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# What insights into the phenomena of cell fate determination and cell migration has the study of the urodele neural crest provided?

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**ABSTRACT In this review we ask whether studies on the development of the urodele neural crest (NC) have provided special insights into the fate and migration of these cells when compared to other amphibian embryos or those of higher vertebrates. We recognize that during the first half of this century and even before, urodele embryos were the favorite objects of experimental embryology for studying the development of mesenchymal derivatives and their participation, together with mesodermal mesenchyme, in the development of the neuro- and viscerocranium. Furthermore, the NC was discovered to be the source of cranial sensory and spinal ganglia, and the influence of the somites on the localization of the latter was clearly pointed out. In addition, pioneering studies were devoted to the NC-derived pigment cells. Investigations in this field concentrated on their migration in the embryo and *in vitro*, and on the mechanisms underlying larval pigment pattern formation. It is mainly in these three areas that the urodele embryo has served as a tool for gaining major results and defining the concepts of classical embryology. Even today, when the interest has shifted towards the molecular biology in *Xenopus*, chicks and mice, the urodele embryo with its large cells, convenient for injections, is a potential model for future lineage studies and knockout experiments. And furthermore, as important concepts of vertebrate development are defined in the urodele, future studies in these embryos may link the disciplines of development and evolution.**

KEY WORDS: *Urodele amphibians, neural crest, migration, differentiation*

## Introduction

The neural crest (NC) represents a transient embryonic structure specific to vertebrates. It consists of a cord of ectomesenchymal cells lying on top of the neural tube. From this site, NC cells migrate into various body regions and give rise to neurons and glial cells of the peripheral nervous system, connective and supportive tissue, pigment cells and a variety of other structures (Weston, 1970; Le Douarin, 1982; Hall and Hörstadius, 1988).

In this review we ask what insights into early embryonic processes, such as cell migration and cell differentiation, have emerged from studies of the NC in urodele amphibians. The urodele species used for experiments are mainly those of the genus *Taricha* (Californian newt), *Triturus* (the European newt) and *Ambystoma* (the Mexican axolotl). The development of these species has been staged in normal tables (*Taricha torosa*: Twitty and Bodenstein, 1962; *Triturus alpestris*: Epperlein and Junginger, 1982; *Ambystoma mexicanum*: Bordzilovskaya *et al.*, 1989).

It is certainly naive to assume that the NC of a particular genus of vertebrates could supply us with major knowledge about the developmental processes of NC cells. Research on embryos within the different classes of vertebrates, whether mammals, birds or amphibians, is so much intermingled and the biological processes are so similar that certain insights and results are often obtained only by comparative observations. To a lesser extent we will be discussing observations of that type. A major part, however, includes experiments which were designed for and carried out only in urodeles, either because this order has served traditionally as an experimental system or because it provides unique advantages over other systems.

Among the experiments first carried out in urodeles were those on the migration and differentiation of the cranial NC and on the formation of cranial ganglia in *Ambystoma punctatum* (Stone, 1926; *punctatum* is *maculatum* today), and those on the three-dimensional reconstruction of the head skeleton (Stone, 1926; Raven, 1931). The first fate map for NC cells was designed in a urodele neurula

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*Abbreviations used in this paper:* NC, neural crest; CSPG, chondroitin sulfate proteoglycan; GFAP, glial fibrillar acidic protein; GAG, glycosaminoglycans; LSM, laser scanning microscope; BM, basement membrane.

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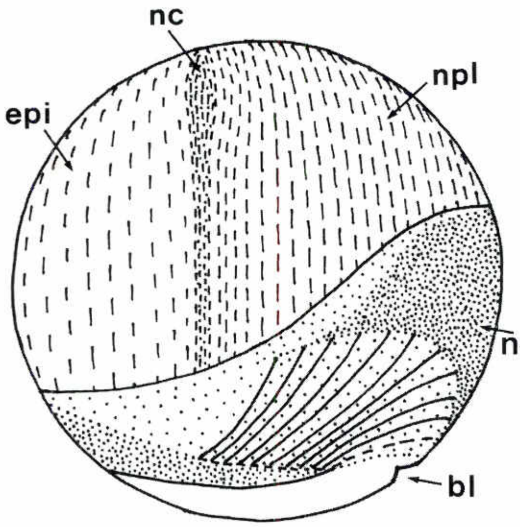


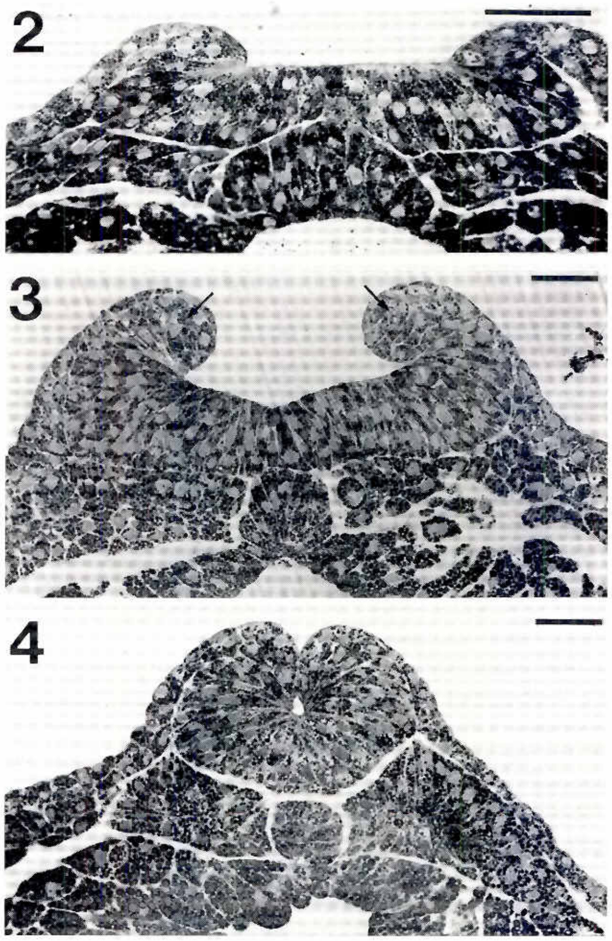
**Fig. 1. Localization of the prospective neural crest (nc) in a urodele gastrula.** *Using vital dyes, the NC has been traced back to this stage. It is situated between the prospective epidermis (epi) and the prospective neural plate (npl). not, notochord; bl, blastopore. Redrawn after Vogt (1929).*

(Hörstadius and Sellman, 1946), and among the first amphibian NC cells in culture were those of *Ambystoma* (Twitty and Bodenstein, 1939; Twitty and Niu, 1948). Apart from this direct involvement of urodele embryos in certain aspects of NC cell research, embryological research generally during the first half of this century was directed towards amphibians. Leading embryologists worked with amphibians: Harrison in the United States in the field of experimental neurogenesis and tissue culture; Spemann in Germany on early organ determination (Hamburger, 1988).

Among those urodele NC derivatives which have attracted special interest are the pigment cells. They have been studied within a long tradition (Twitty, 1936, 1966; Epperlein and Löfberg, 1990) and offer many advantages over pigment cells of chickens and mice. Whereas pigment cells in the latter embryos consist of only the melanocytes, which are often outwardly invisible or visible only with difficulty, the pigment cells in amphibian embryos comprise three different cell types, melanophores, xanthophores and iridophores, which are clearly visible from outside (see Frost-Mason and Mason in this issue). In urodeles, pigment cells are mainly used for studying cell differentiation, cell-matrix interactions and pigment pattern development. Their arrangement in various pigment patterns serves as a model system for studying mechanisms of NC cell differentiation and distribution. These mechanisms could be valid also for other types of pattern, such as the visceral arches formed by NC-derived chondrocytes or the chain of dorsal root ganglia formed by NC-derived neurons and glial cells. These latter patterns develop within the embryo, thus excluding direct observation, but we assume they are subject to similar morphogenetic principles as pigment cells during pigment pattern formation.

## Cell fate determination

One of the most important questions for the genetic and developmental analysis of the NC relates to its lineage and differentiation into various derivatives. *Is the NC already set apart in one defined blastomere, or is it mixed from the material of several blastomeres and present only at later stages, during gastrulation or neurulation?* The only experiments tracing urodele NC cells back earlier than the neurula stage were done by Vogt (1929). Applying vital dyes (externally) to the early gastrula, he defined the prospective NC as a narrow stripe of ectodermal cells between the prospective epidermis and the prospective neural plate (Fig. 1). In the urodele embryo, NC cells were thought to be induced by the lateral margins of the chordamesoderm (Raven and Kloos, 1945), i.e., at the margin of neuralizing and epidermalizing influences (Rollhäuser-ter-Horst, 1977, 1979; Moury and Jacobson, 1989). NC cells in *Ambystoma mexicanum* were shown to develop from both epidermal and neural plate material (Moury and Jacobson, 1990). Before the neural folds become elevated, their inner margins are delineated by a greater number of pigment granules (of maternal origin). Already at this stage the prospective neural fold contains NC material. Still later, the NC cells are located in the apical part of the elevated folds (Figs. 2-4). The identification of NC cells within the folds based solely on morphological criteria is



**Figs. 2-4. Development from neural plate to neural tube.** *Transverse sections through neurula stages (prospective midtrunk region) of Ambystoma mexicanum. Early (stage 14;* ***2****), middle (stage 16;* ***3****) and late neurula (stage 18;* ***4****). Prospective NC material is contained in the apical neural folds (arrows) but cannot be identified solely on morphological criteria. Bars, 100 μm*