The Neural Crest and Neural Crest Cells in Vertebrate Development and Evolution

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Springer

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ISBN 978-0-387-09845-6 e-ISBN 978-0-387-09846-3
DOI 10.1007/978-0-387-09846-3

Library of Congress Control Number: 2008941099

Printed on acid-free paper

springer.com

Preface

Knowledge of the development and evolution of the neural crest sheds light on many of the oldest questions in developmental and evolutionary biology. What is the role of germ layers in early embryogenesis? How does the nervous system develop? How does the vertebrate head arise developmentally, and how did it arise evolutionarily? How did the vertebrate dorsal nervous system, heart, skeleton, teeth (and the neural crest itself) originate? How do growth factors and *Hox* genes direct cell differentiation and embryonic patterning? What goes wrong if development is misdirected by mutations, or if embryos are exposed to exogenous agents such as drugs, alcohol, or excess vitamin A (retinoic acid)?

Twenty years ago, I was instrumental in organizing the publication of a facsimile reprint of the classic monograph by Sven Hörstadius, *The Neural Crest*: *Its properties and derivatives in the light of experimental research*, originally published in 1950. Included with the reprint was an analysis of subsequent studies on the neural crest and its derivatives. A decade later, the first edition of this book was published (Hall, 1999a). The explosion of interest in and knowledge of the neural crest over the past decade prompted me to write this second edition.

As in my 1988 overview of the reprinting of '*Hörstadius*'—as his book is known to many—and as in the first edition of this book, I take a broad approach in dealing with the discovery, embryological and evolutionary origins, migration, differentiation and cellular derivatives of the neural crest. Cells from the neural crest are associated with many developmental abnormalities, many of which have their origins in a defective neural crest (NC) or in defective neural crest cells (NCCs). The book would be incomplete without discussing neurocristopathies—those tumors and syndromes involving NCCs or those birth defects in which NCCs play a role.

The book is organized into three parts.

Part I (Discovery and Origins) begins with a chapter devoted to the discovery of the neural crest and the impact of that discovery on entrenched notions of germ-layer specificity and the germ-layer theory, a theory that placed a straitjacket around embryology and evolution for almost a century. Primary and secondary neurulation and the neural crest as the fourth germ layer are introduced in this chapter.

In Chapter 2, I discuss the embryological origins of the neural crest, including the identification of future NCCs in gastrula-stage embryos; molecular and cellular

markers of future NCCs; neural and neural crest induction; and rostrocaudal patterning of the developing neural tube and neural crest.

Chapter 3 takes NCCs out of the neural tube with discussions of:

- the delamination of NCCs from the neural tube as mesenchymal cells (a process requiring the transformation of epithelial to mesenchymal cells, usually written in the text as EMT or epithelial —> mesenchymal transformation),
- NCC migration and the nature of the extracellular matrices (ECM) through which or along which they migrate, and
- the differentiative potential of NCCs.

Chapter 4 is devoted to the evolutionary origins of the neural crest through an analysis of fossils and of cell types, genes, and gene networks in extant cephalochordates (amphioxus) and in urochordates (chiefly ascidians) in an effort to answer the question 'Is there any evidence of precursors of the neural crest in urochordates or in cephalochordates?' The second aim of Chapter 4 is to examine the origin of neural and skeletal tissues of neural crest origin in the first vertebrates (i.e., chordates with a head), and the origin of the jaws in the transition from jawless to jawed vertebrates.

Part II (Neural-Crest Derivatives) presents an analysis of our knowledge of the cell types into which NCCs differentiate. The organization of this part differs from the first edition in which the chapters were organized by major groups of vertebrates, each of which included a discussion of similar cell types—neural, pigment, and skeletal cells. In this edition, I have organized each of the four chapters around major class of cells and the tissues and organs they form or to which they contribute:

- pigment cells and color patterns (Chapter 5);
- neurons and the nervous system (Chapter 6);
- cartilage, bone, and skeletal systems (Chapter 7); and
- dentine-forming cells and teeth, and the smooth muscle, septa and valves of the heart (Chapter 8).

These chapters cover:

- **trunk neural crest cells** (TNCCs)—Chapter 5;
- the **vagal and sacral neural crest** (VNC, SNC), peripheral nervous system (spinal and cranial ganglia), autonomic and parasympathetic nervous systems (sympathetic and parasympathetic ganglia, enteric ganglia, adrenal chromaffin cells), Schwann and glial cells, and Rohon–Béard neurons—Chapter 6;
- **cranial neural crest cells** (CNCC), chondroblasts and osteoblasts, mesenchyme, the skeletogenic (chondrogenic) neural crest, and epithelial–mesenchymal interactions—Chapter 7;
- the odontogenic neural crest, odontoblasts (dentine-forming cells), tooth formation, and the **cardiac neural crest (CarNC)**, the heart, and development of valves, septa and the aortic arches—Chapter 8.

Part III consists of two chapters, Chapter 9 devoted to tumors of neural-crest origin (neurocristopathies), Chapter 10 to a reconsideration of NCC development in the context of birth defects.

Chapter 9 includes discussions of neuroblastomas, neoplasia, and examples of syndromes based in defective NC or NCCs, the two major examples being APUDomas and DiGeorge syndrome.

Chapter 10 broadens the scope to birth defects (often but not always involving the neural tube to which NCCs contribute) or which are induced by a teratogen—vitamin A and craniofacial defects in this case. Mutations affecting NCCs are discussed as is the ability of NCCs to compensate for lost cells, a developmental property known as regulation and a discussion that brings us full circle to the differing potentials of subpopulations of NCCs and whether any NCCs persist as stem cells in embryos or adults.

To avoid interrupting the flow of the text, I have placed most references and some supporting statements in numbered notes, which are gathered at the end of each chapter, and which serve as an annotated bibliography through which access to the literature may be obtained. I have not included all of the literature published before 1999, much of which is in the first edition (Hall, 1999a*). Otherwise, I have surveyed the literature to early 2008. References marked with an * are significant reviews or analyses. Occasionally, I use footnotes $^{(\otimes)}$ for general points that apply throughout. Similarly, boxes are used for items of general interest, biographies, or interesting case studies. †signifies an extinct taxon. Gene names are italicized and capitalized (*Shh*), proteins are in plain text and capitalized (Shh). Human genes and proteins are capitalized (*SHH*, SHH). As a shorthand expression for a transformation or interaction I use the symbol —>. The text is extensively illustrated and there is a detailed index. A list of abbreviations is provided. From that list, the following are abbreviations for regions of the neural crest (NC) or for populations of neural crest cells (NCCs).

NC	NCCs
CarNC — cardiac neural crest	CarNCCs — cardiac neural crest cells
CNC — cranial neural crest	CNCCs — cranial neural crest cells
NC — neural crest	NCCs — neural crest cells
SNC — sacral neural crest	SNCCs — sacral neural crest cells
TNC — trunk neural crest	TNCCs — trunk neural crest cells
VN — vagal neural crest	VNCCs — vagal neural crest cells

I am grateful to the following experts who provided invaluable comment on individual chapters—chapter reviewed are shown in parenthesis—Marianne Bronner-Fraser (2), Carol Erickson (3), Daniel Meulemans (4), Lennart Olsson (5), Ryan Kerney (7), and Gerhard Schlosser (6, Part). Ryan Kerney and Jennifer Quinn provided helpful comments on stem cells, Cory Bishop comments on ascidians. Tim Fedak prepared 20 of the new figures. Many thanks, Tim. June Hall edited the

manuscript for style and comprehensibility in her inimitable way. Many thanks, June. Individuals who provided figures are acknowledged in the appropriate figure legend. Figure 7.10 is modified from Del Pino and Medina (1998), published by UBC Press, Leioa, Vizcaya, Spain. Financial support for my research program on the neural crest and its derivatives from the Natural Sciences and Engineering Research Council (NSERC) of Canada is gratefully acknowledged.

Halifax and Tempe Brian K. Hall

Contents

Abbreviations

	A
Adam13	a member of the Adam family of membrane-anchoring metalloprotease (named from *a d*isintegrin *a*nd *m*etalloprotease domain)
Amphi	prefix for genes in amphioxus (*Branchiostoma* spp.), for example, *AmphiOtx*
APUDomas	tumors that share *amine precursor uptake and decarboxylation*
	B
BALB/c	an inbred strain of mice that develops numerous tumors in later life
Bdnf	brain-derived neurotrophic factor
bHLH	basic helix–loop–helix transcription factors
Bmp	bone morphogenetic protein family of genes and their product
BmpR	bone morphogenetic protein receptor, for example, BmpR1
	C
CarNC	cardiac neural crest
CarNCCs	cardiac neural crest cells
Cbfa1	Core binding factor alpha 1; see Runx2
Cdh	Cadherin, a family of 20 Ca^{++}-binding transmembrane proteins that function in cell adhesion
ch	*congenital hydrocephalus* mutant mice
CHD7	chromodomain helicase DNA binding protein 7
Chn	*Chinless* mutant zebrafish
Ci	prefix for genes in the sea vase, *Ciona intestinalis* (an ascidian)
cls	*colorless* mutant in zebrafish
CNC	cranial neural crest
CNCCs	cranial neural crest cells
CNS	central nervous system of vertebrates
Col2α1	the gene for the procollagen type II alpha 1 chain.
Con	*Chameleon* mutant zebrafish
CraBP	cellular retinoic acid binding protein
CRKL	*v-crk sarcoma virus CT10 oncogene homolog* [*avian*]*-like*

D

DiI	1,1′-dioctadecyl-3,3,3′,3′-tetramethyl indocarbocyanine perchlorate
Dil	the *dilute* (*dil*) allele in the budgerigar, a mutation in melanocytes
Disp1	the protein dispatched (DISP1), a regulator of *Shh*
Dll	a family of genes Delta proteins, which are type-1 cytokine receptor family protein
Dlx	*distalless* gene family in vertebrates, for example, *Dlx1*
DOPA	3, 4-dihydroxyphenylalanine, a catecholamine precursor
DRG	dorsal root ganglia
Dsh	Disheveled gene and protein product
D–V	dorsoventral axis/polarity of embryonic regions/organ rudiments (e.g., pharyngeal arches, limb bud), organs (e.g., limbs) or organism. Sometimes referred to in the literature as medio-lateral or proximo-distal polarity.

E

ECM	extracellular matrix
EDAR	Ectodysplasin 1, anhidrotic receptor
Edn3	endothelin-3, a mitogenic peptide
Egf	epithelial growth factor genes and their proteins
eIF-4AIII	Eukaryotic translation initiation factor 4AIII
EMT	epithelial-mesenchymal transformation (sometimes shown as epithelial —> mesenchymal transformation)
En	*Engrailed* gene family, for example, *En1*
EphA, EphB	eph receptor tyrosine kinases A and B, members of large subfamilies of receptor protein-tyrosine kinases consists of receptors related to Eph, a receptor expressed in an *e*rythropoietin-*p*roducing human *h*epatocellular carcinoma cell line.

F

Far	*First- arch* murine craniofacial mutation
Fgf	fibroblast growth factor gene and protein family
Fox	*forkhead transcription factor* binding element
Frzb	*Frizzled-related protein precursor*, a secreted antagonist of *Wnt* signaling
FUDR	5-fluoro-2′-deoxyuridine (blocks DNA synthesis)

G

Gdnf	glial-cell-line-derived neurotrophic factor
GFP	green fluorescent protein
GnRH neurons	gonadotropin-releasing hormone (GnRH) neurons; see also LhRH neurons

H

Hh	*Hedgehog* gene family, for example, *Sonic hedgehog* (*Shh*)
H.H.	Hamilton–Hamburger stage of chick embryonic development
HMG	high-mobility group proteins
Hnf3ß	*hepatocyte nuclear factor3ß*
HNK-1	a cell surface carbohydrate (known as CD-57 in immunology) used as a marker for NCCs
Hox	homeotic gene classes in vertebrate, for example, *Hoxd10*
Hr	prefix for genes in the western northern Pacific ascidian *Halocynthia roretzi*

I

Igf	insulin-like growth factor genes and products
Insm1	*insulinoma-associated 1* gene

J

Jag1	gene for the transmembrane protein Jagged1, which functions via the Notch pathway

K

kDa	kilo daltons
Krox20	a gene encoding a zinc-finger transcription factor

L

La-N-5	human neuroblastoma cell line
LhRH neurons	luteinizing hormone releasing neurons; see also GnRH neurons
Lp	the *Loop-tail* mutant mouse, in which hindbrain and spinal cord fail to close and so NCCs fail to migrate
LRD	lysinated rhodamine dextran

M

MAPK	ras/mitogen-activated protein kinase, a signaling pathway involved in the phosphorylation of target molecules such as transcription factors and other kinases in cell membrane, cytoplasm, and nucleus
Mash1	*Mouse achaete-scute homologue 1* gene
Mdkb	midkine-b (a heparin-binding growth factor)
MEN1	*Multiple endocrine neoplasia type 1* in humans—characterized by endocrine neoplasia of the parathyroids, pituitary, and pancreas
MIF	Macrophage inhibitory factor
Mitf	*microphthalmia-associated transcription factor*
Mmp	matrix metalloprotease family
Msh	melanocyte stimulating hormone
Msx	homeobox genes (e.g., *Msx1*, *Msx2*) of vertebrates of the *Drosophila msh* (melanocyte-stimulating hormone) family
MTN	*Mesencephalic Trigeminal Nucleus*
M.W.	molecular weight

N

NC	neural crest
N-CAM	neural cell adhesion molecule
NCCs	neural crest cells
NF1	the *neurofibromin1* gene responsible for von Recklinghausen neurofibromatosis (type 1 neurofibromatosis)
NF1	von Recklinghausen neurofibromatosis (type 1 neurofibromatosis)
NF2	bilateral acoustic neurofibromatosis
Ngf	nerve growth factor family of genes and their products
Nkx	family of transcription factors that function downstream of *Shh*
Nof	*No-fin* mutant in zebrafish, which lacks pectoral fins and gill cartilages
Nrp1, Nrp2	neuropilin1, neuropilin2 (co-receptors for semaphorins)
Nt3	neurotrophin3, a nerve growth factor

O

Oca2	*oculocutaneous albinism2* gene
Osf2	osteoblast-stimulating factor2; see Runx2
Otx	*orthodenticle* family of genes in vertebrates, for example, *Otx1*

P

p27	cell cycle inhibitor
Pax	a family of nine mammalian genes containing a paired-type homeodomain as a DNA-binding motif; for example, *Pax1*, *Pax9*
P–D	proximo-distal axis/polarity of embryonic regions/organ rudiments (e.g., pharyngeal arches, limb bud), organs (e.g., limbs) or organism
Pdgf	platelet-derived growth factor genes and gene products
PdgfR	platelet-derived growth factor receptor genes and gene products
Pitx2	Pituitary homeobox gene-2 in mouse related to *bicoid* in *Drosophila*. Also known as *Ptx.*
PNA	peanut agglutinin lectin
PRE	pigmented retinal epithelium
Ptch	Patched, a binding protein for hedgehog gene products
PTEN	tumour-suppressor gene, *p*hosphatase and *ten*sin homologue
Ptx	*see Pitx*

Q

QCPN	quail non-chicken perinuclear antigen

R

r	rhombomeres, a segment of the hindbrain in vertebrates
RA	retinoic acid, a biologically active form of vitamin A

Raldh2	a gene for retinaldehyde dehydrogenase, required for synthesis of retinoic acid
RaLP	a member of the Src family of tyrosine kinase substrates
R–B	neurons Rohon–Béard neurons
Rhob	a low-molecular-weight GTPase in the Ras protein family
RTK	receptor tyrosine kinase
Runx2	*runt-related transcriptional factor-2*; older names are *cbfa-1* and *osf2*

S

SEM	scanning electron microscopy
Shh	*Sonic hedgehog* gene
Six2	*sine oculis*-related homeobox 2
Snail1, 2	zinc-finger transcription factor-encoding genes, orthologues of *Drosophila Snail homologue 1* and *Snail homologue*
SNC	sacral neural crest
SNCCs	sacral neural crest cells
sof	*short fin*, a zebrafish mutant
Sox	multigene families that encode transcription factors with high-mobility group DNA-binding domains, the acronym coming from coming from *s*ex-determining region homeobo*x*
S phase	the phase of cell division during which DNA is synthesized
Suc	the gene *sucker* in zebrafish, *Danio rerio*, which disrupts Endothelin-1. Also known as *endothelin-1* (*edn1*)

T

T-box	a family of genes encoding transcription factors, for example, *Brachyury*, *Tbx1*, *Tbx5*
Tbx,	a class of genes within the T-box family of transcription factors, for example, *Tbx6*
Tcf/Lef	T-cell specific/lymphoid enhancer binding factor (transcription factors)
TCOF1	*Treacher-Collins Franceschetti syndrome 1* gene coding for nucleolar phosphoprotein Treacle
TEM	transmission electron microscopy
Tgfß	transforming growth factor beta genes and their products
TgfßR	transforming growth factor beta receptors
Timp	tissue inhibitor of metalloprotease
TNC	trunk neural crest
TNCCs	trunk neural crest cells
Trk	a family of tyrosine kinases receptors for neurotropins
Tsp	thrombospondins (family of five glycoproteins involved in cell migration and proliferation)

V

Vegf	vascular endothelial growth factor gene and protein
VER	ventral ectodermal (epithelial) ridge on developing tail buds
Vgr1	older name for *Bmp6*
VMA	vanillinemandelic acid (4-hydroxy-3-methoxymandelic acid, a metabolite of catecholamine
v-myc	a proto-oncogene from retrovirus-associated DNA sequences originally isolated from an avian myelocytomatosis virus
VNC	vagal neural crest
VNCCs	vagal neural crest cells
VNT	ventral neural tube
vt	the *vestigial tail* tailless mouse mutant

W

Wnt	a large gene family orthologous to *wingless* in *Drosophila* that produce secreted molecules involved in intercellular signaling. *Wnt* is a combination of *Drosophila* w*ingless* and *i*nt*1* for *Wnt1*, the first vertebrate (mouse) family member discovered

Part I
Discovery and Origins

The topic of this book is the neural crest—its embryological and evolutionary origins; the multitude of cells, tissues and organs that develop either directly from neural crest cells (NCCs) or under their influence; the ways and means by which those cells migrate and differentiate; and abnormalities resulting from the involvement of the neural crest or of NCCs in tumors, deficiencies or defects.

In Chapter 1, I provide a brief overview of the major phases of investigation into the neural crest and the major players involved, discuss how the origin of the neural crest in vertebrate embryos is intertwined with the development of the nervous system, discuss the impact on the germ-layer theory of the discovery of the neural crest and of secondary neurulation, and present evidence of the neural crest as the fourth germ layer in vertebrates alongside ectoderm, mesoderm, and endoderm.

Chapters 2 and 3 are devoted to the embryological origins, delamination, migration, and potentiality of NCCs and Chapter 4 to the evolutionary origins of the neural crest and NCCs.

Chapter 1
Discovery

The **neural crest** (NC) has long fascinated developmental biologists and, increasingly over the past decades, evolutionary and evolutionary-developmental biologists.

The neural crest is the name given to the fold of neural ectoderm at the junction between neural and epidermal ectoderm in neurula-stage vertebrate embryos (Fig. 1.1). In this sense, neural crest is a morphological term akin to neural tube or limb bud. The neural crest consists of **neural crest cells** (NCCs), a special population(s) of cells that give rise to an astonishing number of cell types and to an equally astonishing number of tissues and organs (Table 1.1).

Major classes of NCCs can be grouped in various ways. Figure 1.2 details the relationships of human NCCs as determined using standard histological criteria and in a cladistic analysis.

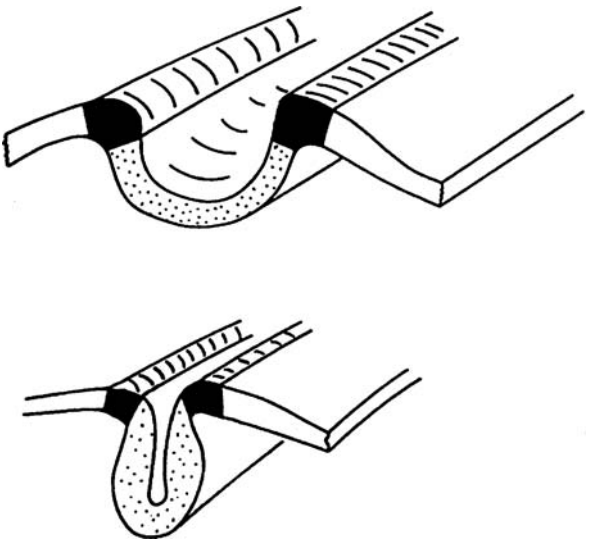

Fig. 1.1 Localization of the neural crest (*black*) at open neural plate (*above*) and closing neural fold (*below*) stages as seen in an avian embryo. Neural crest is located at the boundary between neural ectoderm (*stippled*) and epidermal ectoderm

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Table 1.1 A list of the cell types derived from the neural crest and of the tissues and organs that are entirely neural crest or that contain cells derived from the neural crest

Cell types	
Sensory neurons	Cholinergic neurons
Adrenergic neurons	Rohon–Béard cells
Satellite cells	Schwann cells
Glial cells	Chromaffin cells
Parafollicular cells	Calcitonin-producing (C) cells
Melanocytes	Chondroblasts, chondrocytes
Osteoblasts, osteocytes	Odontoblasts
Fibroblasts (mesenchyme)	Cardiac mesenchyme
Striated myoblasts	Smooth myoblasts
Mesenchymal cells	Angioblasts
Merkel cells	
Tissues or organs	
Spinal ganglia	Parasympathetic nervous system
Sympathetic nervous system	Peripheral nervous system
Thyroid and parathyroid glands	Ultimobranchial body
Adrenal gland	Craniofacial skeleton
Teeth	Dentine
Connective tissue	Adipose tissue[a]
Smooth muscles	Striated muscles
Cardiac septa, valves, aortic arches	Dermis
Eye	Cornea
Endothelia	Blood vessels
Heart	Dorsal fin
Brain	Connective tissue of thyroid, parathyroid, thymus, pituitary, and lacrymal glands

[a] A recent investigation using both murine and Japanese quail embryos has demonstrated the origin of a population of adipocytes (fat cells) from cranial neural crest (Billon *et al*., 2007). Adipocytes have long been thought to arise from cells within mesodermal lineages; indeed, there is a vast literature on the origin of adipocytes, chondro- and osteoblasts from clonal cell lines derived from periosteal or bone marrow cells (Hall, 2005b*). Billon and colleagues demonstrated that cultured mouse neural crest cells form adipocytes, that cultures of quail NCC can be induced to differentiate as adipocytes, and that a subset of adipocytes associated with the developing ear—confirmed by visualization of perilipin, a lipid marker—in mouse embryos arise from the neural crest during normal development. In another analysis, Takashima *et al*. (2007) demonstrated that mesenchymal stem cells capable of producing adipocytes, chondro- and osteoblasts arise from $Sox1^+$-neuroepithelial cells (i.e., NCCs) as well as from paraxial mesoderm.

NCC contribution may be direct (providing cells) or indirect (providing a necessary, often inductive, environment in which other cells develop). The enormous range of cell types produced provides an important source of evidence of the NC as a germ layer, bringing the number of germ layers to four: ectoderm, endoderm, mesoderm, and neural crest.

This chapter provides a brief overview of the major phases of investigation into the neural crest and the major players involved, discusses how the origin of the neural crest relates to the origin of the nervous system in vertebrate embryos, discusses the

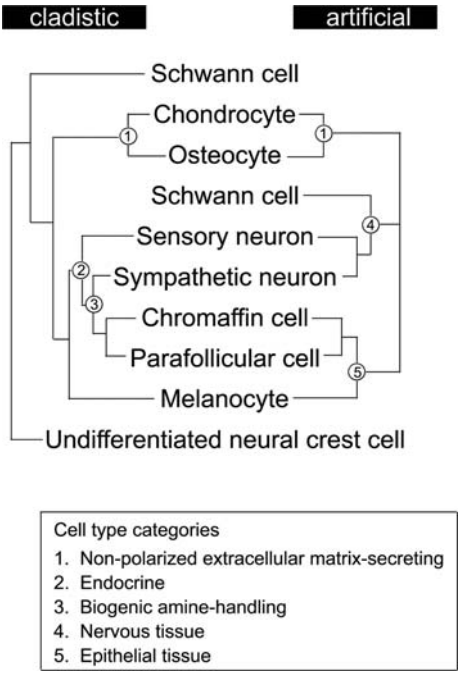

Fig. 1.2 Two classification schemes for human neural crest cells showing the different relationships obtained in a cladistic analysis (cladistic) and in an analysis using standard histological criteria (artificial). Of five cell-type categories (1–5) shown, only the skeletal cells (chondrocyte, osteocyte) separate out in both schemes, although chromaffin cells of the adrenal glands and parafollicular cells of the thyroid glands cluster as a subgroup. Note that neural crest-derived neurons (sensory, sympathetic) do not cluster as an individual (monophyletic) group, reflecting their origins from different NCC populations (see Chapter 6). See Vickaryous and Hall (2006) for further analysis and for the 19 characters used in the cladistic analysis. From Vickaryous and Hall (2006)

impact on the germ-layer theory of the discovery of the neural crest and of secondary neurulation, and presents evidence of the neural crest as the fourth germ layer.

This chapter begins with the story of how this amazing embryonic region was discovered.

Zwischenstrang

Wilhelm His (1831–1904), professor of anatomy and physiology in Basel, Switzerland, was the first to provide a causal explanation for embryonic development, basing his explanation on mechanics (embryos as rubber tubes), developmental physiology, and predetermined organ-forming germinal regions, each of which contained cells with specified fates.

In 1868, His identified a band of cells sandwiched between the developing neural tube and the future epidermal ectoderm as the source of spinal and cranial ganglia in chicken embryos. He named this band *Zwischenstrang*, the intermediate cord.[1] In 1874, His included *Zwischenstrang*—the **neural crest** as we now know it—as one of the organ-forming germinal regions.

As far as can be determined, the term ‘neural crest’ was first used in a paper on the development of the olfactory organ published in 1879. The author, Arthur Milnes Marshall (1852–1893), was a professor of zoology at Owens College in Manchester, where he remained until his death at age 41 in a fall from Scafell Pike, England’s

highest peak.[2] In a paper on the development of the cranial nerves in chicken embryos, Marshall (1878) used the term **neural ridge** for the cells that give rise to cranial and spinal ganglia. Realizing that this term was less descriptive than was desirable, a year later he replaced neural ridge with neural crest. As told in his own words,

> I take this opportunity to make a slight alteration in the nomenclature adopted in my former paper. I have there suggested the term neural ridge for the longitudinal ridge of cells which grows out from the reentering angle between the external epiblast and the neural canal, and from which the nerves, whether cranial or spinal arise. Since this ridge appears before closure of the neural canal is effected, there are manifestly two neural ridges, one on either side; but I have also applied the same term, neural ridge, to the single outgrowth formed by the fusion of the neural ridges of the two sides after complete closure of the neural canal is effected, and after the external epiblast has become completely separated from the neural canal. I propose in future to speak of this single median outgrowth as the neural crest, limiting the term neural ridge to the former acceptation.
>
> (Marshall, 1879, p. 305, n. 2)

A Brief Overview of the Past 120 Years

1890–1950s

His and Marshall independently identified the NC as the origin of cranial and spinal ganglia and neurons, an origin that was easy for others to accept because of the relationship of these cell types and of the neural crest to the dorsal neural tube, the source of the dorsal nervous system.

In the 1890s, however, Julia Platt claimed that the cartilages of the craniofacial and pharyngeal arch skeletons⊕ and the dentine-forming cells (odontoblasts) of the teeth of the mudpuppy, *Necturus maculosus*, arose from the ectoderm adjacent to the neural tube. Although supported by several contemporary researchers, Platt's conclusion was not accepted. In fact, her proposal of an ectodermal origin of the pharyngeal arch skeletons raised major controversies.

Why?

Because her conclusions ran completely counter to the entrenched **germ-layer theory**, according to which skeletal tissues arose from mesoderm, not ectoderm (see the section on germ-layer theory below).

Because an NC origin for skeletal tissues was so contentious, there was a 40-year gap between Platt's papers and independent studies in the 1920s and 1930s by Stone, Raven, and Holtfreter demonstrating the NC as a major source of mesenchyme, connective tissue, and cartilage (see Chapter 7).[3] Even more detailed reports by Sven Hörstadius, Sven Sellman, and Gavin de Beer were published in the 1940s. de Beer (1947) also thought it probable that NCCs differentiated into the osteoblasts of

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⊕ Three terms—pharyngeal, visceral, and branchial—are often used interchangeably for the multiple arches that arise from the pharynx in vertebrates. I use the term *pharyngeal arches*, except when referring to gills in urodeles or fish, when gill arch seems more appropriate.

dermal bones in *Ambystoma*, although the evidence was less convincing than was his evidence of the NC origin of cartilage and teeth.[4] Nowadays, not only has the skeletogenic capability of the cranial neural crest (CNC) been documented in all classes of vertebrates (see Chapter 7) but also the NC and its cells occupy a central position in studies of vertebrate development and evolution (see Chapters 2 and 4).

Despite these studies on the skeletogenic neural crest but because of the entrenched germ-layer theory, the focus of interest until the 1940s and 1950s was the NC as a source of pigment cells (chromatophores) and neural elements, such as spinal ganglia (see Chapters 5 and 6). Amphibian embryos were the embryos of choice.◊

Standing as a milestone on the road to understanding the NC is *The Neural Crest*: *Its properties and derivatives in the light of experimental research*, a monograph by Sven Hörstadius (Box 1.1). Published in 1950, 82 years after the discovery of the neural crest, it was reprinted in 1969 and again in 1988. 'Hörstadius,' as the monograph is known, was based on a series of lectures delivered during 1947 at the University of London at the invitation of Professor (later Sir) Gavin de Beer, then head of the Department of Embryology at University College and later director of the British Museum (Natural History). As noted above, de Beer had just completed his extensive experimental study of the NC origin of craniofacial cartilages and dentine in the Mexican axolotl and had provided suggestive evidence of a NC contribution to the splenial, a membrane bone of the skull.

Box 1.1 Sven Otto Hörstadius (1898–1996)

Born in Stockholm on 18 February 1898, Hörstadius began his academic career at Stockholm University, from which he graduated in 1930 and where he was appointed first lecturer and then associate professor of zoology. Early marked for recognition, in 1936, Hörstadius was awarded the Prix Albert Brachet by the Belgian Academy of Science. From 1938 to 1942 he directed the Department of Developmental Physiology and Genetics at the Wenner-Gren Institute of Experimental Biology. In 1942, Hörstadius became professor of zoology at the University of Uppsala, a position he occupied for 22 years, retiring in 1964 as Professor Emeritus.

A pioneering embryologist, brilliant lecturer, and an expert ornithologist, Hörstadius had a reputation for producing some of the best and among the earliest close-up photographs of difficult-to-photograph birds. The numerous

◊ Although studies on anuran (frogs, toads) and urodele (newt, salamander) embryos are often discussed together, bear in mind that relationships between these two groups of amphibians are not fully resolved. Indeed, use of the term amphibian at all is contentious; in current terminology, amphibian would imply a monophyletic group, which amphibians clearly are not (Hall and Hallgrímsson, 2008).

honors he received reflect his standing in the European scientific circles and the breadth of his interests and accomplishments.[a]

Our knowledge of the most fundamental aspects of echinoderm development derives from his studies (Hörstadius, 1928, 1939). Hörstadius was the first to demonstrate a fundamental feature of life now taken as a given, namely, *nuclear control of the species-specific characteristics of organisms*. By enucleating an egg from one species of sea urchin and fertilizing it with the sperm from another, Hörstadius created the first chimeric sea urchin embryos. The characteristics of the resulting embryos were those of the species providing the nucleus, not the species providing the cytoplasm. Hörstadius' experimental studies culminated in a book, *Experimental Embryology of Echinoderms*, published in 1973.

Based on the work undertaken with Sven Sellman, Hörstadius published two large papers devoted to an extensive experimental analysis of the development of the neural crest (NC)-derived cartilaginous skeleton of *Ambystoma* (see Chapter 7). This experimental verification of Platt's observations on the mudpuppy ran counter to the dogma enshrined in the germ-layer theory, according to which skeletal tissues develop from mesoderm and from no other germ layer. According to one assessment of Hörstadius' experimental work on the NC, 'This is the first time that the phenomenon of complex and additive inductive action by different structures has been demonstrated under experimental conditions' (*Nature* 1950, 169, p. 821).[b] A symposium to mark the centennial in 1998 of his birth, to honor his contributions to the developmental biology of echinoderms and the NC, and to chart future directions for developmental biology, was organized by Carl-Olof Jacobson and Lennart Olsson at the Wenner-Gren Research Institute in Stockholm. A handsome publication based on the symposium, the only volume devoted to studies on echinoderm embryology and the NC, was published in 2000 under the editorship of Olsson and Jacobson.

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[a] Hörstadius was a member of the Royal Swedish Academy of Sciences and the Academia Pontifica (Vatican); Fellow of the Royal Society of London, the Royal Institution of Great Britain and the Societé Zoologique de France; and held honorary doctorates from the Université de Paris and Cambridge University. Active in scientific administration at the international level, Hörstadius was Secretary-General and organizer of the Xth International Ornithological Congress in Uppsala in 1950 and edited the proceedings, to which he contributed a paper on Swedish ornithology (Hörstadius, 1951). A founding member of the Council of The World Wildlife Fund, Hörstadius was also chairman of the European Section of the International Council for Bird Preservation, president of the Swedish Ornithological Society, president of the International Union of Biological Sciences (1953–1958) and president of the International Council of Scientific Unions (1962–1963).

[b] See Ebendal (1995) and O. Jacobson (2000) for details of his scientific work and Olsson (2000) for a bibliography.

1960s–1970s

The 1960s ushered in investigations of mechanisms of NCC migration and a move away from amphibian and toward avian embryos as the organisms of choice. The floodgate was opened by the seminal studies of Jim Weston (1963) and Mac Johnston (1966) on migrating trunk and cranial neural crest cells (TNCCs, CNCCs)[⊕] in chicken embryos, by Pierre Chibon (1964) with his studies on the skeletogenic NC in the Spanish ribbed newt, by the discovery and exploitation of the quail nuclear marker by Nicole Le Douarin (1974*), and by an influential review by Weston (1970) on the migration and differentiation of NCCs.

Detailed maps of the fate of NCCs appeared during the 1970s. The microenvironment encountered by these cells was revealed as a major determinant of their migration, differentiation, and morphogenesis in normal embryos and in embryos with abnormalities resulting from mutations or the consequences of exposure to teratogens, such as alcohol or drugs (see Chapters 9 and 10).

Syndromes[◊] involving one or more cell types derived from the NC were recognized as separate and identifiable entities and classified as **neurocristopathies** (see Chapter 9). Monoclonal antibodies against individual populations or types of NCCs were developed in the 1980s and used to analyze cell determination, specification, lineage, and multipotentiality (see Table 1.2 for definitions of terms such as multi- and pluripotentiality). Even mammalian embryos, which are difficult to study, began to yield the secrets of their NCCs to skilled and persistent experimental embryologists.[5]

1980s to the 21st Century

Homeotic transformations, and a code of *Hox* genes that patterns the major axis of most (and all bilaterally symmetrical) animal embryos, were discovered and analyzed in some detail during the 1980s and 1990s (Box 1.2). The NC, which had earlier been shown to be divisible into **cranial** and **trunk** regions, was further subdivided following the recognition of:

Table 1.2 Terms used in the text for the potentiality of individual cells

Totipotent	Able to generate all cell types—stem cells
Pluripotent	Able to generate cell types from all four germ layers—stem cells
Multipotent	Able to generate cell types within a germ layer—germ layer-restricted cells
Bipotential	Able to generate two cell types—typical of many embryonic cells
Unipotent	Able to generate a single cell type—committed progenitor cells

[⊕] CNCC migrate from the developing brain, TNCC from the developing spinal cord.

[◊] A syndrome is a group of symptoms that collectively characterize an abnormal condition or disease state.

- subpopulations in the hindbrain associated with specific **rhombomeres** (r)[6] and segmental patterns of expression of *Hox* genes;
- a **vagal** and a **sacral neural crest** in the neck and caudal body, respectively, from which arise the enteric ganglia and the neurons of the parasympathetic nervous system of the intestine and blood vessels (see Chapter 6); and
- a **cardiac neural crest** that contributes cells to the valves, septa, and major vessels of the heart (see Chapter 8).

Box 1.2 *Hox* genes

Recognition of the importance of *Hox* genes marks a fascinating episode in the history of the search for relationships between animals, the origin of body plans associated with individual phyla and, with respect to our topic, the origin of chordates and vertebrates.

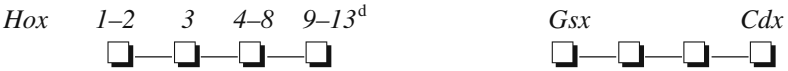
Vertebrate homeobox (Hox) genes with sequence homology to such gene complexes as *Ultrabithorax* and *Antennapedia* in the fruit fly *Drosophila* are orthologs of a series of transcription factors organized as homeobox clusters throughout the animal kingdom. As in *Drosophila*, the order of *Hox* genes within a cluster is paralleled by an anterior–posterior sequence of gene expression. Conservation of the roles of these genes in vertebrates and in *Drosophila* is demonstrated by research showing that, for example, after being transfected into *Drosophila*, the mouse *Hoxb6* gene elicits leg formation in the place of antennae.

Considerable information now is available on the evolution of the genes leading to the *Ultrabithorax* and *Antennapedia* gene complexes. The scenario (outlined below[a]) is that a single protoHox gene (□) duplicated to produce a ProtoHox cluster of four genes (❑) arranged in an anterior–posterior (A–P) sequence,

A----------P

ProtoHox

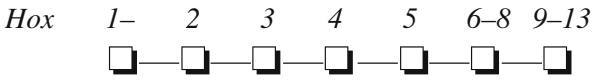
Duplication of this ProtoHox cluster produced two clusters of four genes each, four Hox and four ParaHox.



Hox ParaHox

Further tandem duplications of three of the Hox cluster at the origin of the bilaterally symmetrical animals (the Bilateria) produced the Hox clusters found

in bilaterians; Hox cluster 3 remained as a single gene in both Hox and ParaHox clusters.[b]



Bilaterian Hox cluster

The number of Hox clusters varies among vertebrates: four clusters of 39 genes in mice, three clusters in lampreys, and up to seven in teleost fish. "Duplication of the genome" at the origin of the chordates is the most likely current explanation for the four clusters; duplication sets up the possibility of future structural and functional divergence and *specialization of function among copies of the genes*.

Four possibilities, which are not mutually exclusive, could explain evolutionary changes in gene function:

- **Two involve change in gene number**, either (i) the number of Hox gene clusters or (ii) the number of genes per cluster. Duplication of Hox clusters *before* the teleosts arose—perhaps associated with duplication of large portions of chromosomes or entire chromosomes or genomes—would have taken the number from four to eight in teleosts. This, coupled with subsequent loss of one cluster, would explain the seven clusters in zebrafish.
- **The other two possibilities involve altered function,** either (iii) modification of individual *Hox* genes through regulatory or other changes or (iv) increasing the complexity of interaction between gene networks, either of which could come about by alteration in the upstream and/or downstream regulation of a Hox gene(s).[c]

  The *patterning role* carried out by *Hox* genes is demonstrated by studies in which knocking out or knocking in a *Hox* gene to eliminate or enhance its function in mice is followed by the transformation of skull, vertebral, or other features into a more anterior element in the sequence. Such a transformation is known as **homeotic**, a term introduced into biology by William Bateson in the early 20th century. In the tadpoles of some species of frogs, an amputated tail can be made to transform homeotically into the duplicated posterior portion of the body, including hindlimbs and a pelvic girdle, rather than regenerating a tail. Developmental and evolutionary transformations of middle ear ossicles are discussed in Boxes 7.1 and 10.2. Meckel's cartilage and the ossicles are duplicated following *Hoxa2* knockout, essentially because the second pharyngeal arch fails to form. Instead, a second set of first-arch elements forms more anteriorly than the normal position of