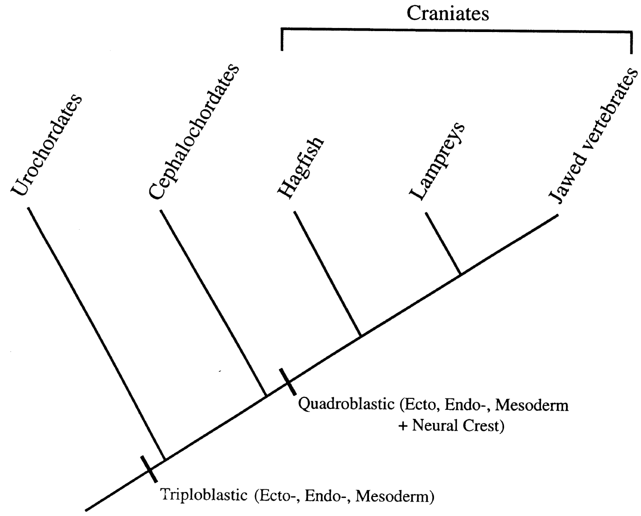

Fig. 3. The origin of the neural crest, and therefore of the quadroblastic grade of organization, preceded the origin of the craniates (shown as the extant groups, hagfishes, lampreys, jawed vertebrates) from a protochordate ancestor. Two extant protochordate groups (urochordates, cephalochordates) are shown.

The evolution of mesoderm permitted the origin and enormous diversification of triploblastic metazoans (Fig. 2). Neural crest arose at the origin of the craniates from a protochordate ancestor (Fig. 3). Possession of a neural crest is a synapomorphy for craniates and provided the basis for the evolution of unique craniate/vertebrate tissues and organs (Smith and Hall 1990, 1993; Hall 1998b, 1999).

In recognition of the neural crest as a fundamental cell layer in all craniate embryos and to reflect the absence of this layer in all other metazoans, the neural crest should be regarded as a germ layer and craniates as quadroblastic not triploblastic.

Acknowledgments

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Note added in proof: Comments are invited. As the first comment, I would add that, contrary to the claim by Richardson et al. (1999) in their paper in the first issue of this journal, I did not reject the universality of traditional germ layers. Indeed, in my 1998 paper, I concluded an extensive series of arguments with the statement that: "While germ layers remain as essential components of metazoan embryos, the germ-layer theory falls by the wayside. It is germ layers 4; germ-layer theory 0" (Hall, 1998a p. 171).

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