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Pentimento: Neural Crest and the origin of mesectoderm

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ABSTRACT

The Neural Crest, a transient epithelium in vertebrate embryos, is the source of putative stem cells known to give rise to neuronal, glial and endocrine components of the peripheral (sensory, autonomic and enteric) nervous system (PNS) and pigment cells in the skin. The Neural Crest is also widely believed to be the source of mesectodermal derivatives (skeletogenic, odontogenic, connective tissue and smooth muscle mesenchyme) in the vertebrate head [see (Bronner and LeDouarin, 2012; Le Douarin, 2012; Le Douarin and Kalcheim, 1999); see also (Hörstadius, 1950; Weston, 1970)]. This conventional understanding of the broad developmental potential of the Neural Crest has been challenged over the past few years (Breau et al., 2008; Lee et al., 2013a, 2013b; Weston et al., 2004), based on recognition that the definition of the embryonic epithelia that comprise the Neural Crest may be imprecise. Indeed, the definition of the embryonic tissues understood to constitute the Neural Crest has changed considerably since it was first described by Wilhelm His 150 years ago (His, 1868). Today, the operational definition of the Neural Crest is inconsistent and functionally ambiguous. We believe that more precise definitions of the embryonic tissues involved in Neural Crest development would be useful to understand (1) the range of cellular phenotypes that actually segregate from it, (2) when this lineage diversification occurs, and (3) how diversification is regulated.

In this idiosyncratic review, we aim to explain our concerns with the current definitions in this field, and in the chiastic words of Samuel Johnson (1781), "… make new things familiar and familiar things new".[1] Then, we will try to distinguish the developmental events crucial to the regulation of Neural Crest development at both cranial and trunk axial levels of vertebrate embryos, and address some of the implicit assumptions that underlie the conventional interpretation of experimental results on the origin and fates of Neural Crest-derived cells. We hope our discussion will resolve some ambiguities regarding both the range of derivatives in the Neural Crest lineage and the conventional understanding that cranial mesectodermal derivatives share a common Neural Crest-derived lineage precursor with components of the PNS.

"Theories have four stages of acceptance: i) This is worthless nonsense; ii) This is an interesting, but perverse, point of view, iii) This is true, but quite unimportant; and iv) I always said so." [from a book review by J.B.S. Haldane (Haldane, 1963)]."

Historic Background

His (His, 1868) first recognized and described a distinct band of cells that lay between the dorsal neural tube and the epidermal epithelium at the neurula stage of avian embryo development. He operationally named this transient structure *Zwischenstrang* and suggested, from morphological studies, that its cells were the source of peripheral ganglia. Subsequently, Marshall (Marshall, 1879) named this domain of cells the *Neural Crest*. He described the Neural Crest as the "outgrowth" of cells formed by the fusion of the longitudinal "neural ridges" after they meet in the embryonic midline to form the *neural tube* (which he referred to as the "neural canal") and after the overlying *epidermal epithelium* (which he called the "external epiblast") separates from the neural tube. Importantly, Marshall explicitly distinguished the Neural Crest, which appears to be part of the dorsal epithelium of the nascent

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[1] Samuel Johnson's words were paraphrased by Mardy Grothe [drmardy.com] from Johnson's Lives of the Poets [1781]. Discussing Alexander Pope's poem, *The Rape of the Lock*, Johnson wrote, "In this work are exhibited in a very high degree the two most engaging powers of an author: New things are made familiar, and familiar things are made new."

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neural tube, from its antecedent, the paired neural ridges, what we now call the *dorsal ridges* of the embryonic neural folds. He described these ridges as the "reentering angle between the external epiblast and the neural canal"—clearly indicating that the folds included both epidermal and medullary (neural) epithelia. As we will discuss further in the Formation of the Neural Crest section, the Neural Crest might more accurately be considered a portion of the dorsal neural tube epithelium that forms after the paired neural folds fuse in the embryonic midline to create the neural tube and after the neural epithelium separates from the overlying epidermal epithelium.

Julia Platt is often credited as the first to suggest, 120 years ago, that the Neural Crest was the source of skeletal and connective tissue derivatives in the head of amphibian embryos (Hall, 1999). Platt's detailed histological descriptions (Platt, 1891a, b; Platt, 1893, 1894) led her and a few others to infer that cells forming the cranial skeleton originated from a lateral epithelial domain of the embryonic neural folds. Her main point—that skeletogenic mesenchyme ("Mesectoderm") arose from ectodermal epithelium—caused a serious controversy in the field of comparative morphology. This controversy, nicely summarized by Landacre (Landacre, 1921), arose because it contradicted a major tenet of the Germ Layer Theory, which had stipulated that mesoderm was the embryonic germ layer that produced skeletal and connective tissues, as well as muscle, blood and vascular tissues. Although Platt's audacious challenge to the classical Germ Layer Theory was manifestly deleterious to her scientific career, it is important to emphasize that she was not responsible for making the Neural Crest "famous" as the source of skeletogenic mesenchyme [see (Hall, 1999)]. Rather, she seems to have claimed only that the ectoderm of the neural folds, including the dorsolateral and epibranchial epithelium, produces mesectodermal connective tissue as well as peripheral ganglia.[2] The assertion that the Neural Crest was the source of skeletogenic mesenchyme should, instead, more appropriately be attributed to the numerous pioneers in the newly emerging field of experimental embryology who undertook to establish the developmental fates of embryonic cells and map them to epithelial locations in developing embryos.

Fate-mapping

Various experimental approaches have been used to test the normal developmental fates of cells in early embryonic epithelial domains. The details and limitations of the classical cell marking and fate-mapping studies have been critically reviewed elsewhere (Le Douarin and Kalcheim, 1999; Weston, 1970), but they all primarily used amphibian and avian embryos and employed one or more of the following three basic experimental procedures: (1) surgically ablating specific embryonic regions followed by an assessment of the resulting structural lesions; (2) marking specific locations of embryonic epithelia with vital dyes or other extrinsically applied substances followed by analysis of the fates of marked cells; and (3) observing the fates of cells derived from transplanted tissues of embryonic donors whose cells had been labeled with intrinsic or applied markers. The validity of the inferences from all these procedures depends on the specificity of the marking method. Such specificity, in turn, depends on knowing *accurately* what tissues were ablated or transplanted, assuring that extrinsic markers were *precisely* applied to known embryonic locations and not transferred to adjacent cells, and finally, assuring that intrinsic markers were *not expressed* in tissues or regions other than the one designated. As we shall discuss below, problems of interpretation arise when these criteria are not fulfilled.

The experimental embryologists confirmed the various derivatives of putative Neural Crest, and their studies—including the controversial suggestion that Neural Crest derivatives included cranial (visceral arch) structures, odontoblasts, and osteoblasts of dermal bone—were considered in an influential descriptive paper by de Beer (de Beer, 1947), who pronounced that such studies showed "unequivocally" that Neural Crest was the source of skeletogenic mesenchyme. He went on to name these putative crest-derived precursors "Ectomesenchyme".[3] These early experimental analyses of Neural Crest development culminated in Hörstadius' influential review (Hörstadius, 1950), who summarized the work of his student Sellman and other workers who had mapped the developmental history and fates of the cells thought to originate from the Neural Crest of amphibian embryos.

It is important to recognize, however, that the original marked domains in all of the relevant fate-mapping studies included not only the Neural Crest itself, as described by Marshall (Marshall, 1879), but also the adjacent, lateral non-neural epithelia of the neural folds (see Fig. 1). In his review (Hörstadius, 1950), Hörstadius explicitly acknowledged the ambiguity of whether the Neural Crest was an outgrowth from the spinal cord or a separate rudiment, and noted discrepancies in the comparative morphology literature about the "position of the crest material in relation to the thick neural plate and the thinner presumptive epidermis." These discrepancies are illustrated in Fig. 2. Significantly, Hörstadius followed Raven's (Raven, 1931) specific conclusion that the entire ectoderm of the dorsal neural fold (see Fig. 2A) consists of presumptive Neural Crest cells, and explicitly dismissed potential problems of interpretation by asserting that

> "…these discrepancies are of minor importance for the experimental worker, as in any case crest cells in Urodeles at the stages used for operation are situated in the ridges that are extirpated or transplanted."

This conclusion affected the interpretation of experiments in this field for the next half-century. Thus, in the years that followed Hörstadius' review, the operational definition of Neural Crest was implicitly changed to include not only the dorsal ridges of the neural epithelium but also the lateral non-neural epithelium of the embryonic neural folds. Subsequent "neo-classical" grafting studies (Johnston, 1966; Noden, 1975; Weston, 1963) largely confirmed the various derivatives that had previously been attributed to the trunk and cranial Neural Crest, and began to map more precisely the timing and pathways of the migration of Neural Crest-derived cellular precursors of these derivatives, using tritiated-thymidine as a precise and relatively durable marker of donor cell nuclei. Conforming with these earlier mapping studies in amphibian embryos, pharyngeal cartilages and dermal bone were seen to be populated by graft-derived cells at rostral axial levels but, where neural fold grafts at all axial levels gave rise to connective tissue derivatives, grafted tissues from trunk axial levels of amniote embryos failed to contribute cells to skeletogenic mesenchyme in the trunk.

[2] Platt's actual, rather convoluted, summary statement (1894) was: "Die aus der Neuralleiste und aus der dorsolateralen und epibranchialen Verdickungen des Ektoderms ausgehenden Zellen bilden nicht allein Nerven, denn eine jede dieser Anlagen trägt sowohl zur Bildung des mesektodermalen "Bindegewebes" wie zur Bildung der Ganglien bei, und nachdem Ganglien und "Bindegewebe" sich von einander getrennt haben schliessen sich Ektodermzellen noch weiter den beiden Abtheilungen des Mesektoderms an."

[3] Strictly speaking, the name "Ectomesenchyme" should include any population of mesenchymal cells, regardless of their subsequent developmental fate, that delaminate from ectodermal epithelium. To distinguish mesoderm-like, skeletogenic mesenchyme from mesenchyme that originates from "authentic" Neural Crest, therefore, it would probably be better to refer to the former as "Mesectoderm" (Le Douarin et al., 2004; Le Douarin and Kalcheim, 1999).