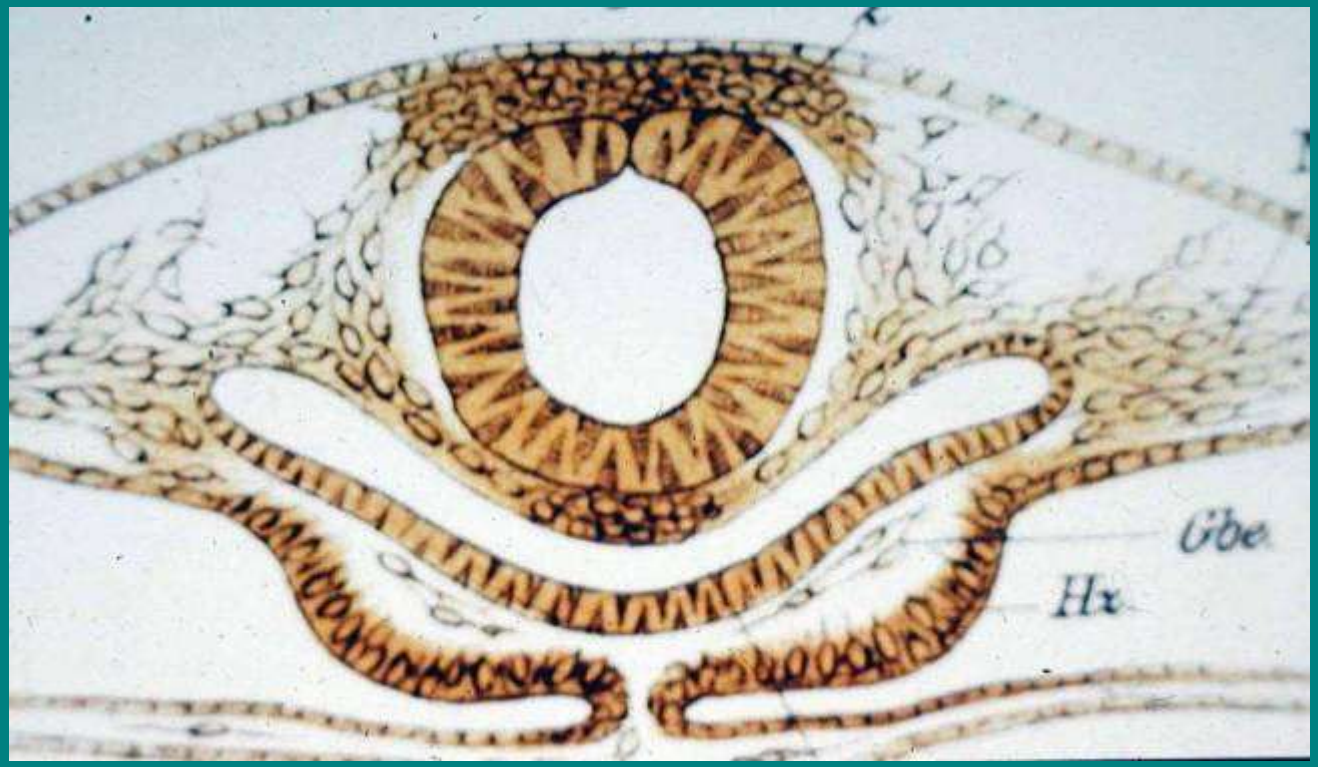
The Neural Crest: its Derivatives and Stem Cells

Miloš Grim

Institute of Anatomy, First Faculty of Medicine, Charles University in Prague

New Trends…6th May 2015



W. His (1868)

The structure of the lecture:

1) Why to study the neural crest (NC)
2) Origin and development of the NCCs (epithelo-mesenchymal transition, migration, differentiation)
3) Methods of labeling of the NC cells
4) Cell types differentiated from the NC
5) Developmental disorders of the NC
6) NC cells in epidermis
7) Neural crest stem cells (NCSCs) in the hair follicle

Why to study the neural crest (NC) cells?

- Cells of NC participate in development of almost all organs originating from ectoderm, mesoderm and endoderm
- NC formation enabled the expansion of vertebrates "... shift from filter feeding to active predation.
- NC is the 4th embryonic layer of craniates
- NC is a model system for studies of embryonic induction, cell determination, differentiation and epithelial-mesenchymal transition
- NC is a model system for the study of cell invasivity – development of carcinoma metastasis
- Defective development of the NC leads to developmental malformations

Origin and development of the NC

Induction of the NC

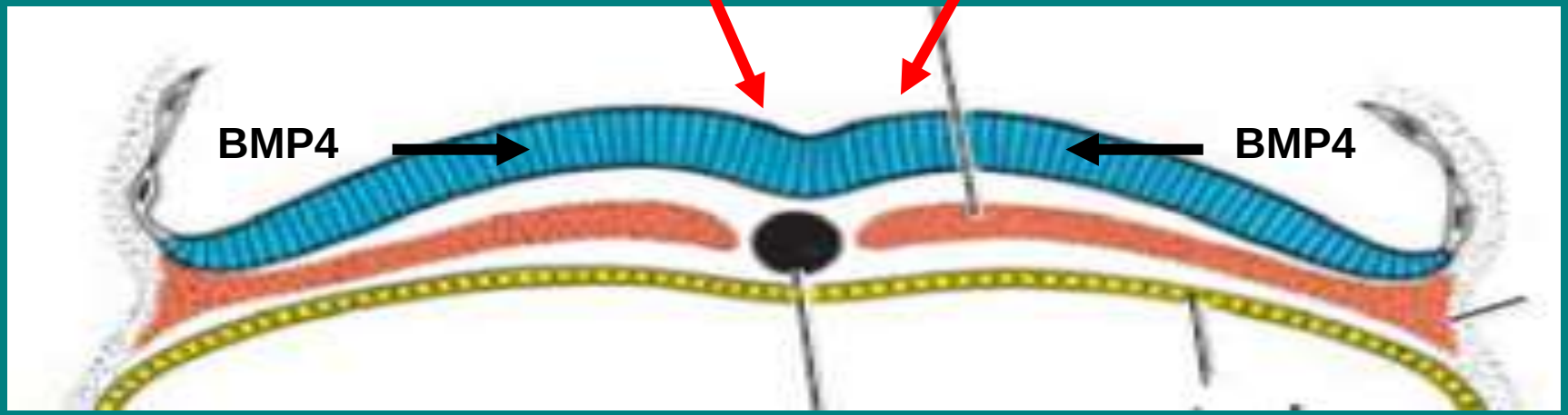
Epithelial-mesenchymal transtition

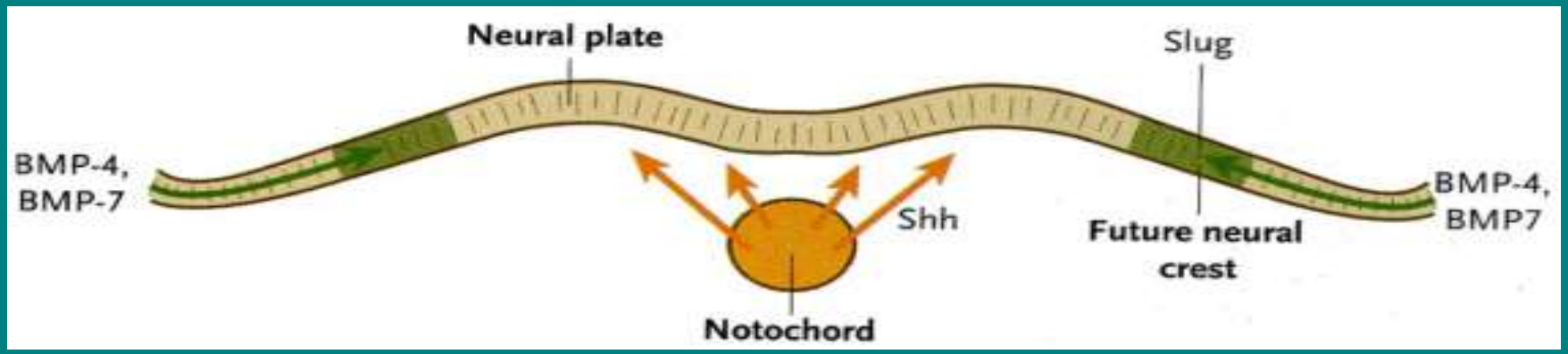
Migratiom

Differentiation

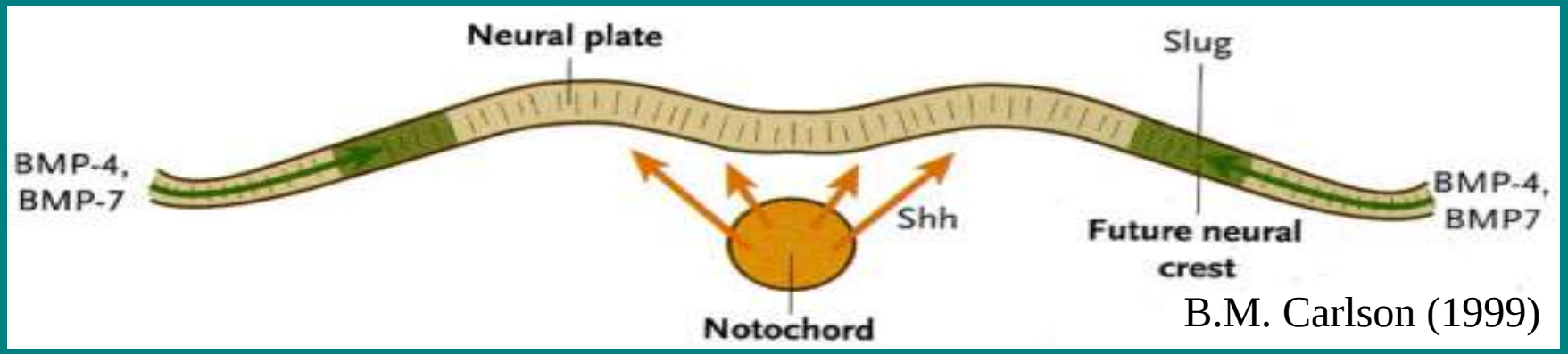
Induction of the neural plate and epithelial-mesenchymal transition of NC cells

Noggin, Chordin, Follistatin

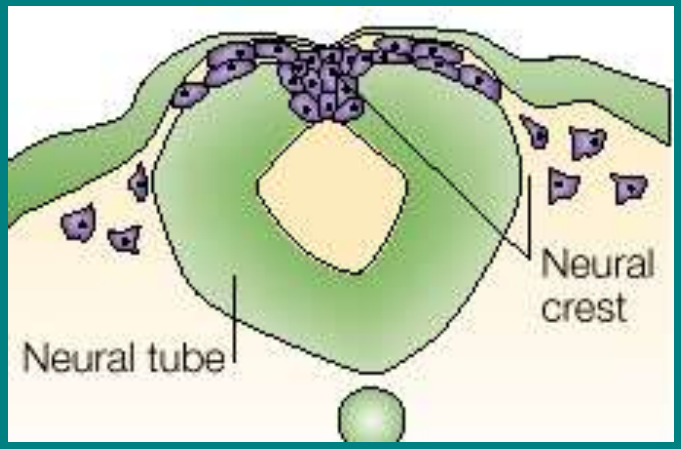




Induction and epithelial-mesenchymal transition of NC cells

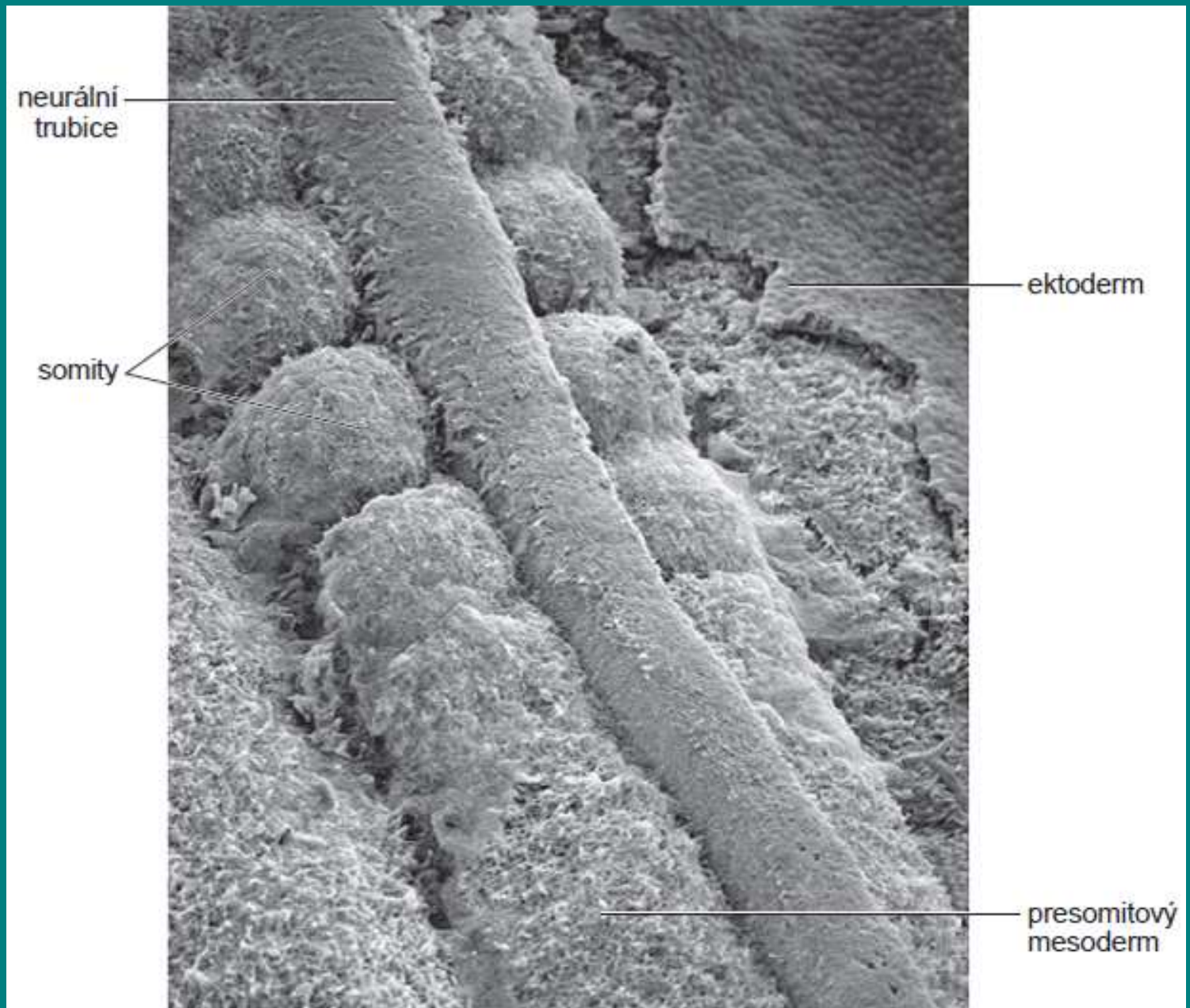


Epithelial-mesenchymal transition of NC cells

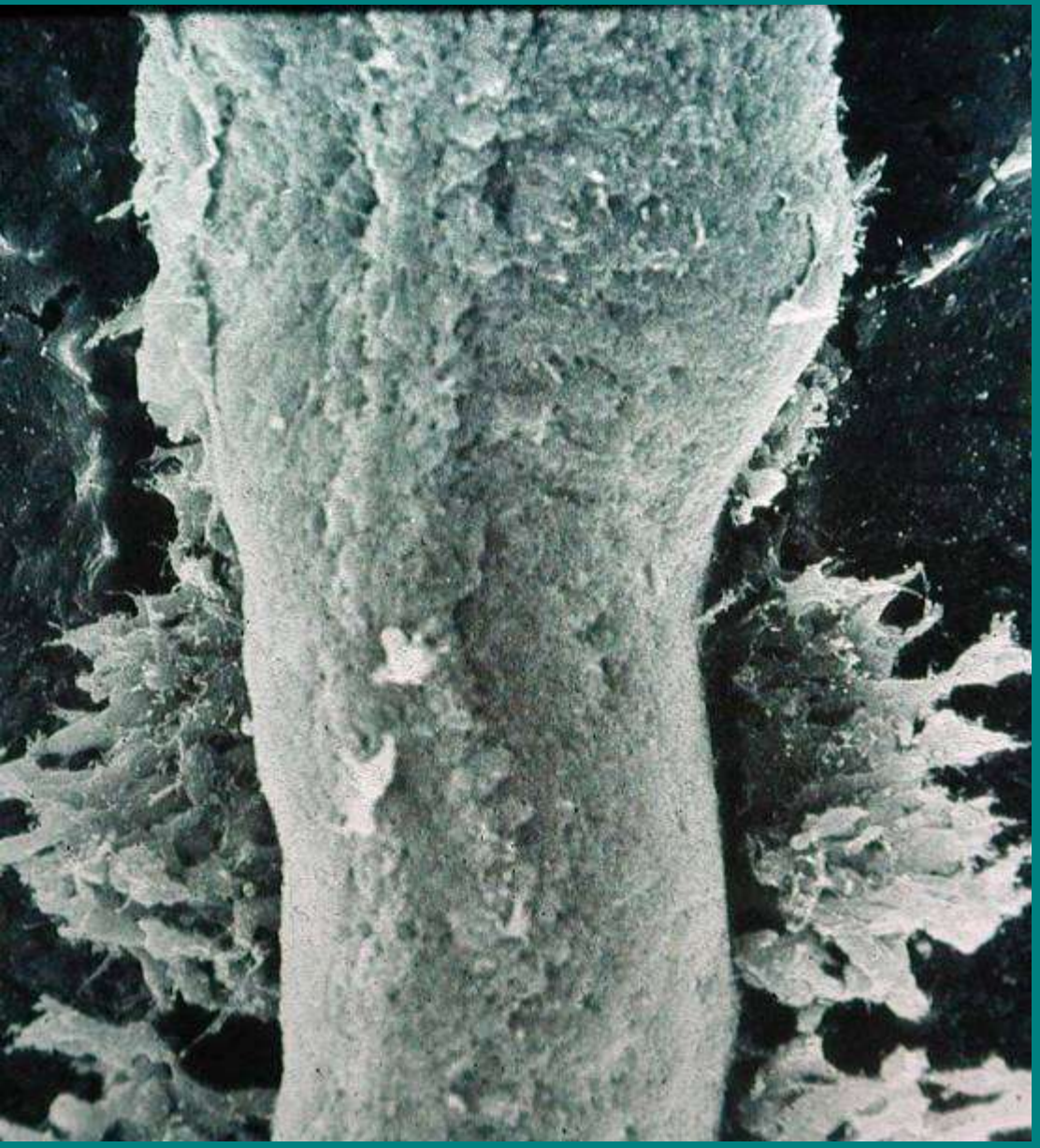


ISH of mRNA for Slug

Beginning of migration of neural crest cells

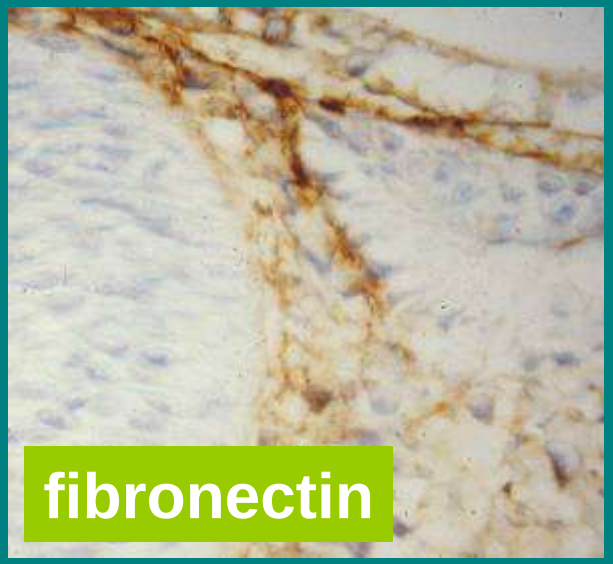


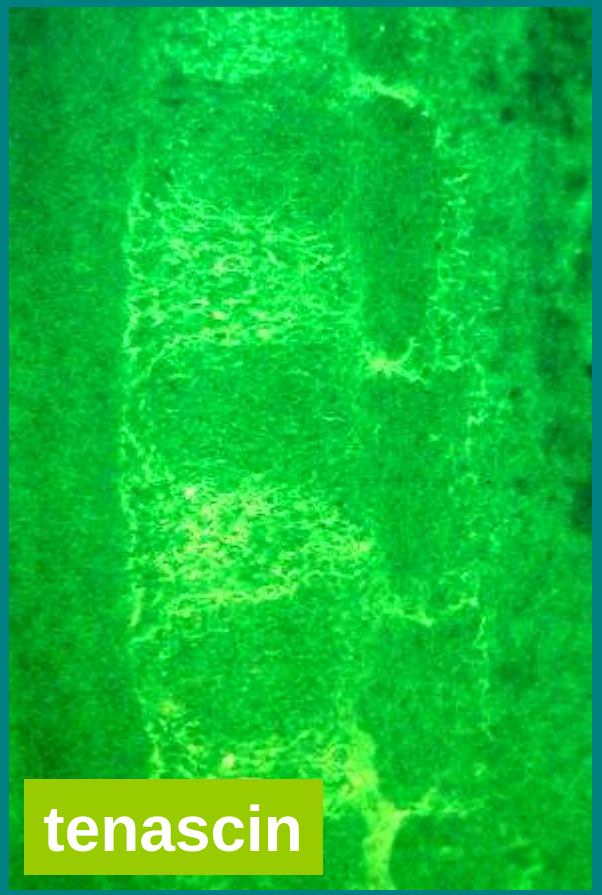
Migration of NC cells *in vivo* and *in vitro*

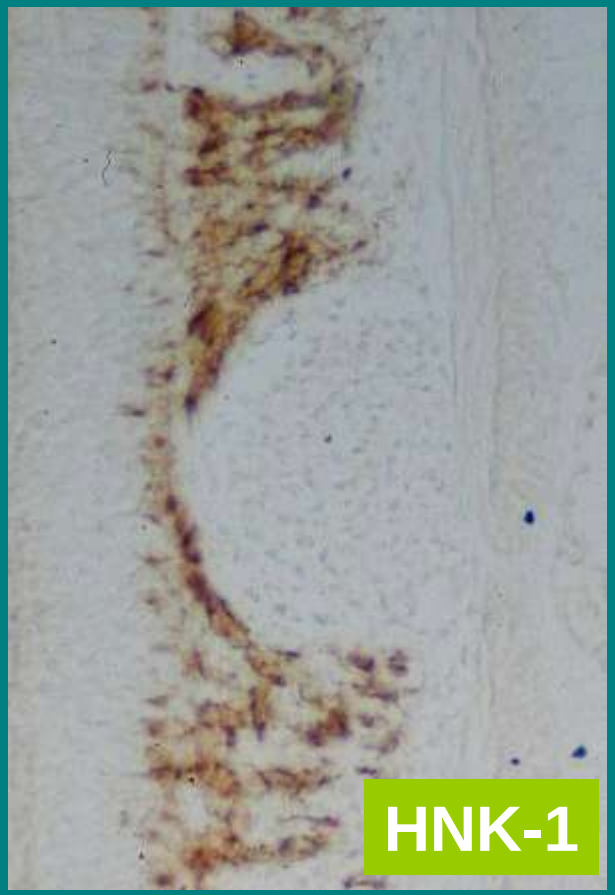


Molecular mechanisms of NC cells migration

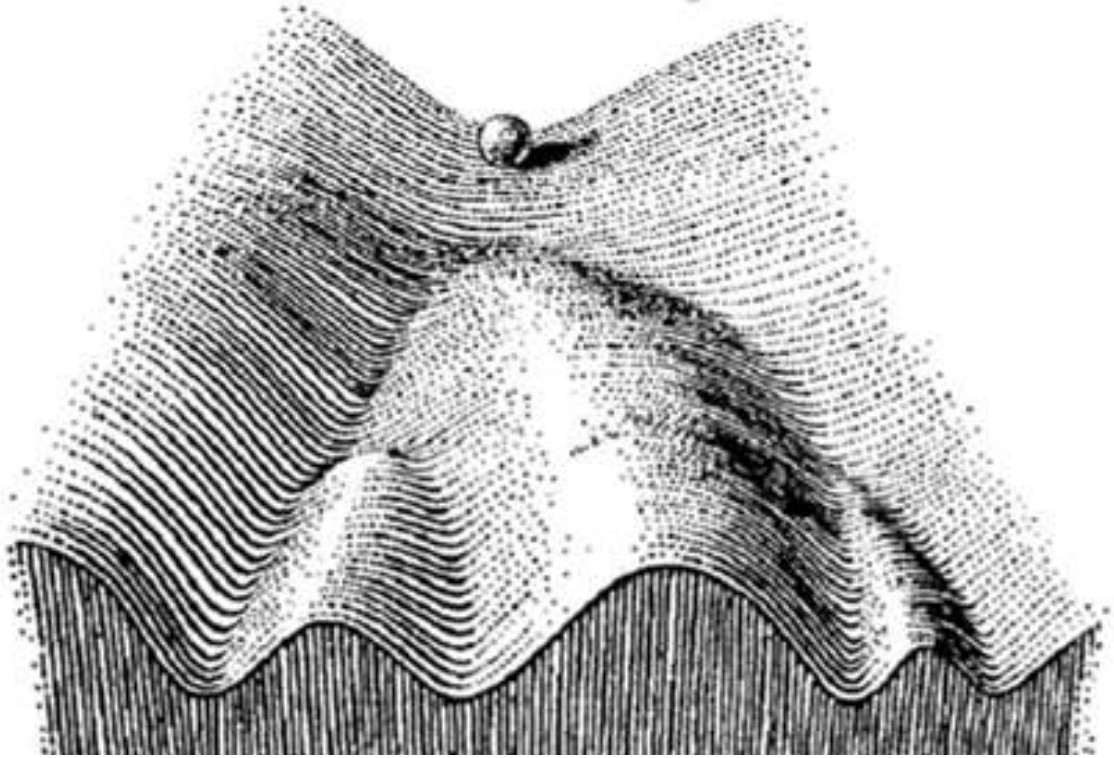
- Permisive contact-guidance + chemorepelent molecules

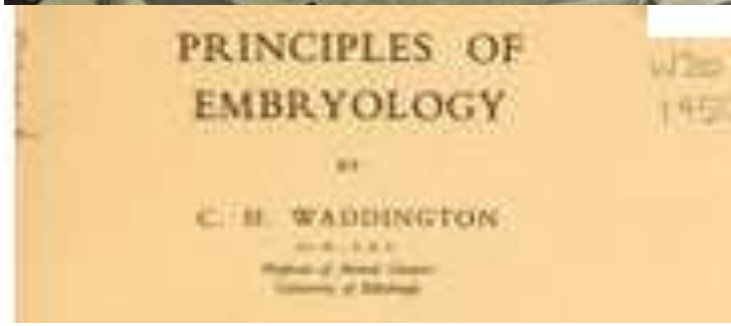






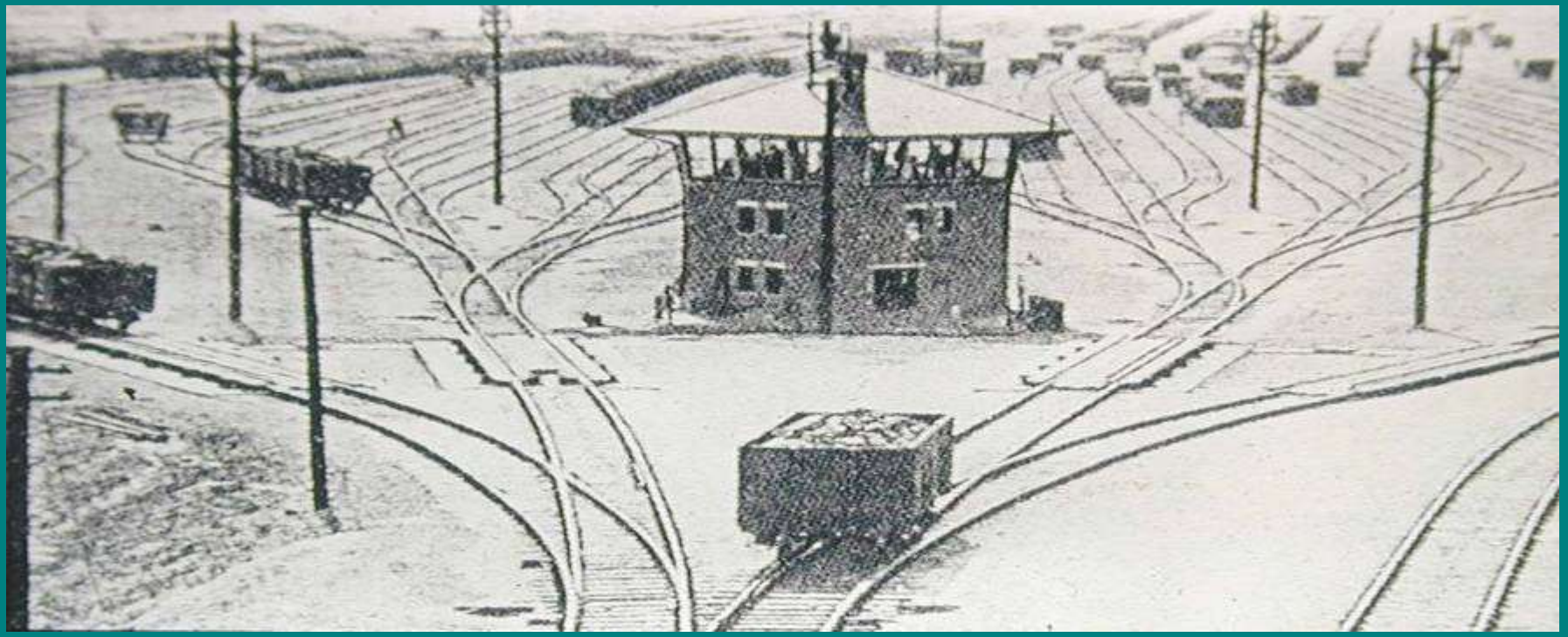
- Paracrine signaling systems:
  - Scatter factor /c-met receptor, Pax3
  - Steel factor (stem cell factor) /c-kit receptor
  - Chemokin SDF-1 / CXCR4 receptor





Epigenetic landscape was originally a metaphor for biological development. Its originator, **Conrad Hal Waddington (1905-1975)**, said that cell fates were established in development much like a marble rolls down to the point of lowest local elevation.

Regional identity and differentiation of NC cells



Local signaling during migration of NC cells induces differentiated gene expression. Pluripotent NC cells successively differentiate in bipotent and unipotent cell types (marshalling yard as a metaphor for cell differentiation)

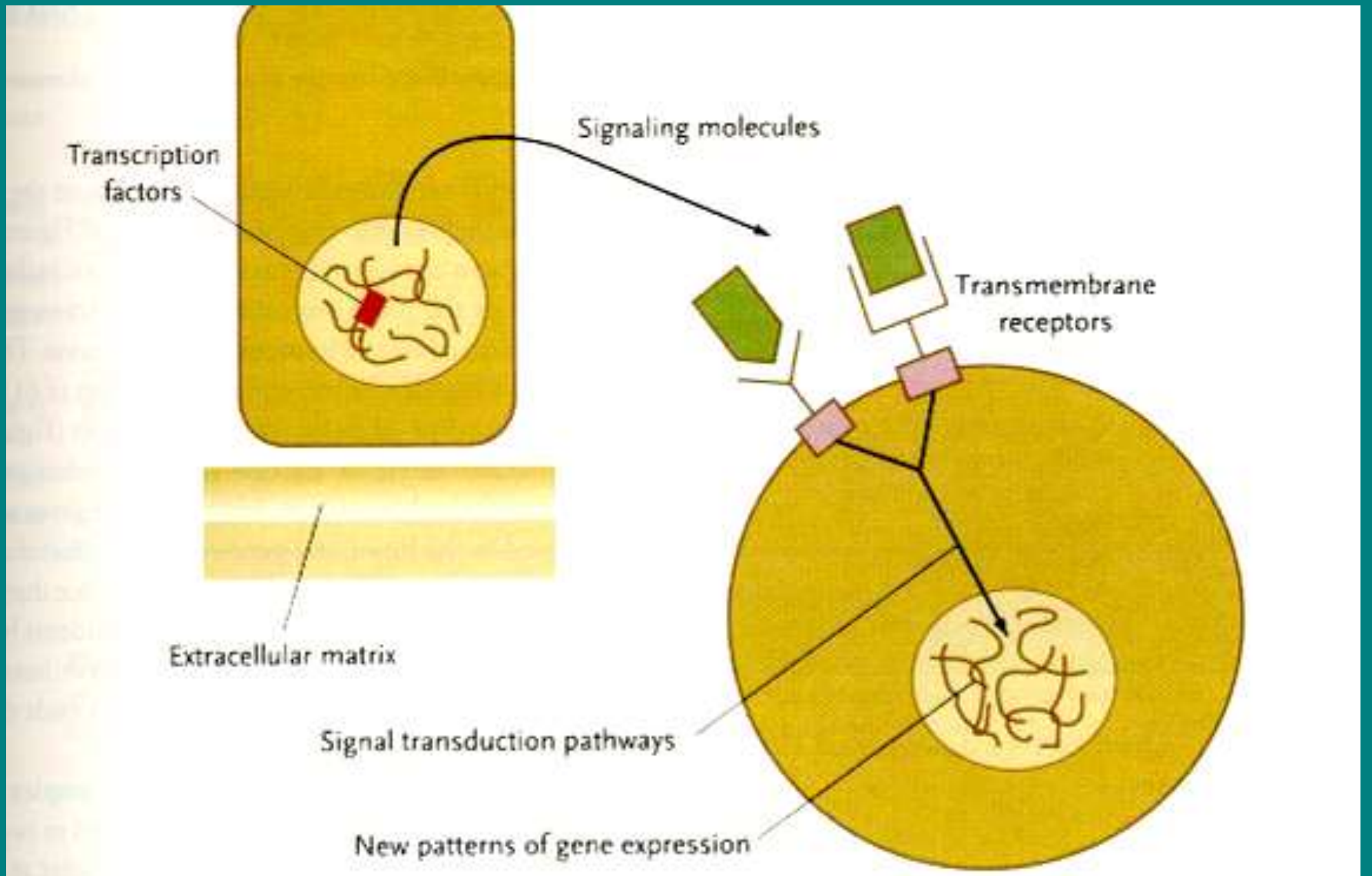
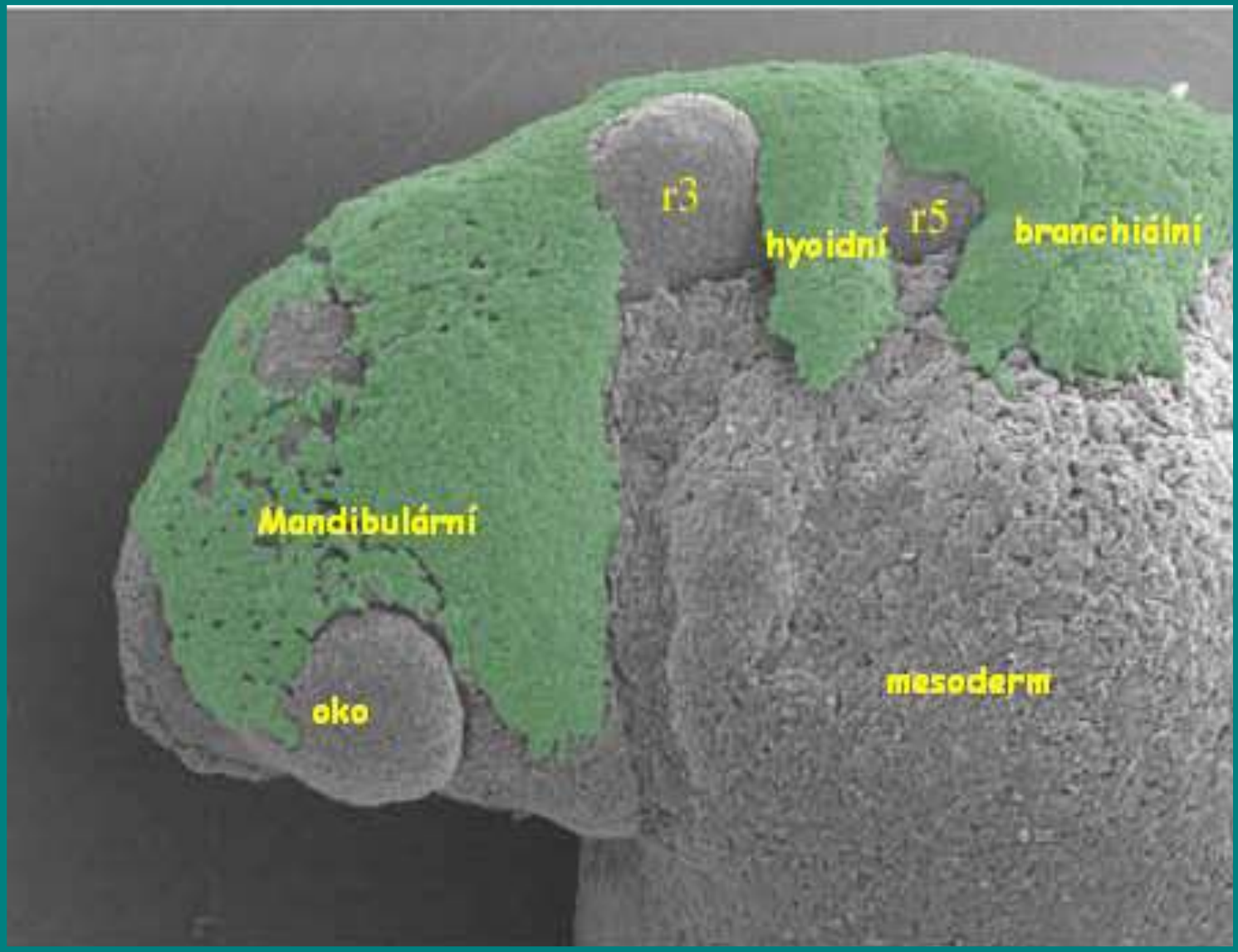


Figure 5-2 Schematic representation of types of developmentally important molecules and their sites of action.

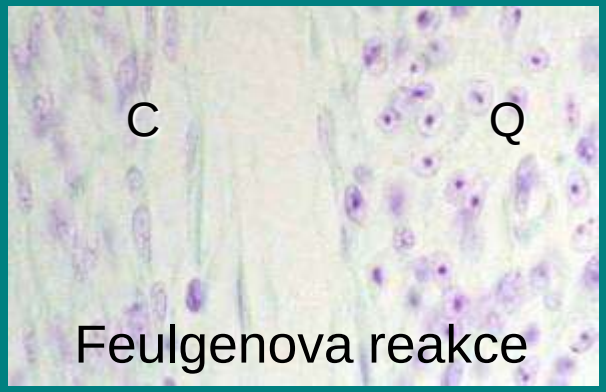
Methods of labeling of the NC cells

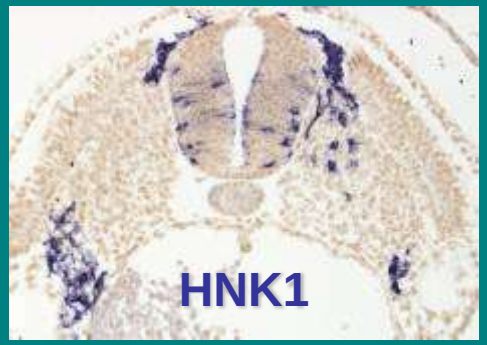


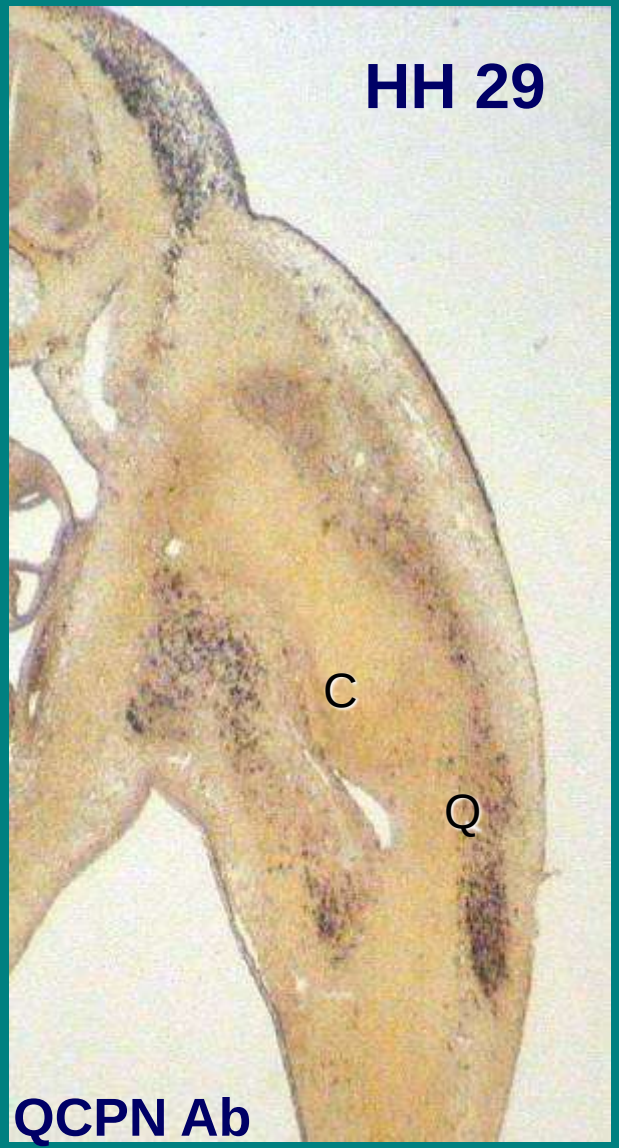
Ambystoma mexicanum (Černý et al., 2004)

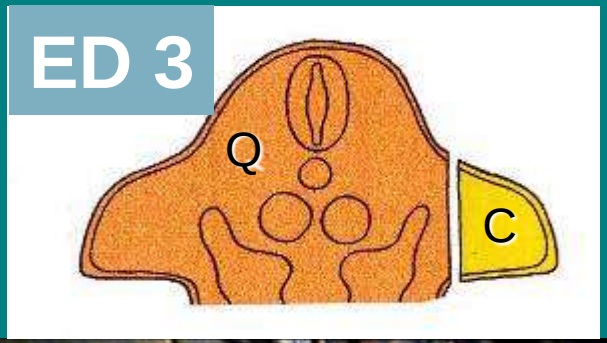
Neural crest cells in embryonic chimeras of Japanese quail and white Leghorn chicken

Embryonic himeras of Japanese quail (Q) and white Leghorn chick (C)

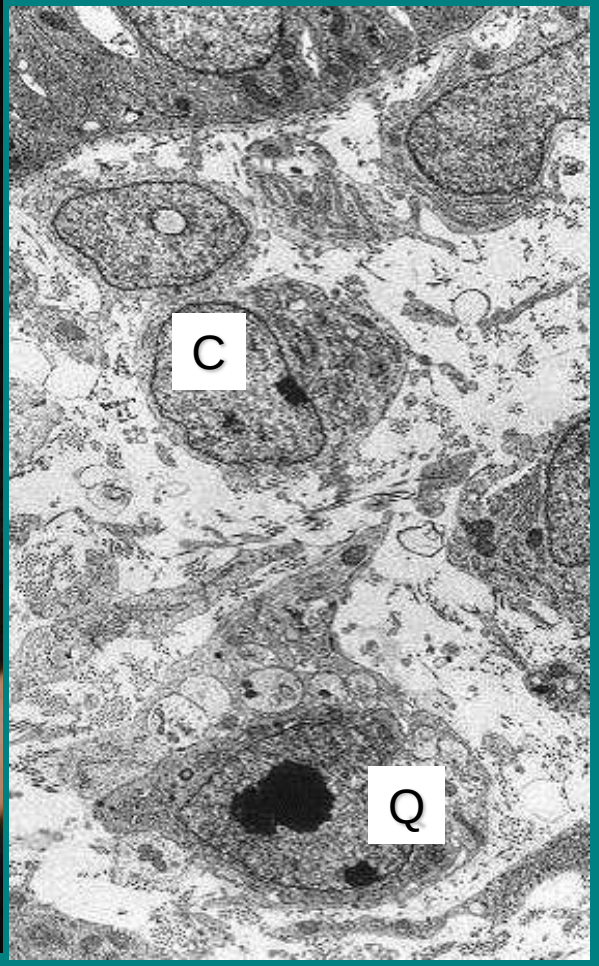






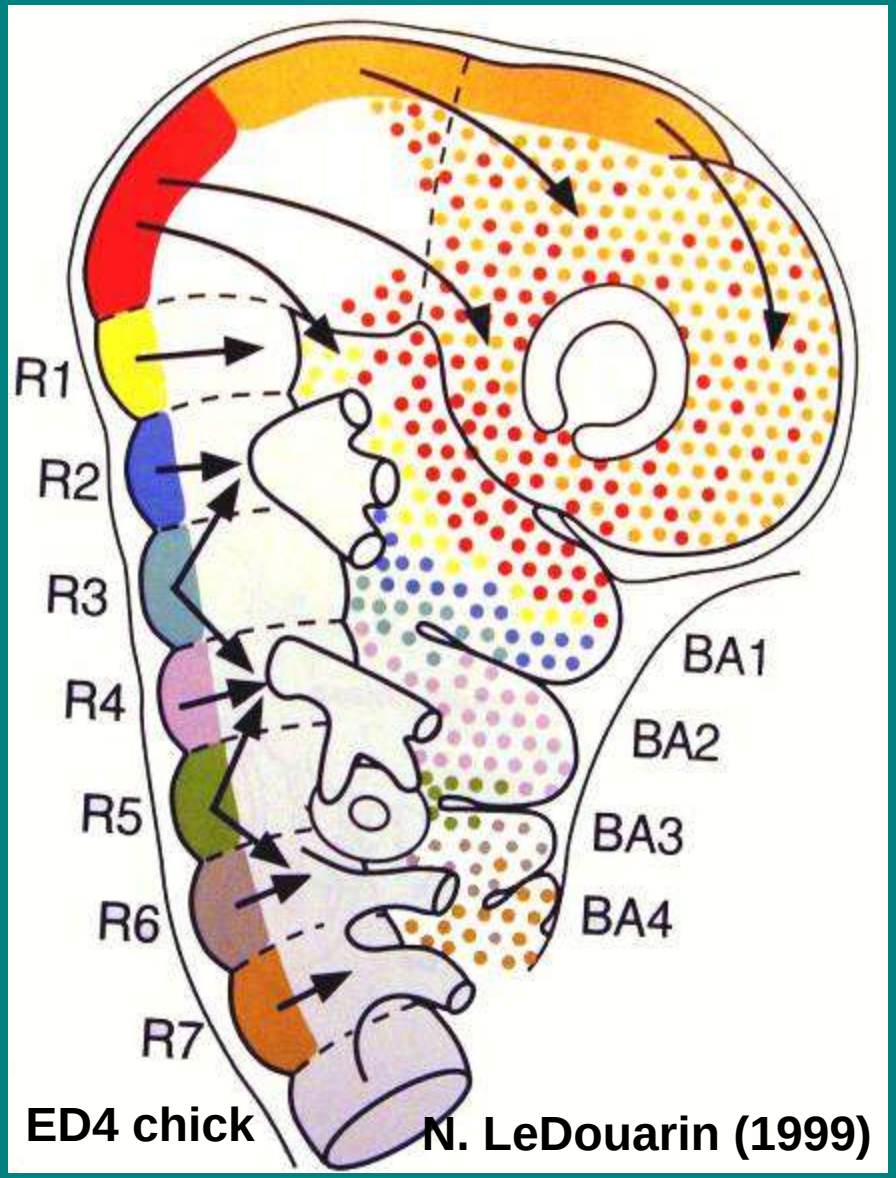


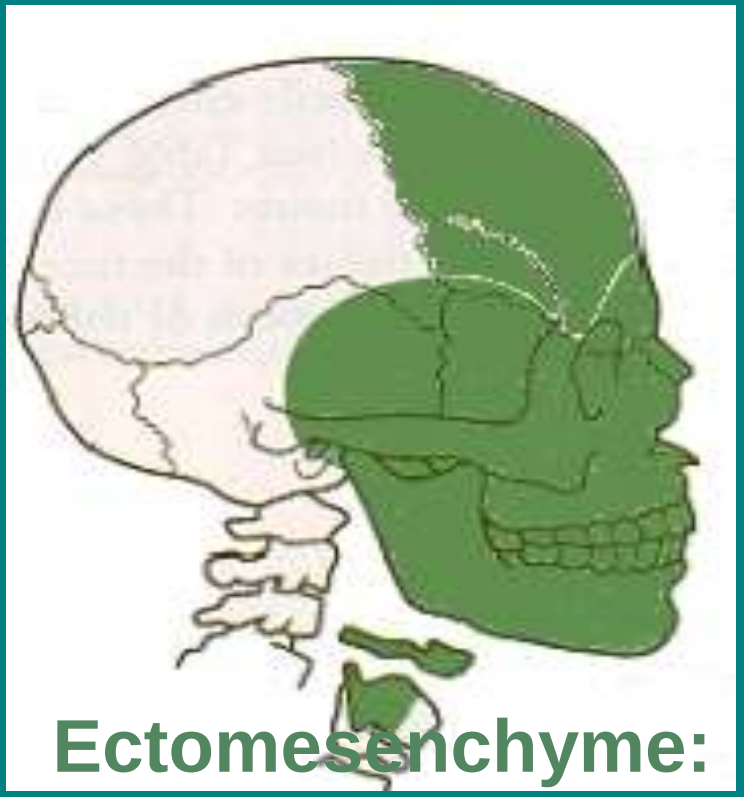
Labeling system exploring different organisation of perinucleolar chromatine in chick (C) and Japanese quail (Q) in their embryonic chimeras



Grim M, Halata Z. Anat Embryol 202: 401, 2000

Migration of NC cells of the head





Ectomesenchyme:

osteoblasts, fibroblasts, chondroblasts, smooth muscle cells, odontoblasts

Cardiac NC (R4-R8): for cardiac outflow tract

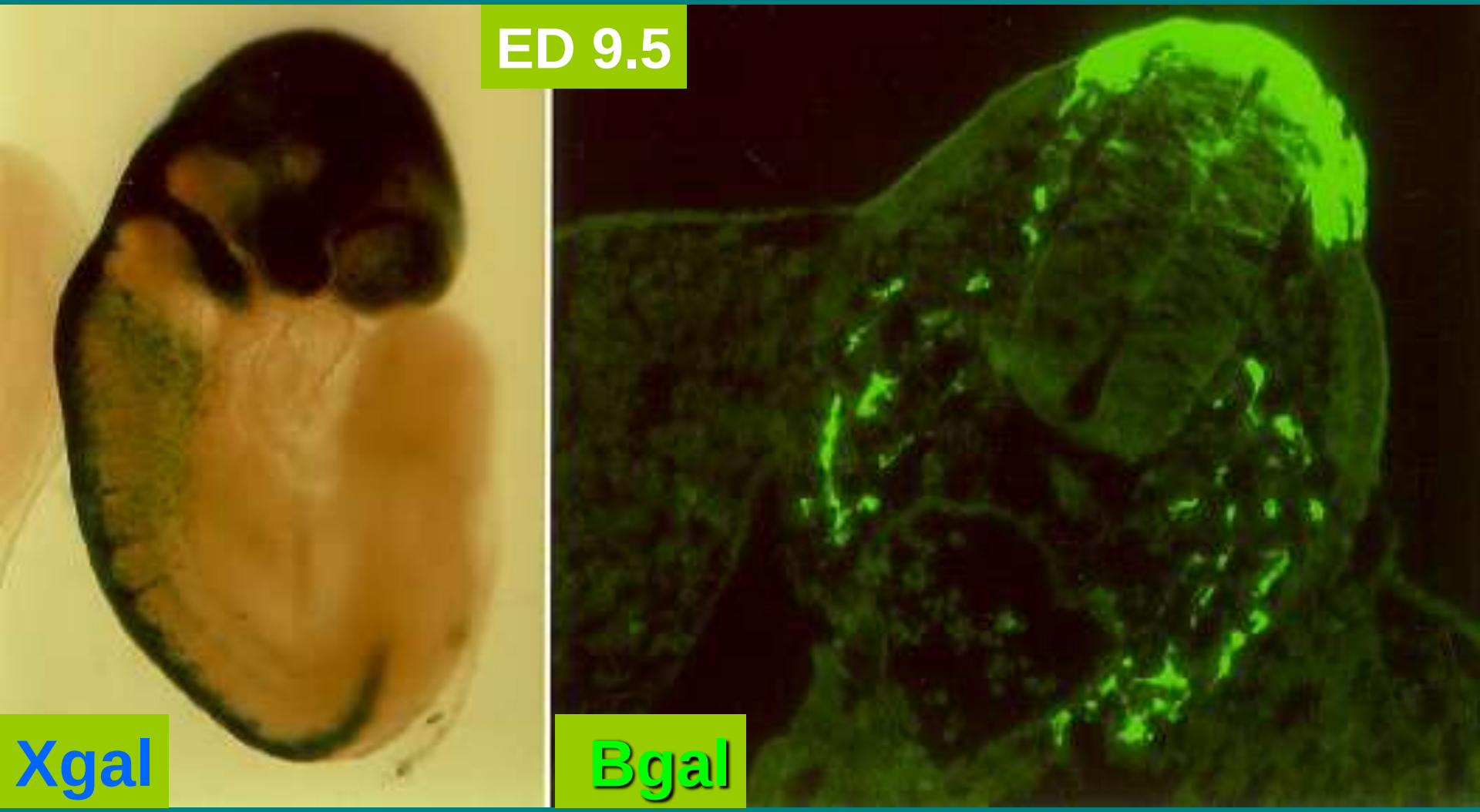
Labeling of NC cells in mammals

- using anti p75 Ab in mouse
- using reporter gene *lacZ* (*E. coli) in* transgenic mouse *Wnt1/lacZ*
- permanent expression of *lacZ gene* in cre-lox system in transgenic mouse *Wnt1-cre*/R26R

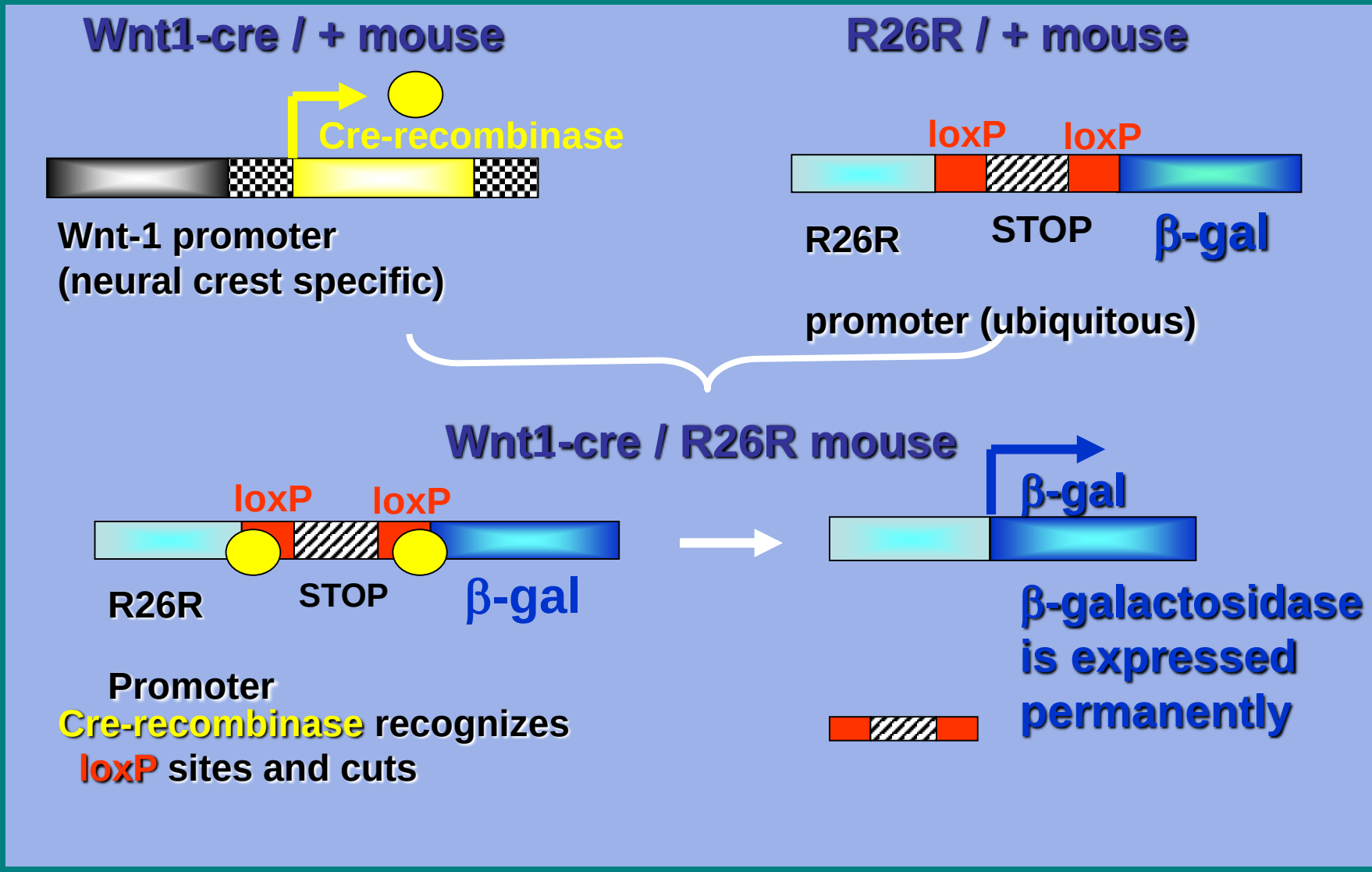
- detection of *lac*Z gene expression indigogenic method (Xgal) at pH 7.5 immunohistochemicaly (Bgal) (rabbit anti-*E. coli* β-galaktosidase; Chemicon)

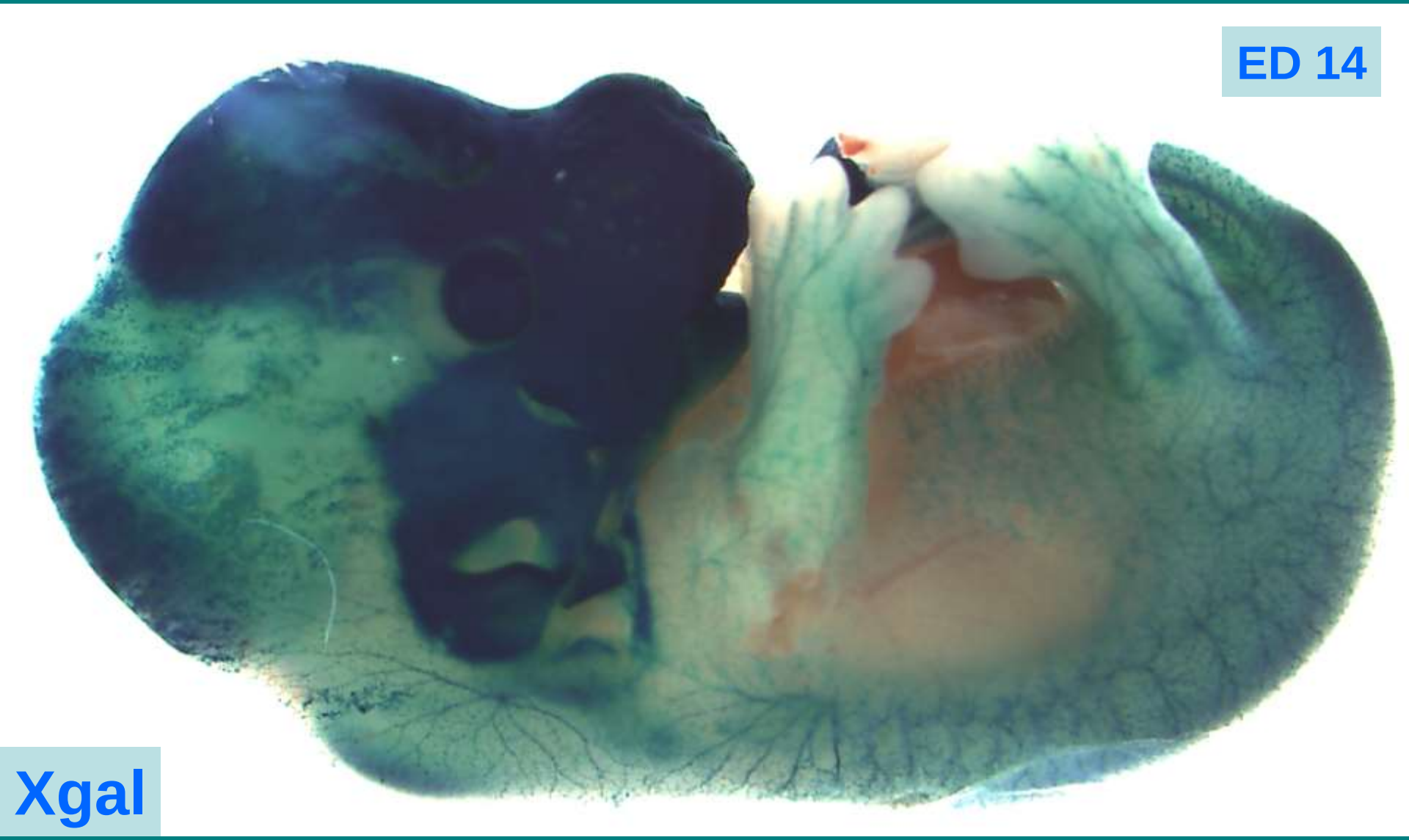
Labeling of neural crest cells

in *Wnt1-lacZ/ +mouse*



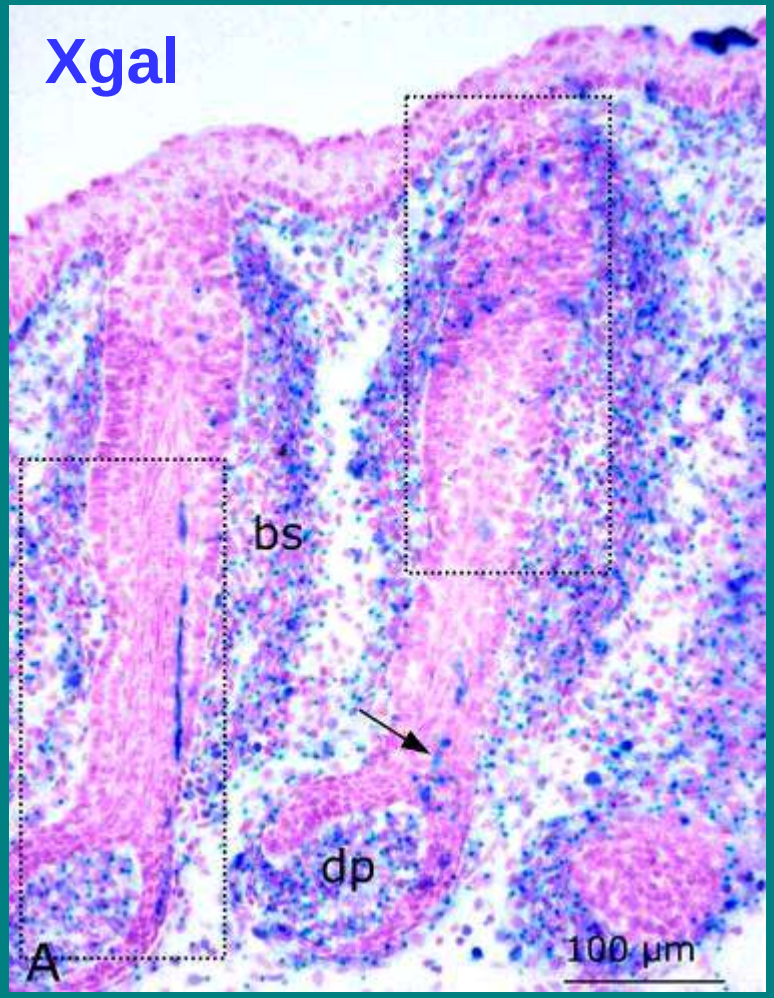
The Wnt1-cre / R26R two-component system to label neural crest cells



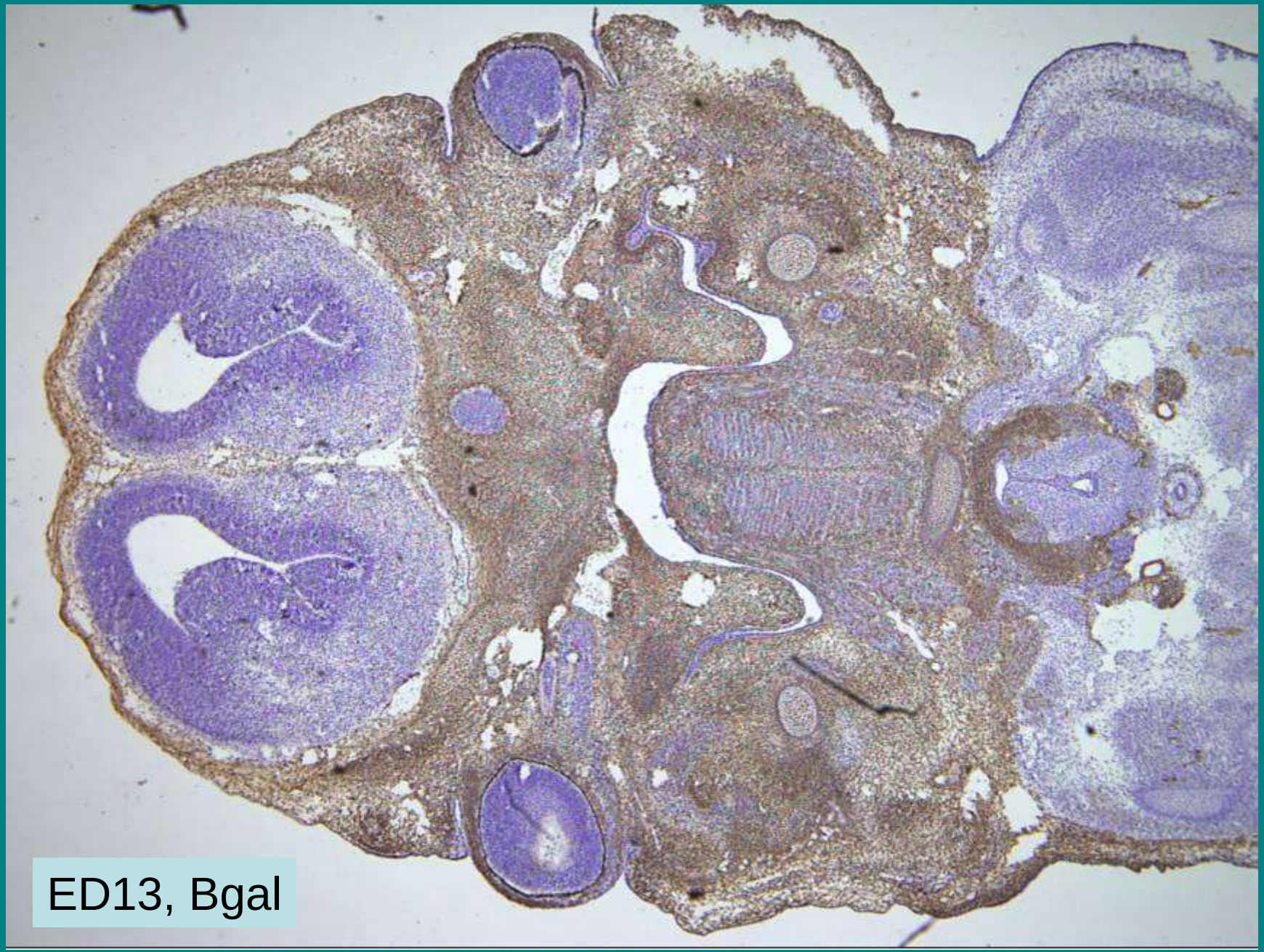

ED 14
Xgal

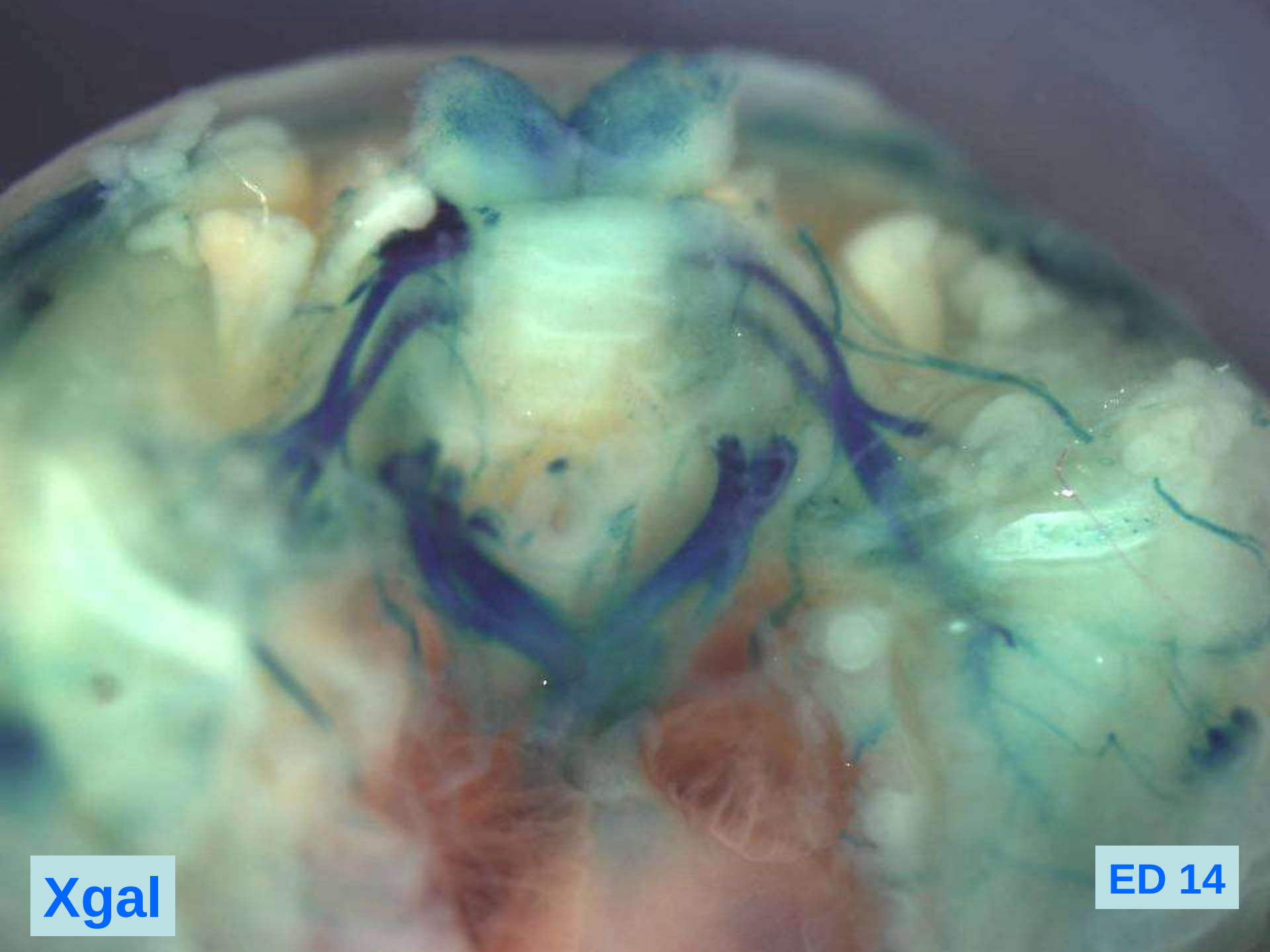
NCCs in *Wnt1-cre*/R26R mouse

ED13, X gal



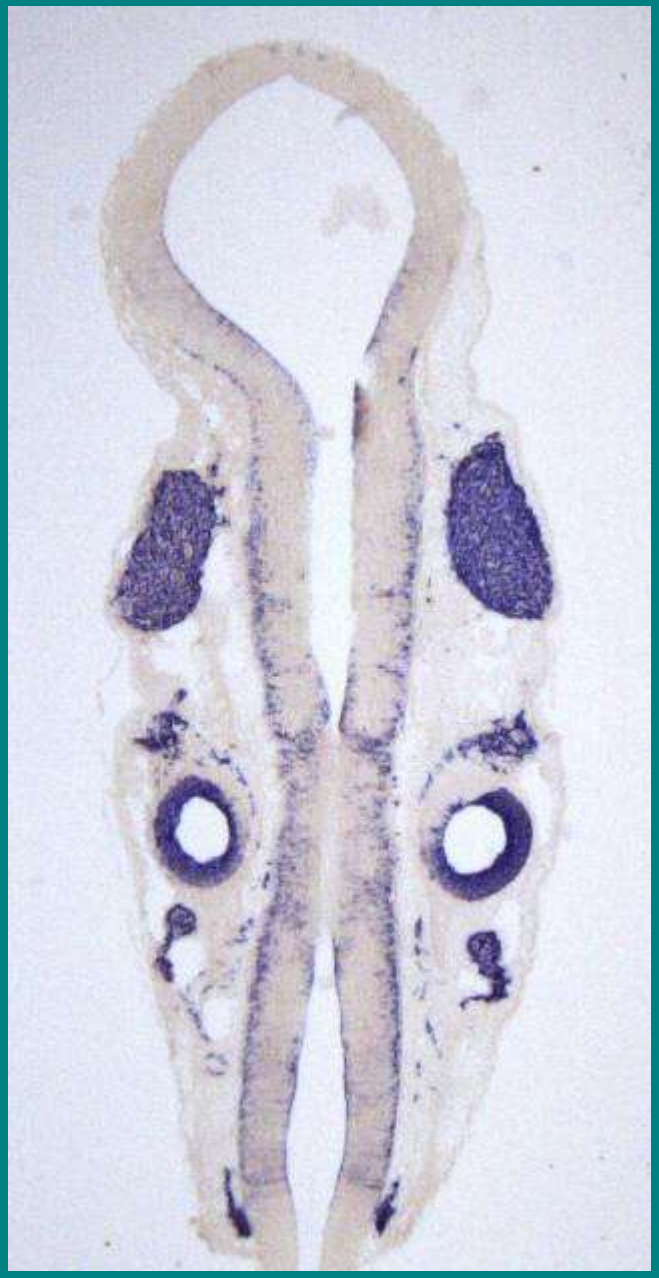
Hair follicles, ED 17.5


ED13, Bgal

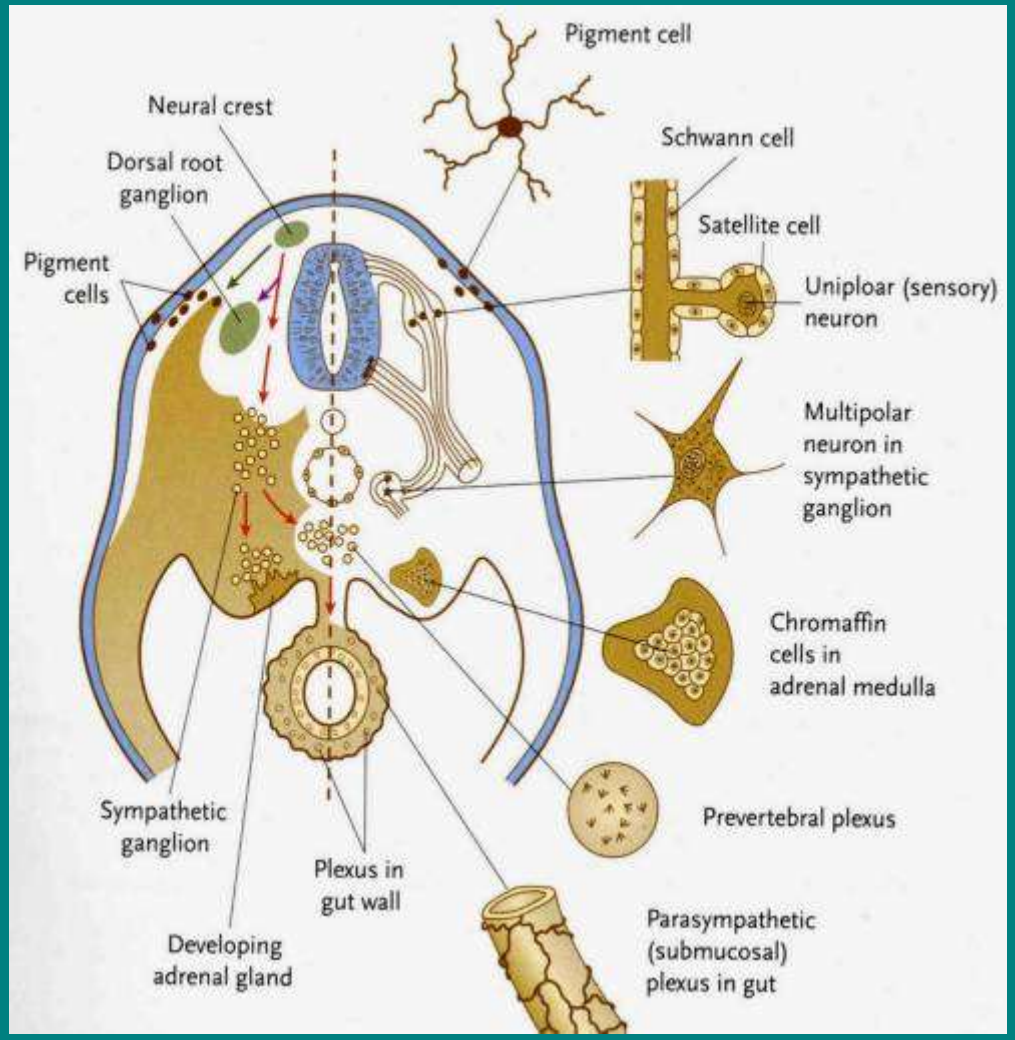

Xgal
ED 14

Cell types differentiated from the NC

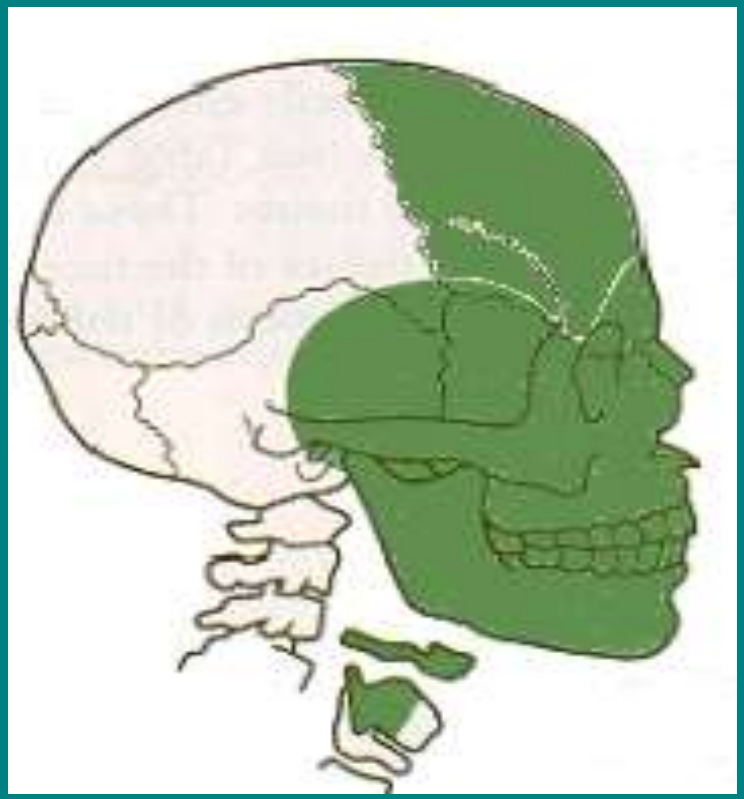
Derivatives of trunk NC: HNK-1 Ab, chick embryo



Derivatives of trunk and head NC



Neurons of spinal and autonomic ganglia, enteric eurons, Schwann cells, pigment cells, cells of adrenal medulla



Ectomesenchyme:

osteoblasts, fibroblasts, chondroblasts, smooth muscle cells, odontoblasts

cardiac NC (R4-R8): for cardiac outflow tract

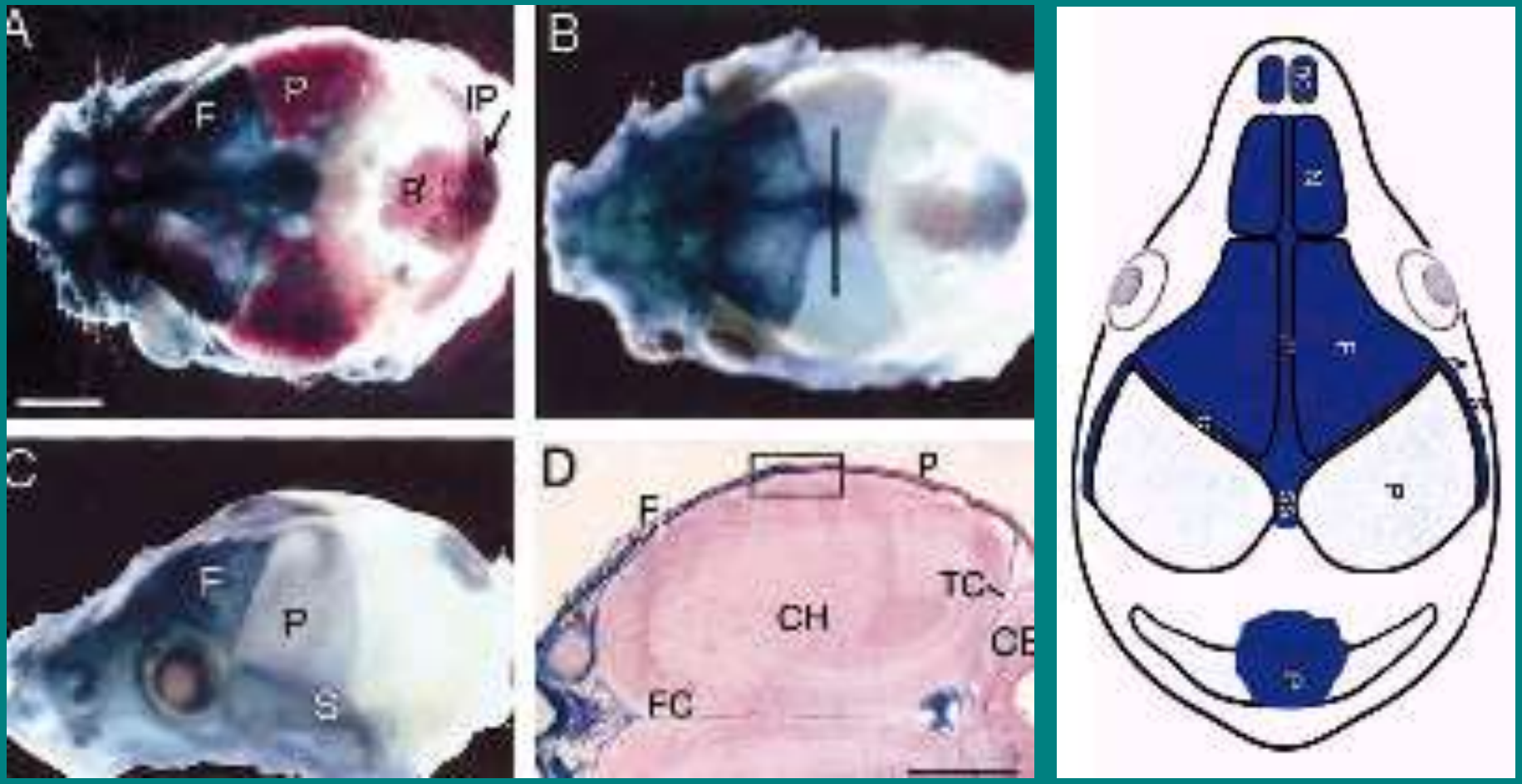
Derivatives of the NC in the head

- a part of sensory ganglia of V., VII., IX., X.
- parasymp. ganglia and their satellite cells
- Schwann cells of glomus caroticum
- parafolicullar C – cells (calcitonin),
- melanocyte,
- **Ectomesenchyme** – osteoblasts, fibroblasts, chondroblasts, smooth muscle cells in anterior part of the head, odontoblasts, pia mater, arachnoidea, stromal cells of cornea, smooth muscle cells of iris stromal cells of thymus, thyroid and parathyroid gland, salivary glands, lacrimal gland
- **Cardiac neural crest** – outflow tract, wall of large branches of aortic arch

Migration of cranial neural crest cells

B.M. Carlson (1999)

***Wnt1-cre*/R26R mouse**



Jiang et al., Dev Biol 241:106, 2002

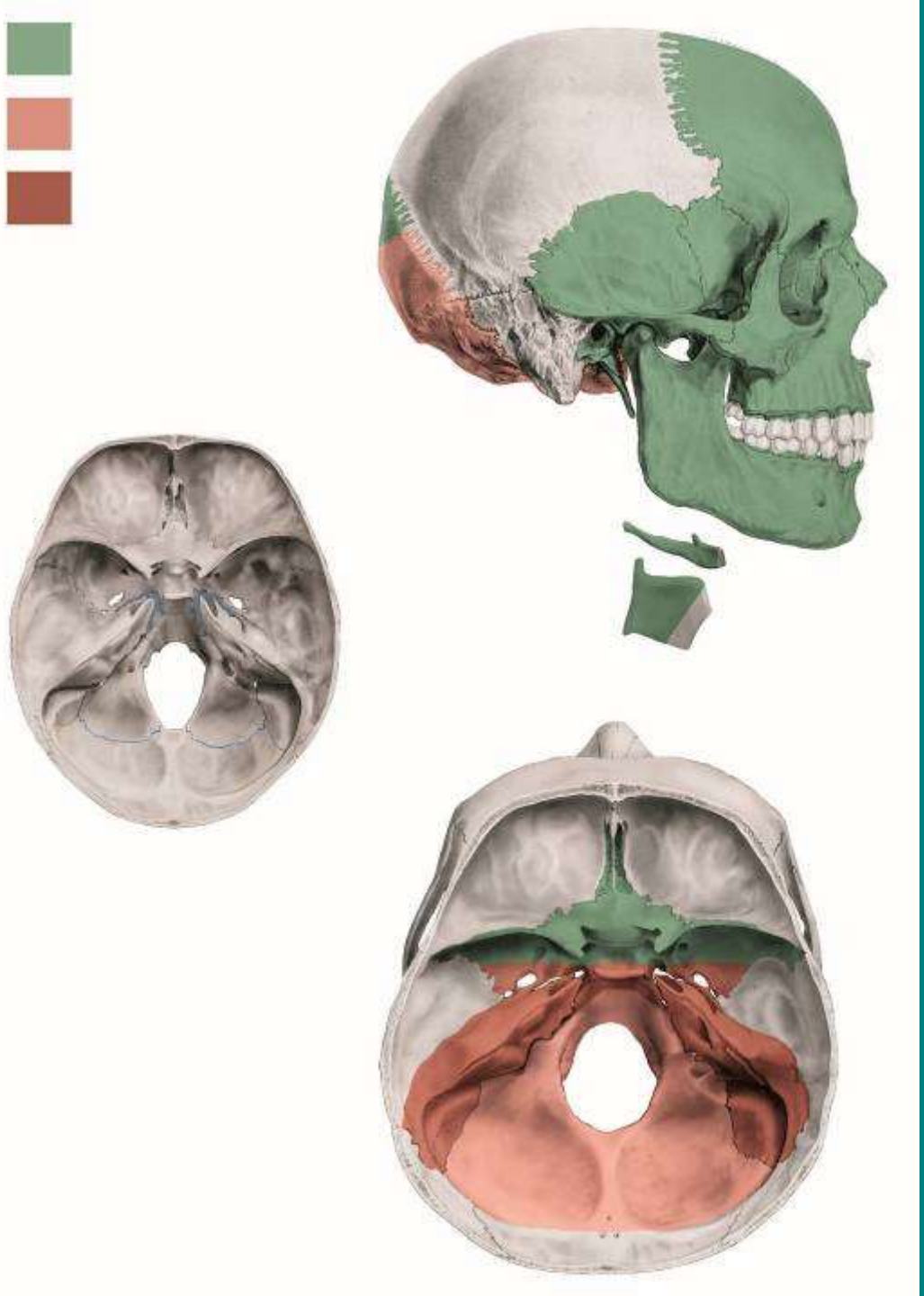
Developmental origin of the skull bones:

from the neural crest (green),

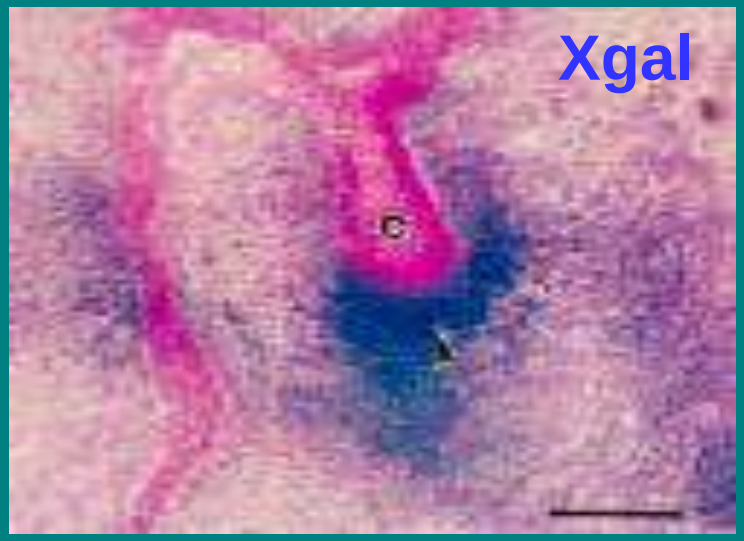
from occipital somites (pink)

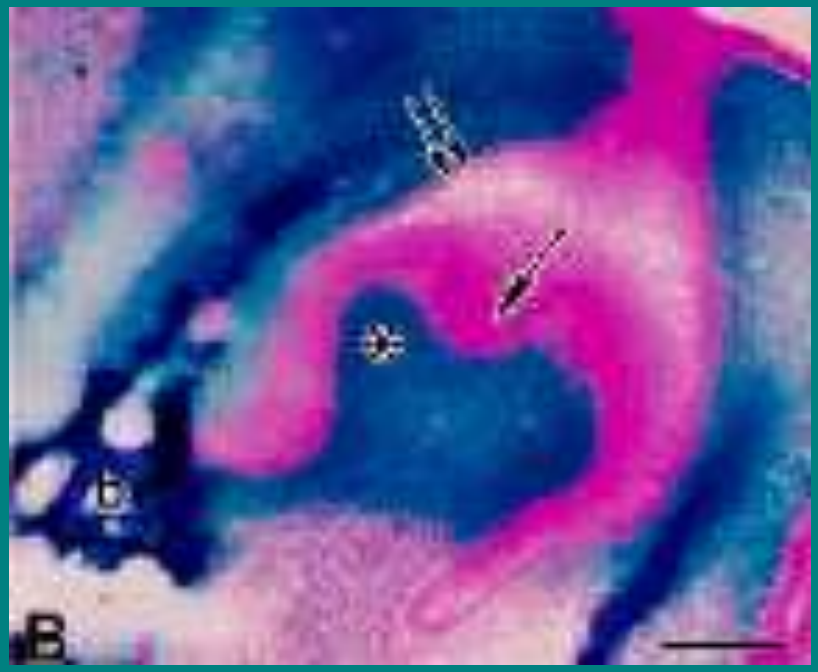
from nonsegmented paraxial mesoderm (red)

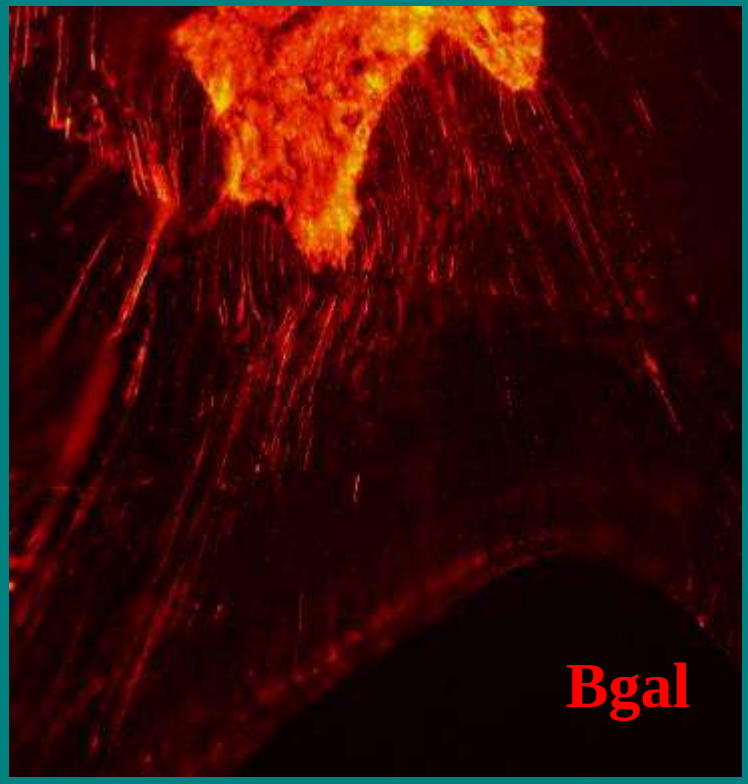
membranous ossification (grey).

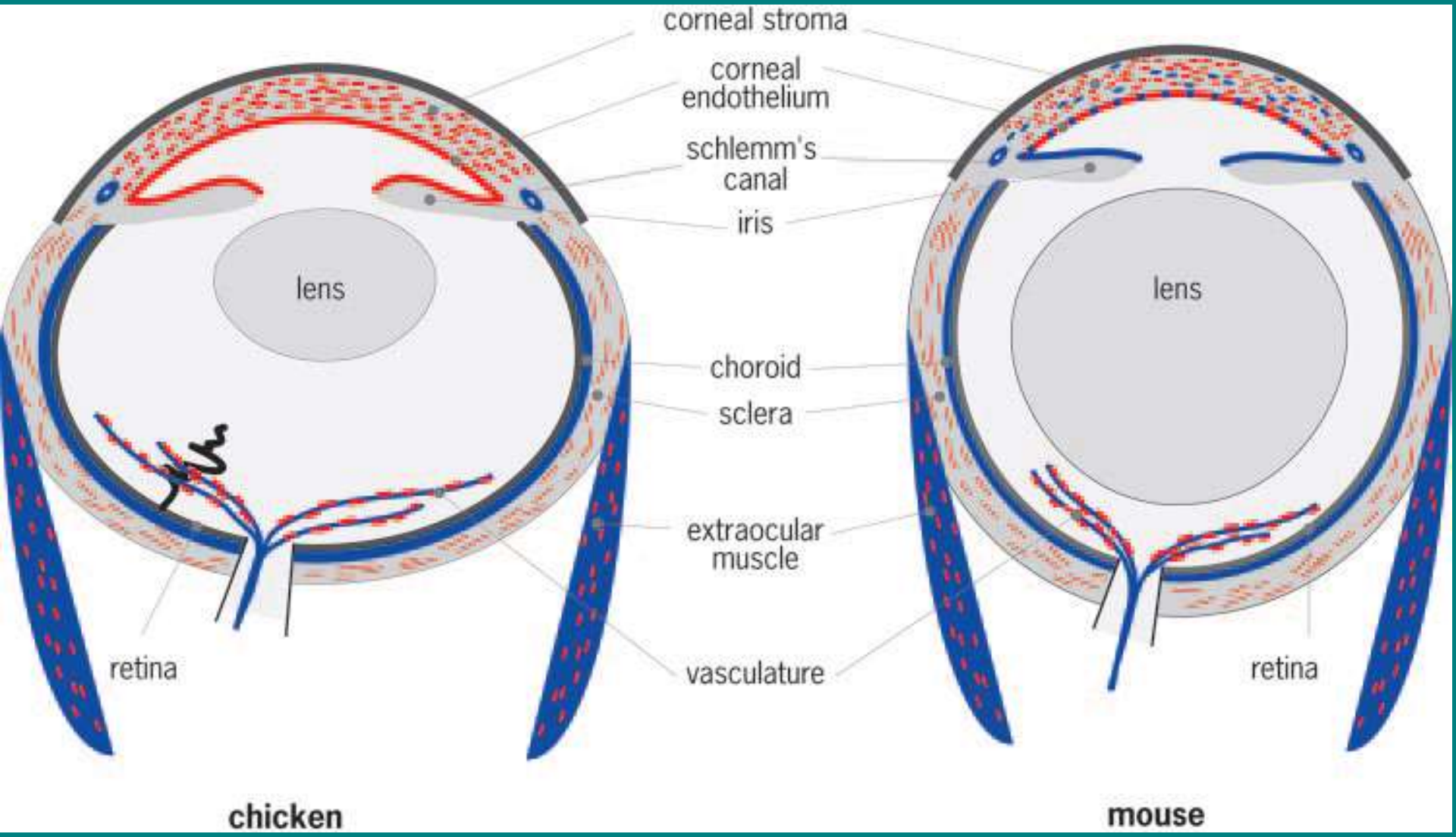


Tooth development
(*Wnt1-cre*/R26R) (Chai et al. Development 127:1671, 2000)
From the NC originate odontoblasts, cementoblasts, periodontium





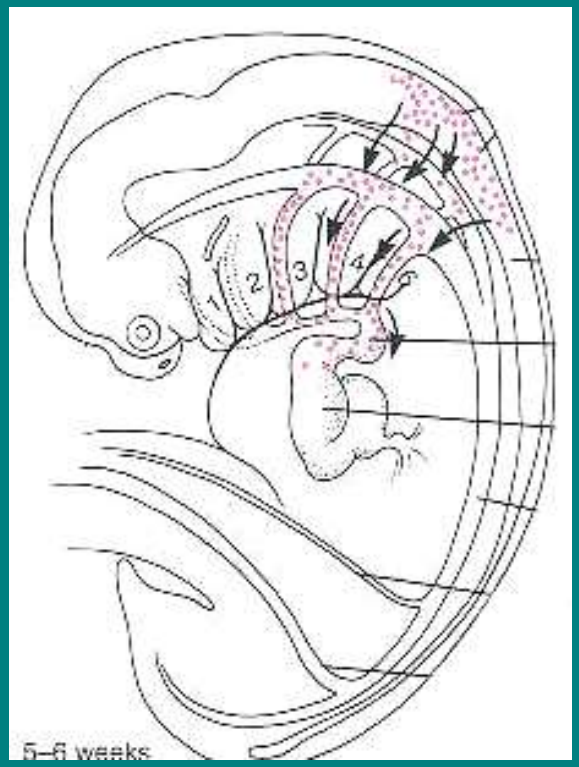


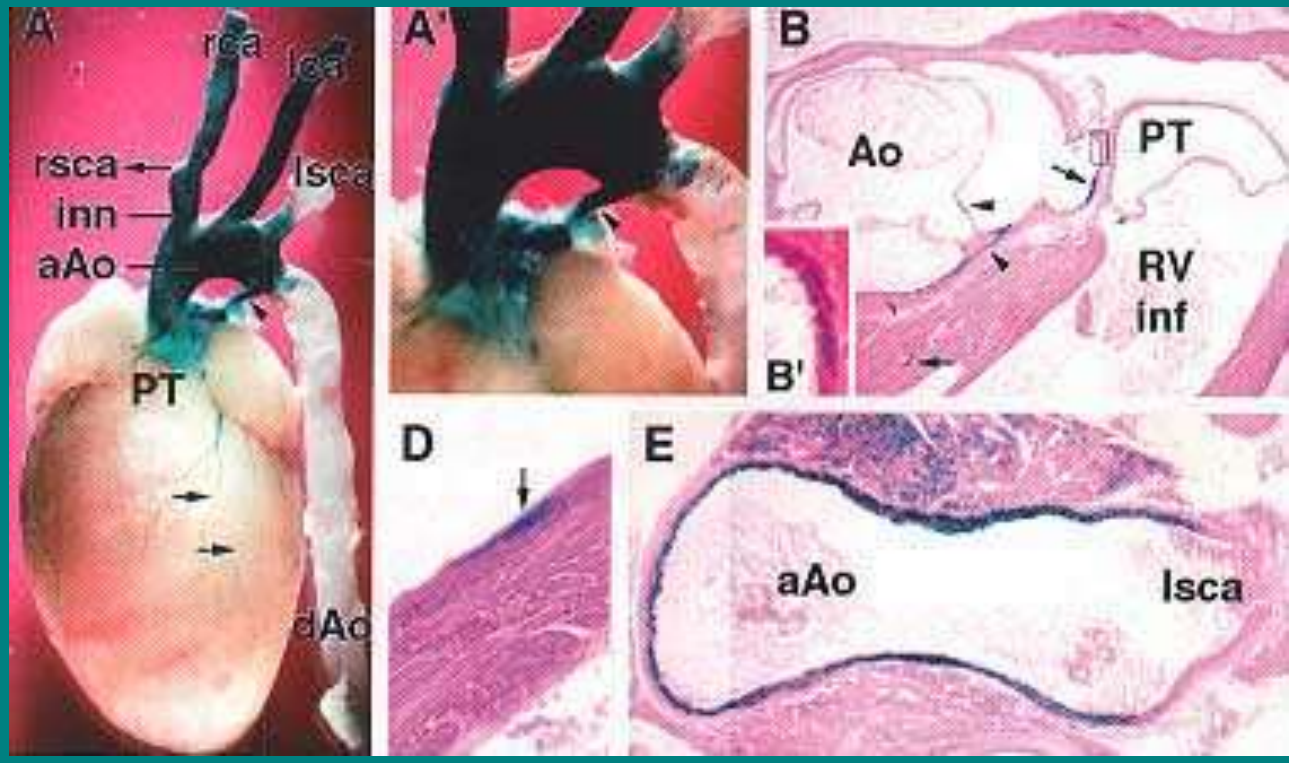


Fate Maps of Neural Crest (red) and Mesoderm (blue) in the Mammalian Eye. P. J. Gage, W. Rhoades, S. K. Prucka and T. Hjalt, Invest Ophthalm & Vis Sci. 46:4200 – 8, 2005

Cardiac neural crest

R 4. – R 8.





***Wnt1-cre*/R26R transgenic mouse, Xgal , 7. – 9. week**

Jiang et al.: Development 127:1607, 2000

Developmental disorders of the NC

Developmental defects of the NC

- CHARGE syndrom (Coloboma iridis, Heart defects, Atresia choanae, Retardation of development, Genital hypoplasia in males, Ear anomalies)
- DiGeorge syndrom (hypofunction of parathyroid and thyroid gland, thymus hypoplasia, defects of septation of aorta and pulmonary trunk
- anomalies of teeth • albinism
- Waardenburg syndrom (*Pax3* mutation – pigmentation defects, defects of limb muscles, cleft palate, cardiovascular defects, hypertelorism)
- Hirschsprung disease • Piebaldism

Pax3 mutation in mouse (splotch mutation)

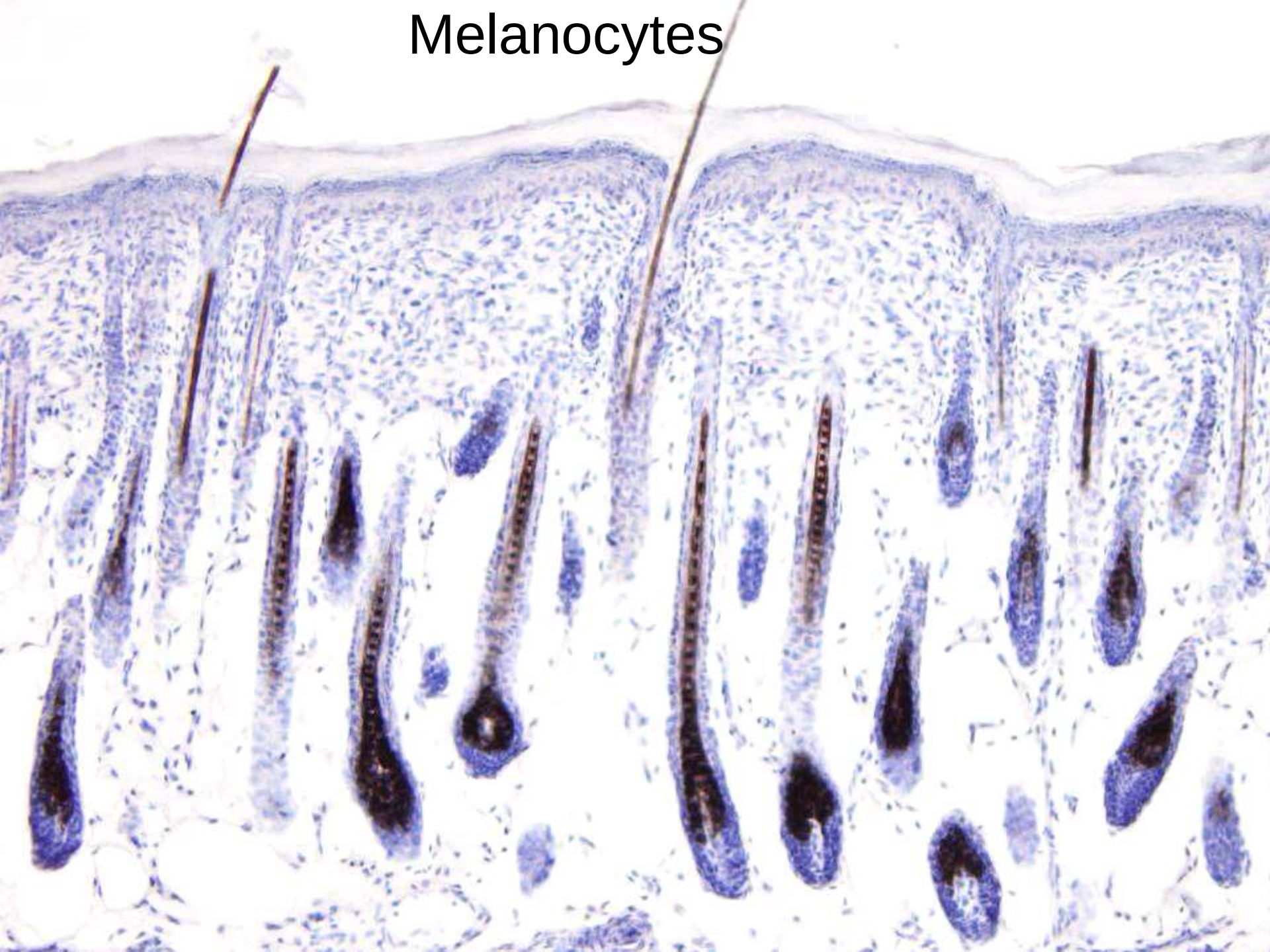
$Sp^{1H}/+$

Sp^{1H}/ Sp^{1H}

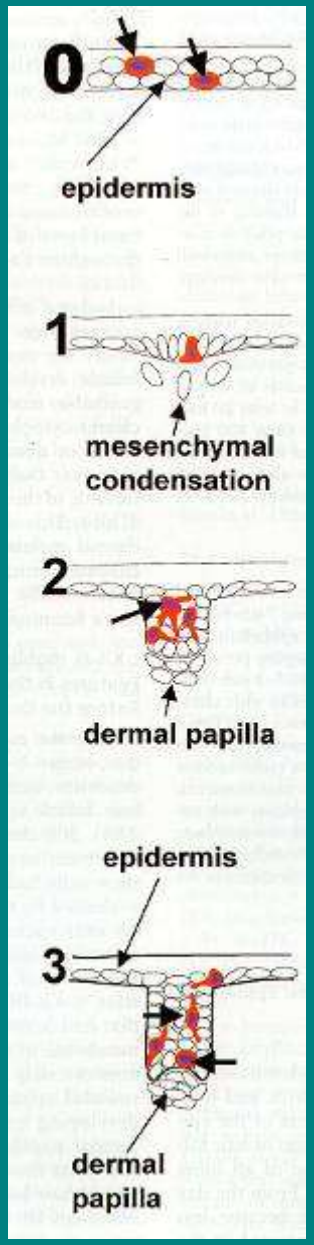
Piebaldism (pigmentation defects, sterility, anemia)
mutation of *KIT* gene in man and *Kit* gene in mouse

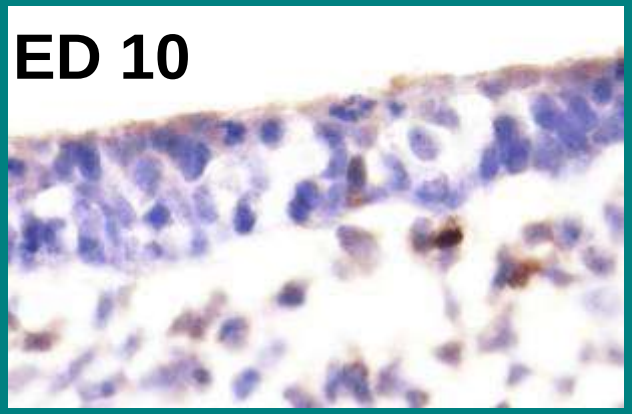
Neural crest cells in epidermis

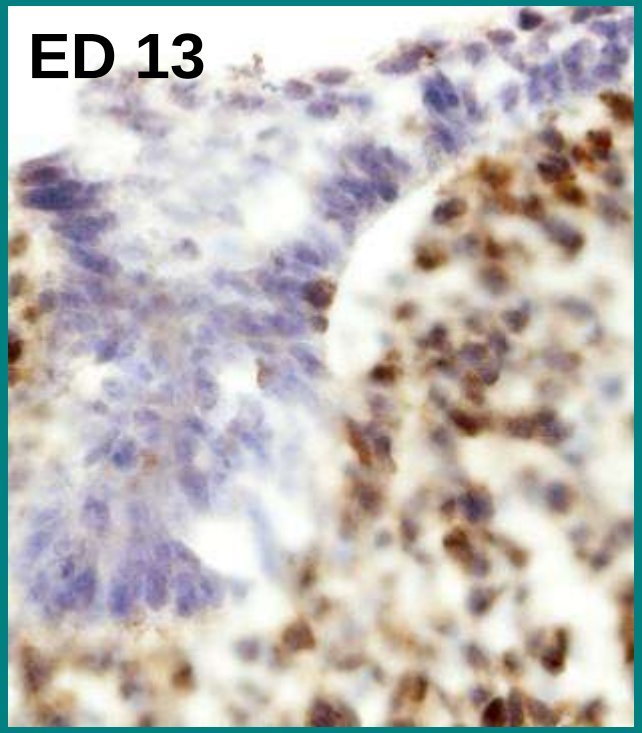
Merkel cells, melanocytes, stem cells

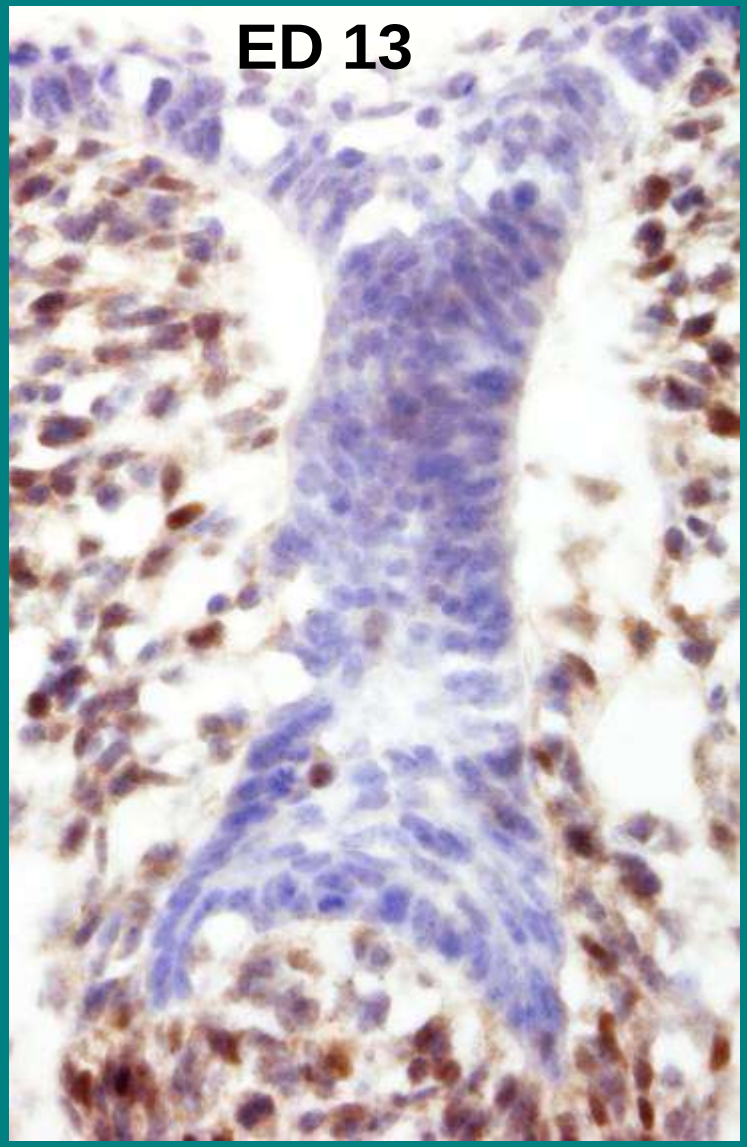

Melanocytes

Neural crest cells in epidermis – in hair follicle



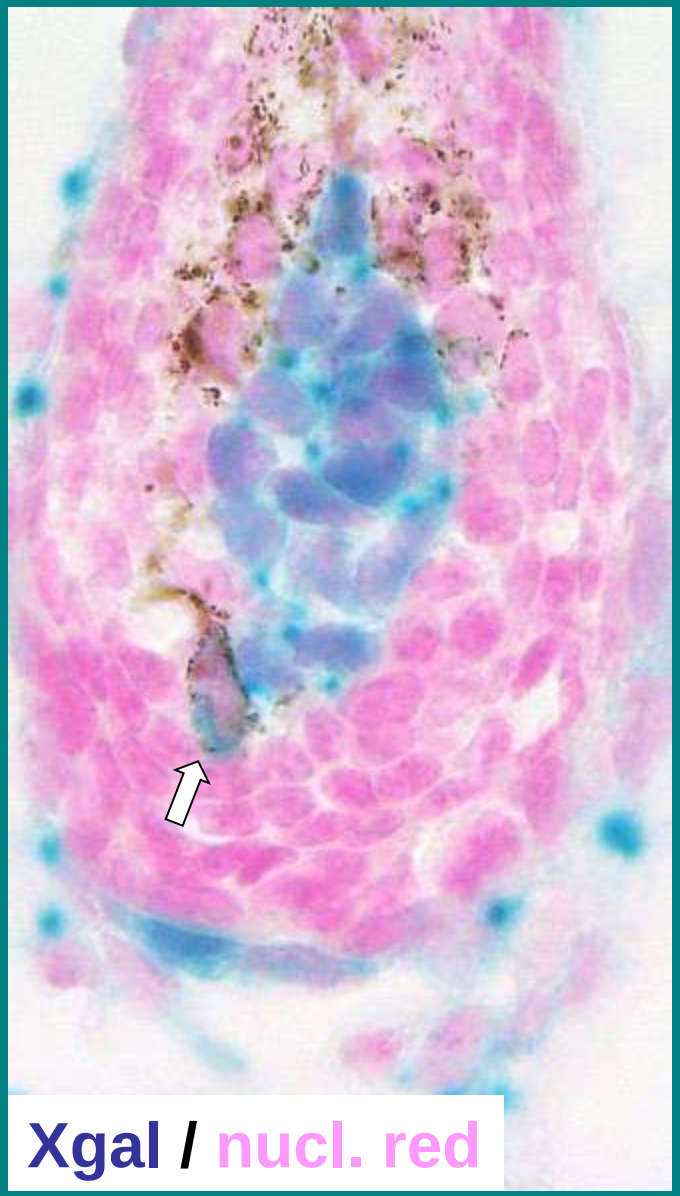


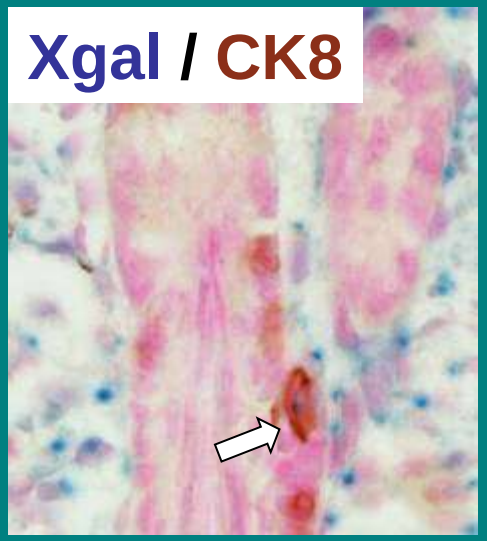


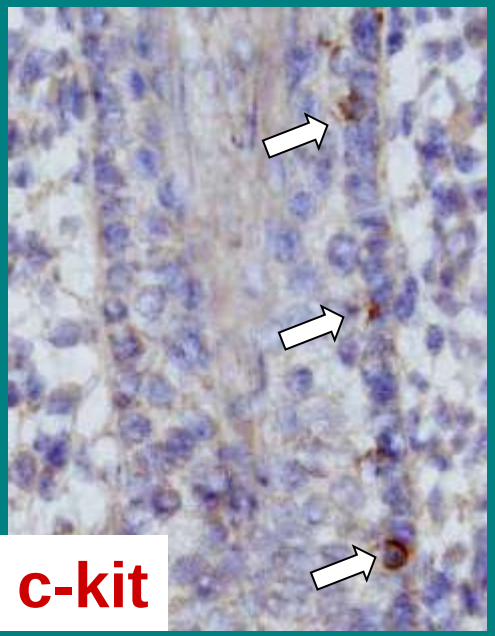


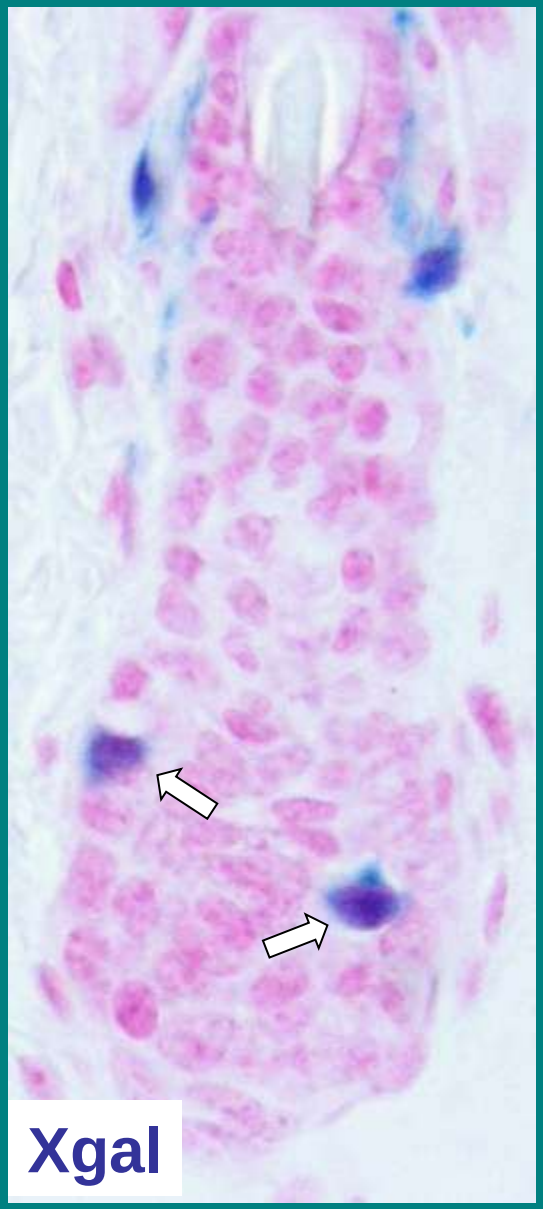
Bgal/H Wnt1-cre/R26R

NCCs in hair follicles, back skin, Wnt1-cre/ R26R mouse

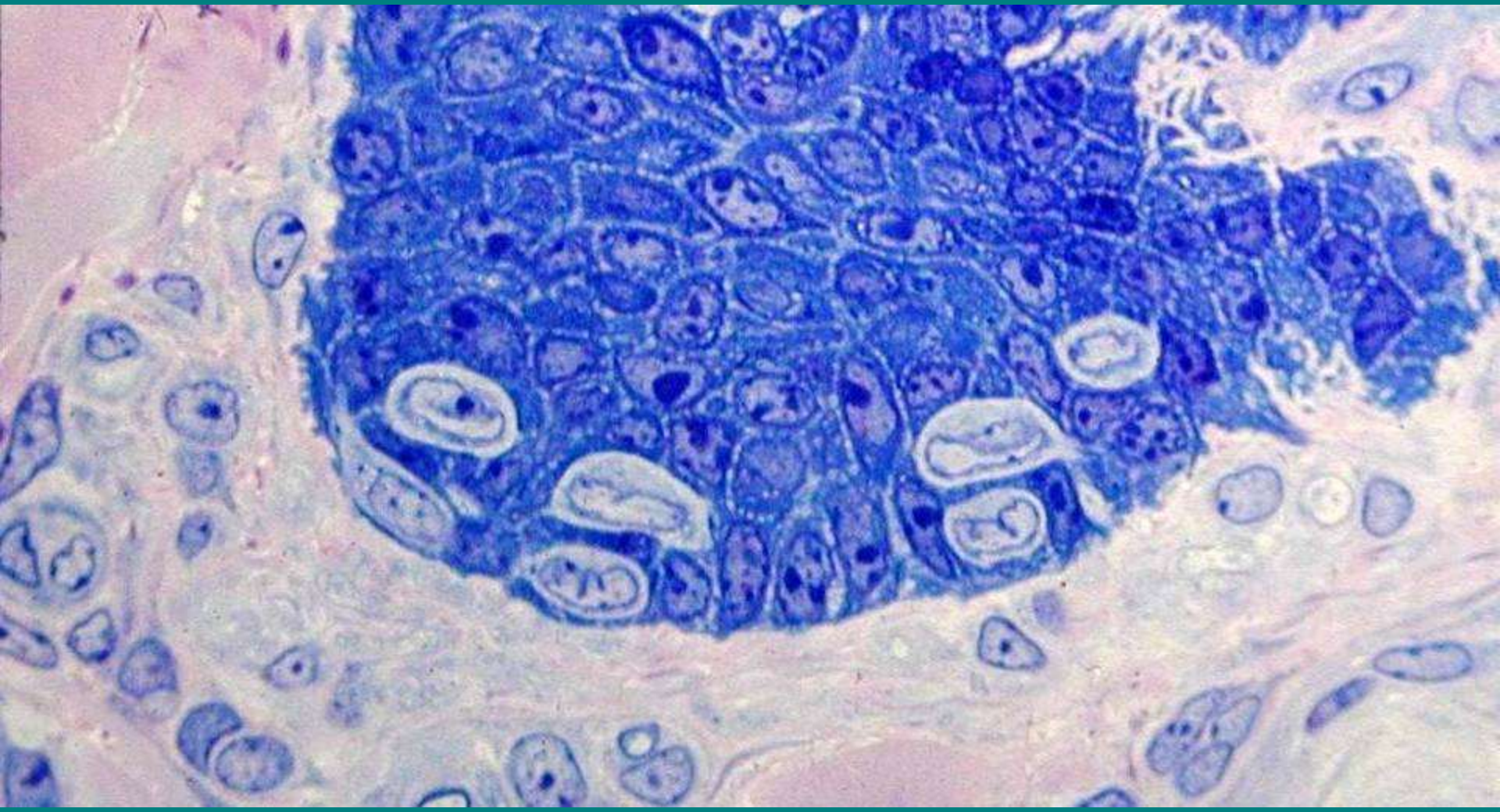




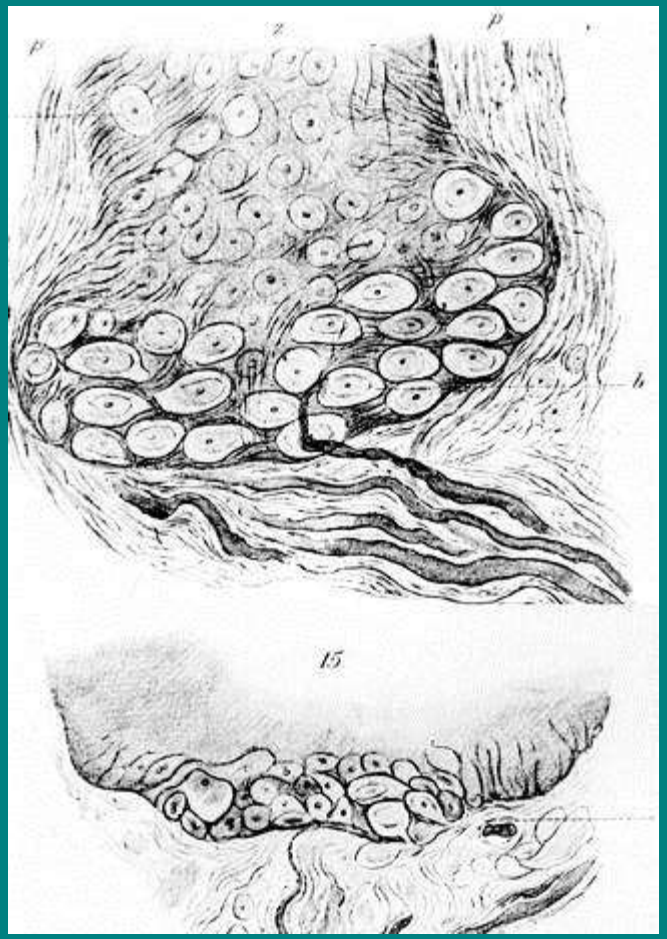


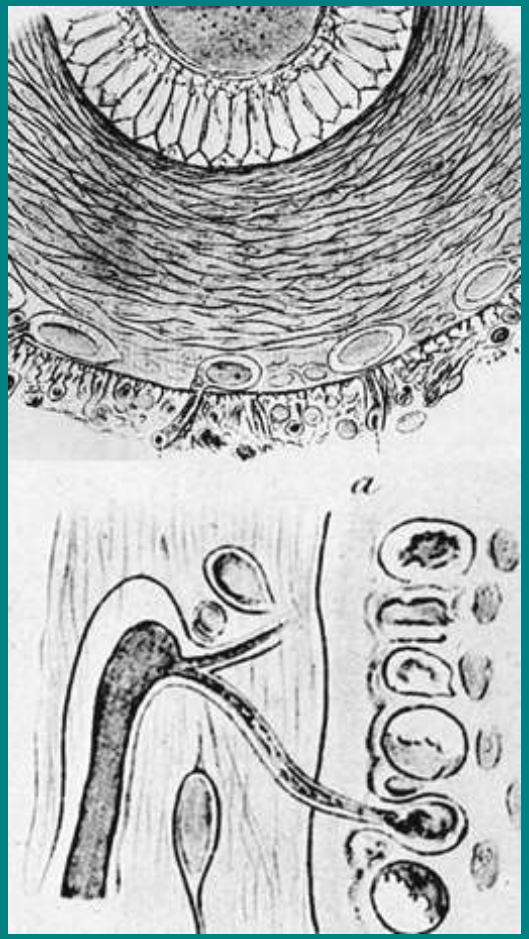


Merkel cells - large light cells in the basal layer of epidermis and in mucous membranes of ectodermal origin in vertebrates

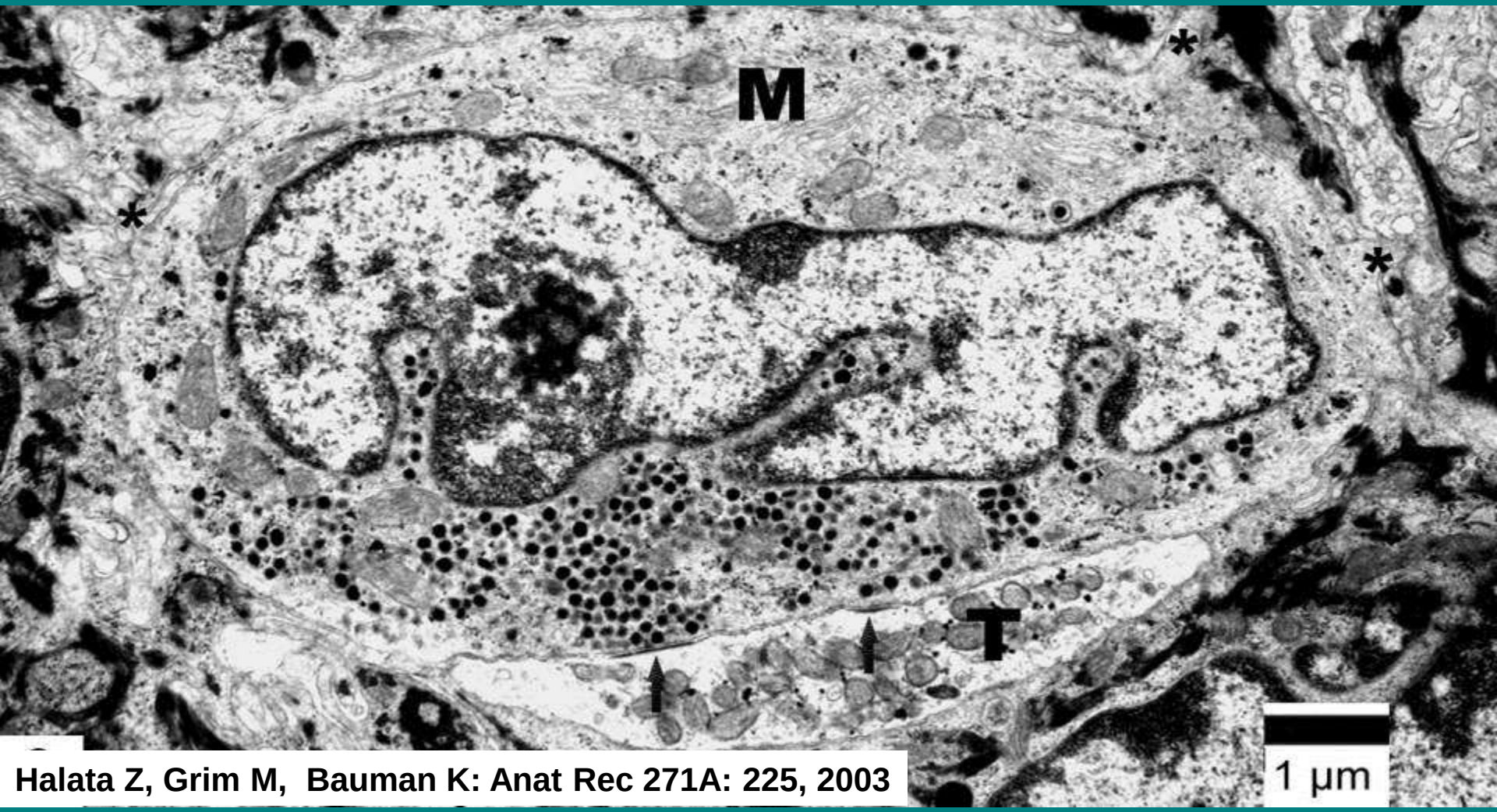


F. S. Merkel (1875) :
„Tastzellen“ of the skin of birds and mammals





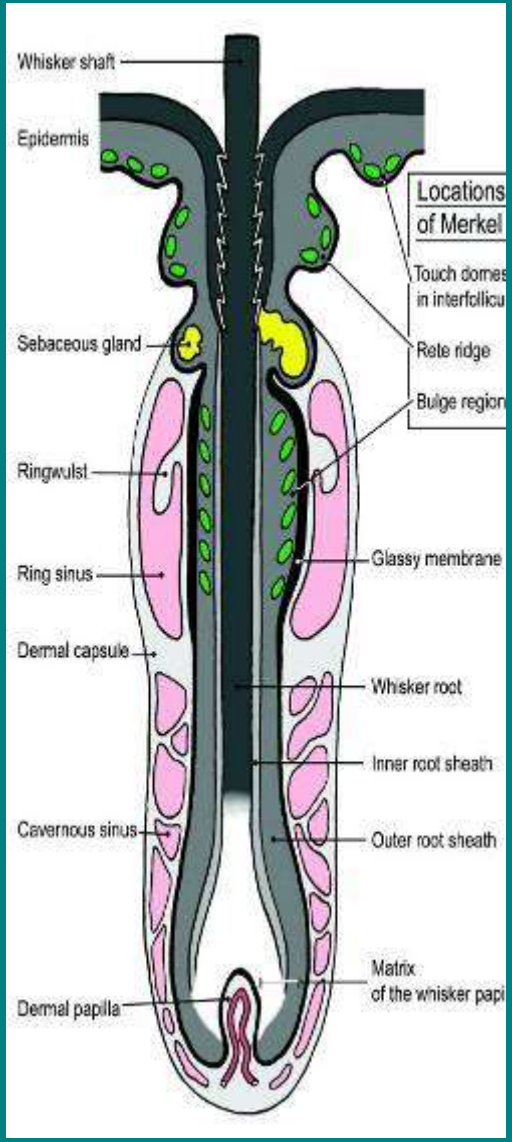
Merkel cells are transducers of tactile stimuli in slowly adapting mechanoreceptors of the skin

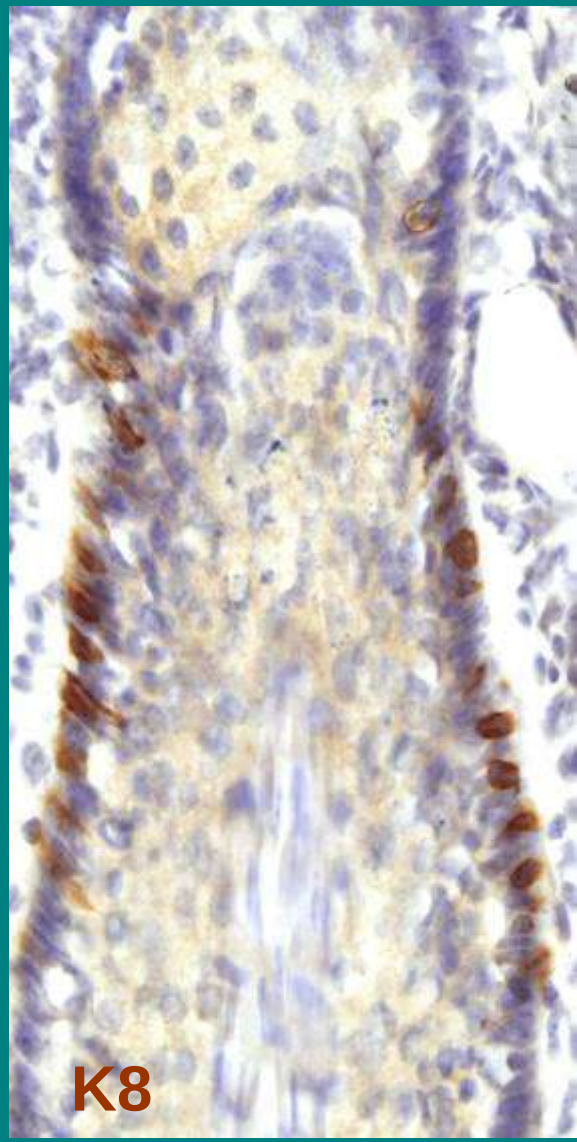


Halata Z, Grim M, Bauman K: Anat Rec 271A: 225, 2003

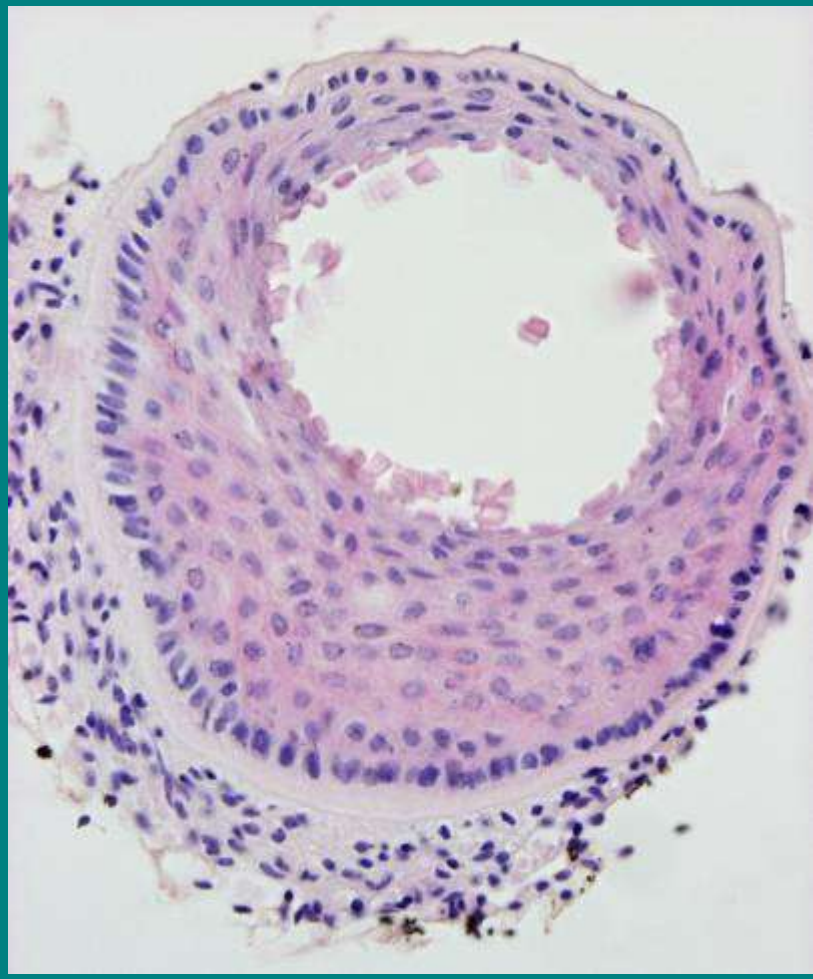
Human MCs represent 3.6 - 5.7% of basal epidermal cells from glabrous and hairy skin (Fradette et al., 2003)

Merkel cells in whisker hair follicle





Whisker hair follicle, blood sinus, innervation, bulge



Merkel cell carcinoma

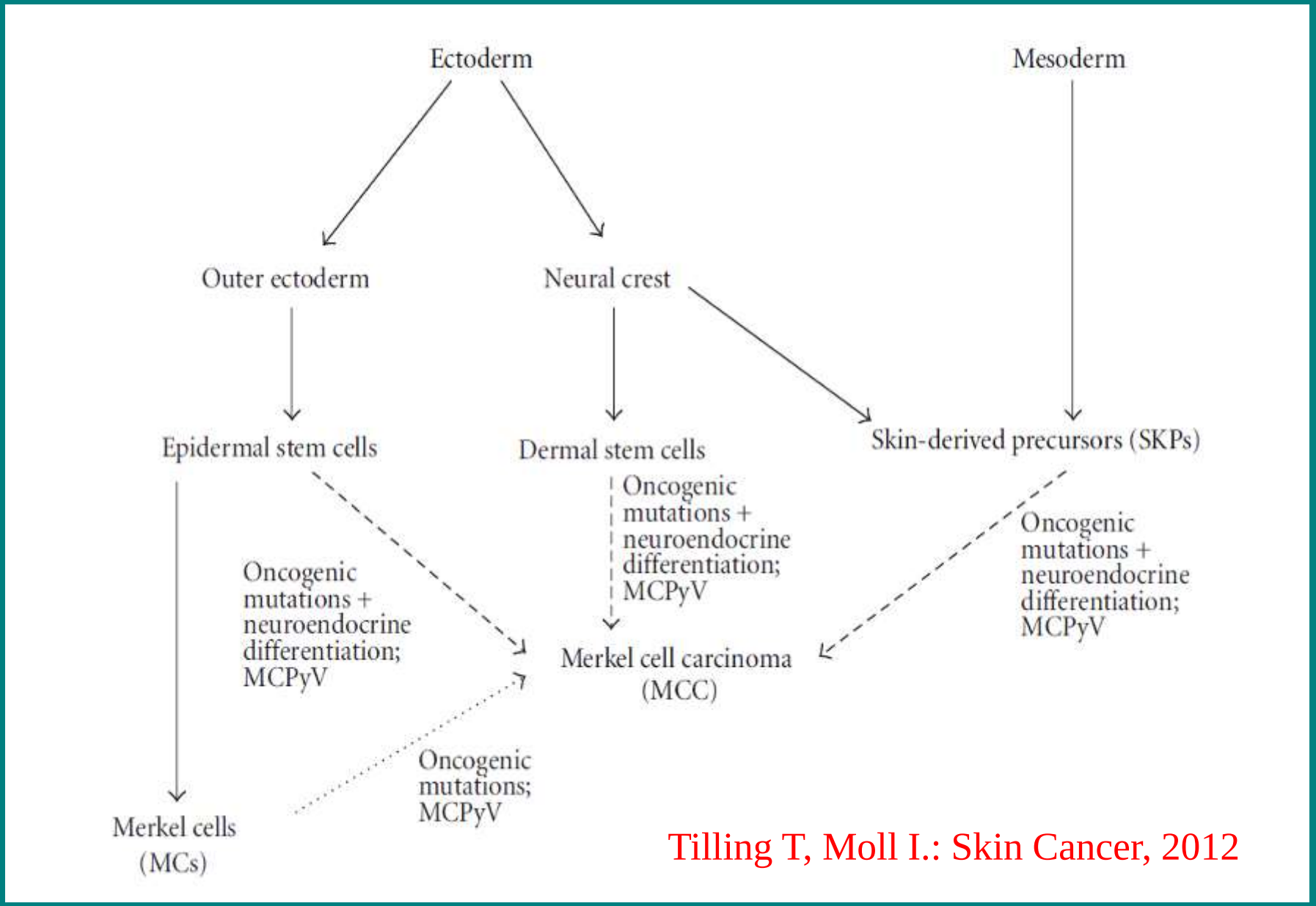
Merkel cell carcinoma

Merkel cell carcinoma is a rare and highly aggressive skin cancer, which, in most cases, is caused by the Merkel cell polyoma virus discovered at the University of Pittsburg 2008.

It is also known as cutaneous neuroendocrine carcinoma of the skin, primary small cell carcinoma of the skin, and trabecular carcinoma of the skin.

It has 4 times greater mortality than the melanoma and its incidence is rising. Now accounts for 5-6 cases per 1 million inhabitants.

MCC cells express cytokeratins 8, 18,19 and 20, and neuroendocrine markers as Merkel cells..

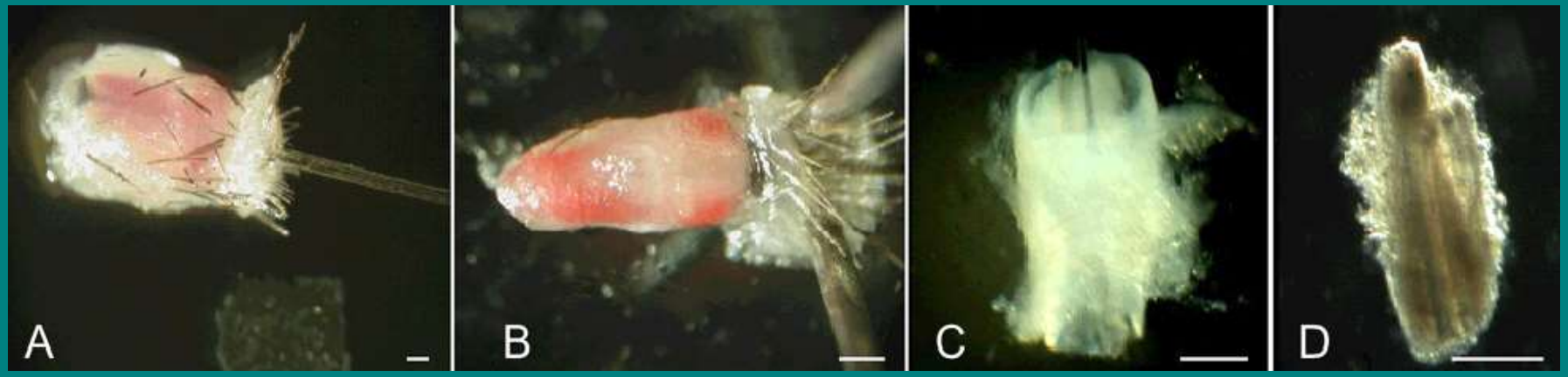


Tilling T, Moll I.: Skin Cancer, 2012

Scheme of potential cells of origin of Merkel cell carcinoma (MCC) from an ontogenetic perspective. Arrows: hypothetic lineage relationships.

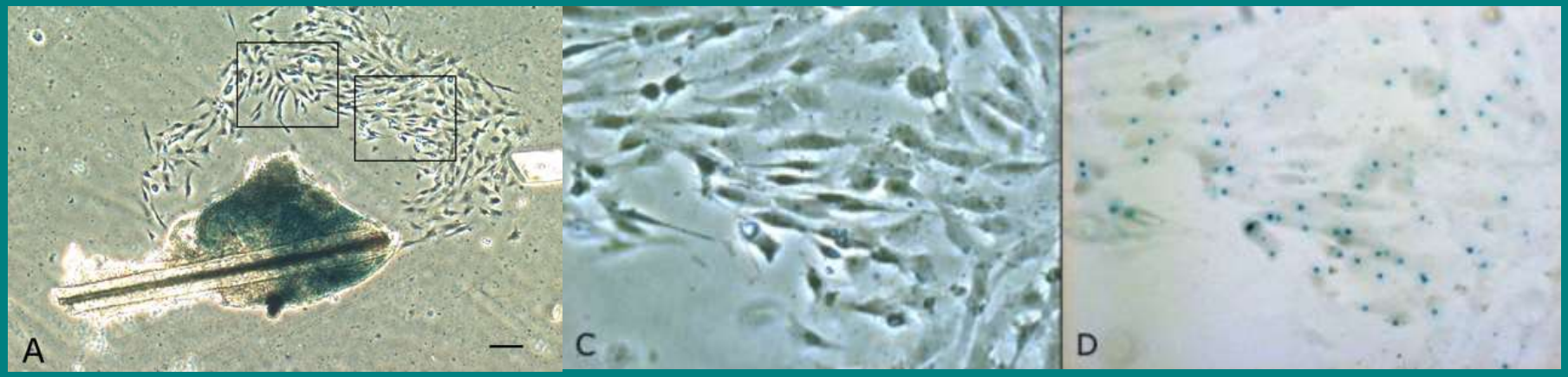
Neural crest stem cells in hair follicles of the mouse

Dissection of the bulge from adult whisker follicle

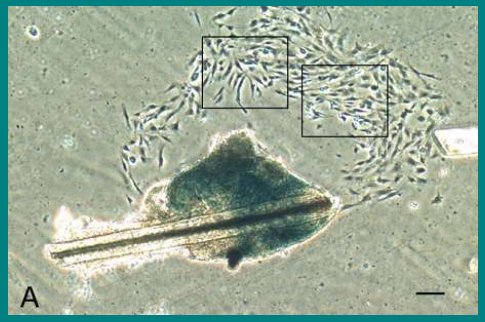


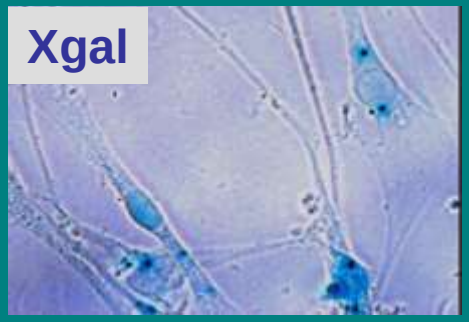
Dev Dyn 231:258-269, 2004;

Xgal + NCCs emigrated 4 days after explantation

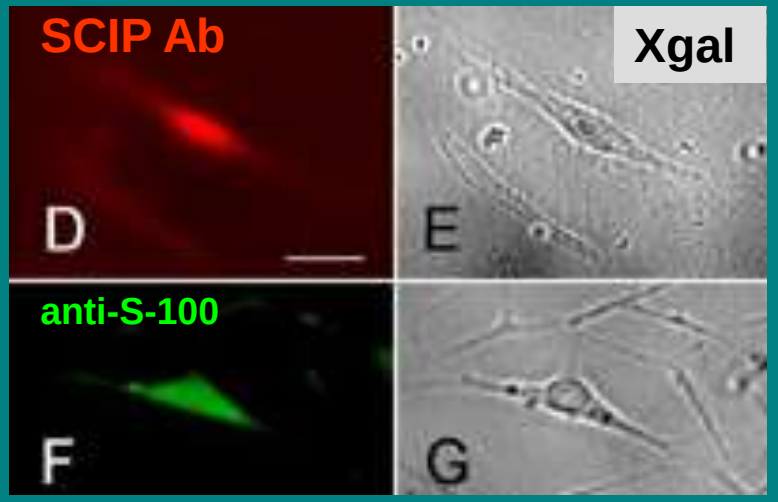


Bulge explant-derived NCCs are pluripotent

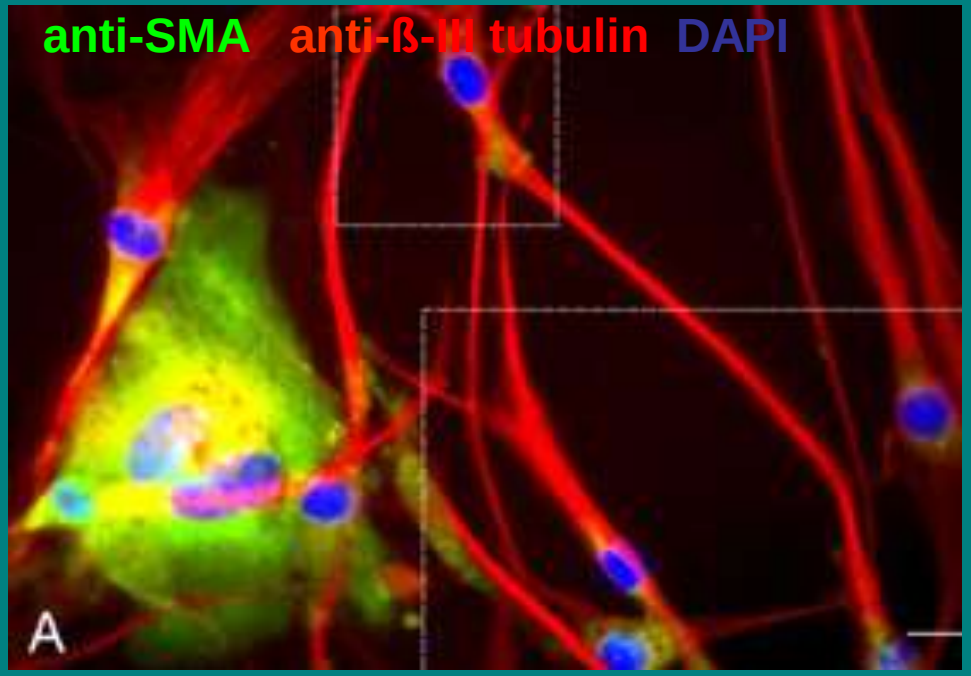




Schwann cells



Smooth muscle cells Neurons

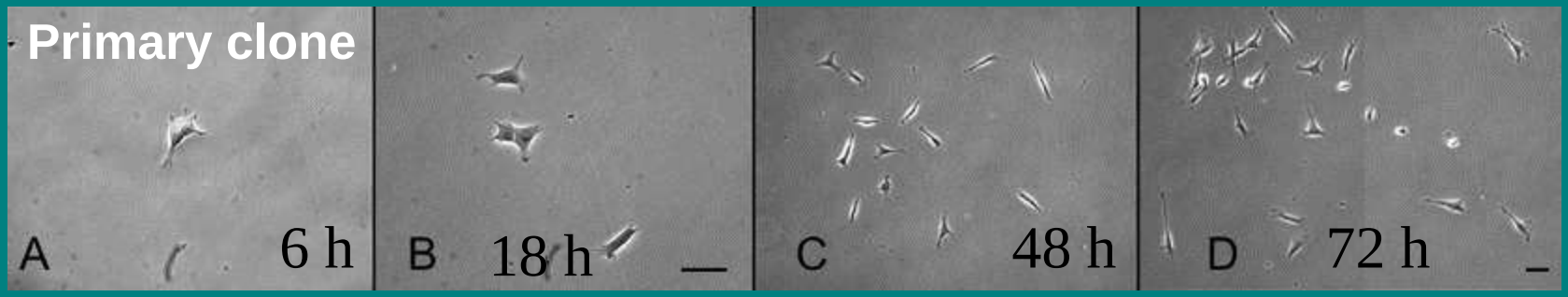


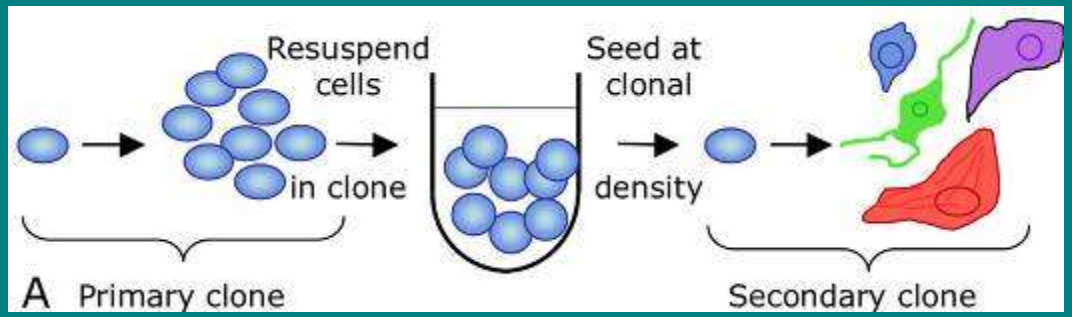
Melanocytes Chondrocytes

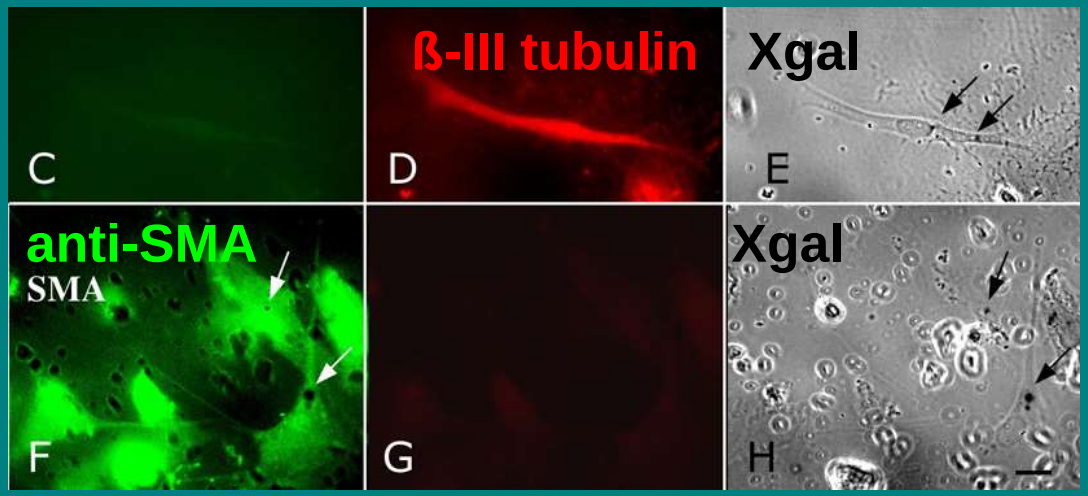
Dev Dyn 231:258-269, 2004; Embryo Today 72:162-172, 2004
Supp. by LN 00A065 and VZ 111100003-3

Bulge-derived NCCs undergo self-renewal

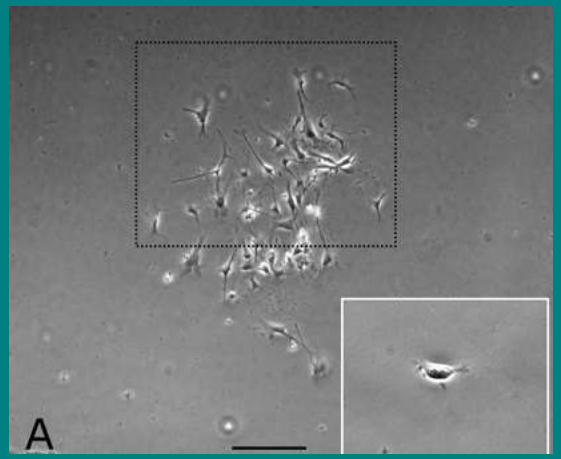
(determined by serial cloning)





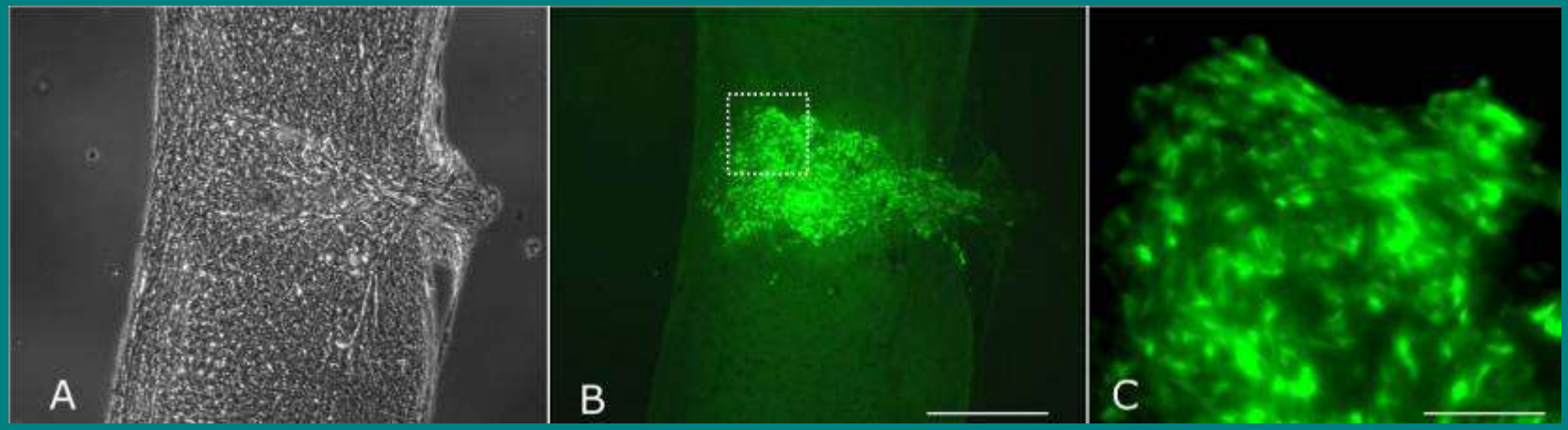


5-day-old secondary clone

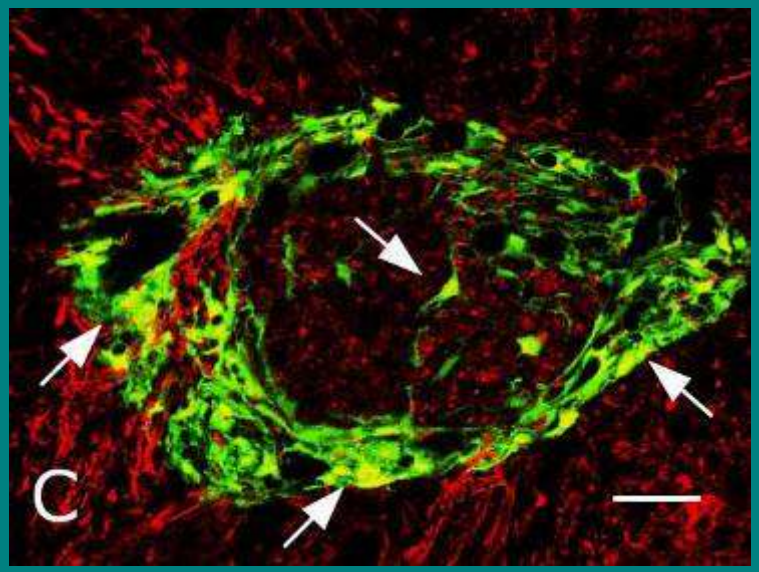


Dev Dyn 231:258-269, 2004;

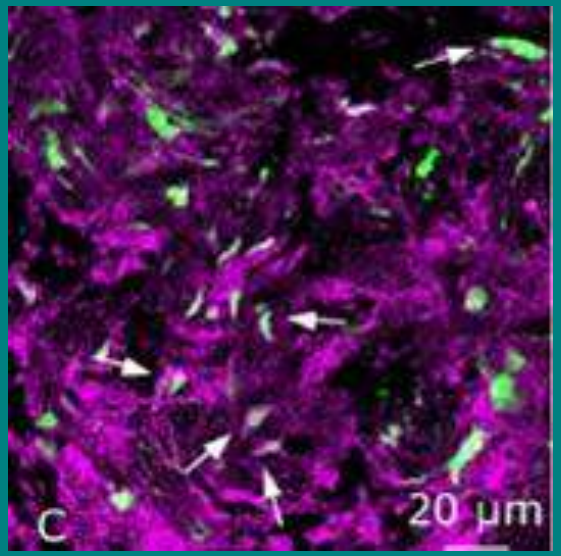
cells from 2-weeks secondary clones



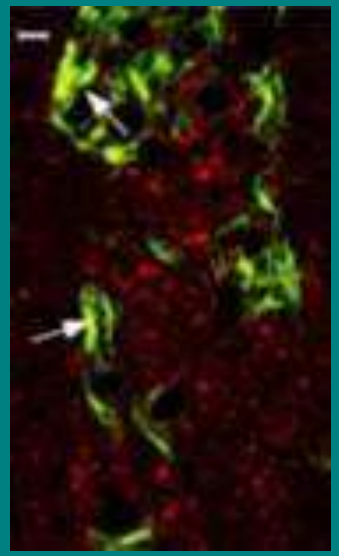
Morphology of EPI-NCSC implants in the lesioned spinal cord



Nestin



GAD67



RIP

Mol Cell Neurosci 32: 67 - 81, 2006

US008030072B2

(12) **United States Patent**
Sieber-Blum et al.

(10) **Patent No.: US 8,030,072 B2**
(45) **Date of Patent: Oct. 4, 2011**

(54) **METHOD OF ISOLATING EPIDERMAL NEURAL CREST STEM CELLS**

(75) Inventors: **Maya Sieber-Blum**, Brookfield, WI (US); **Milos Grim**, Prague (CZ)

(73) Assignees: **Newcastle University**, New Castle Upon Tyne (GB); **Univerzita Karlova V Praze**, Prague (CZ)

Claudinot, S., et al., "Long-term renewal of hair follicles from clonogenic multipotent stem cells," PNAS 102:14677-14682 (2005).
Fernandes, K.J., et al., "A dermal niche for multipotent adult skin-derived precursor cells," Nature Cell Biology 6:11:1082-1093 (2004).
Ito, M., et al., "Stem cells in the hair follicle bulge contribute to wound repair but not to homeostasis of the epidermis," Nature Medicine 11:12:1351-1354 (2005).
Kruger, G.M., et al., "Neural Crest Stem Cells Persist in the Adult Gut but Undergo Changes in Self-Renewal, Neuronal Subtype

Primary Examiner — Thaian N Ton
(74) *Attorney, Agent, or Firm* — Quarles & Brady LLP; Sara D. Vinarov

(57) **ABSTRACT**

The present invention describes novel methods for isolating a substantially pure cell population of non-embryonic epidermal neural crest stem cells from the bulge-region of mammalian hair follicles. Also disclosed is the substantially pure cell population of follicular bulge-derived neural crest stem cells for medical research and therapeutic use.

The use of NCSCs in regenerative medicine 2009 – 2014

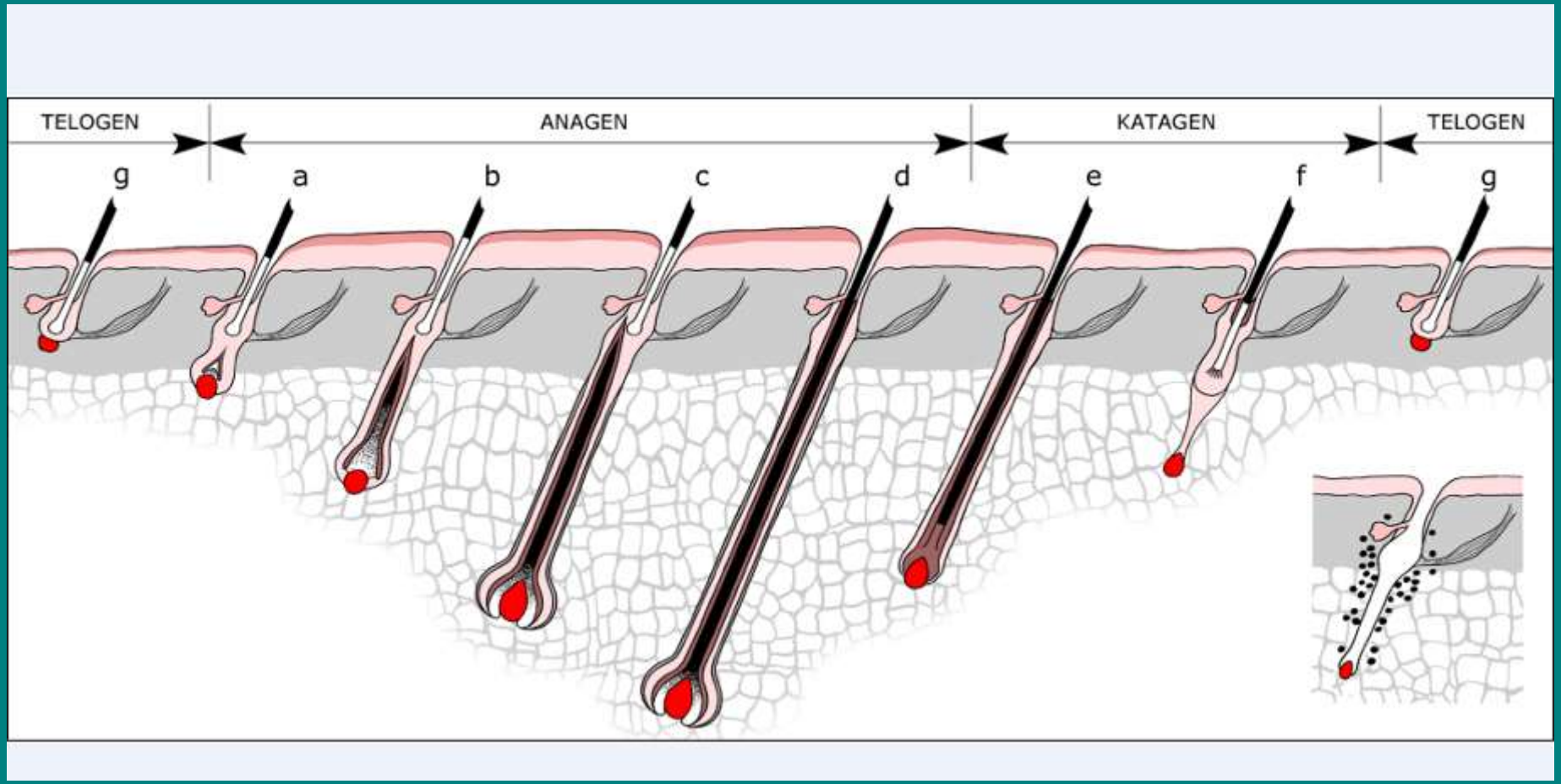
Epidermal NCSCs are capable of differentiating into cells of mesodermal and ectodermal cell line.

Epidermal NCSCs express neurotrophins, angiogenic factors and metalloproteinases.

Their transplantation in damaged mouse spinal cord show improvement in sensory function ... but there is no use of NCSCs in clinical medicine up to now...

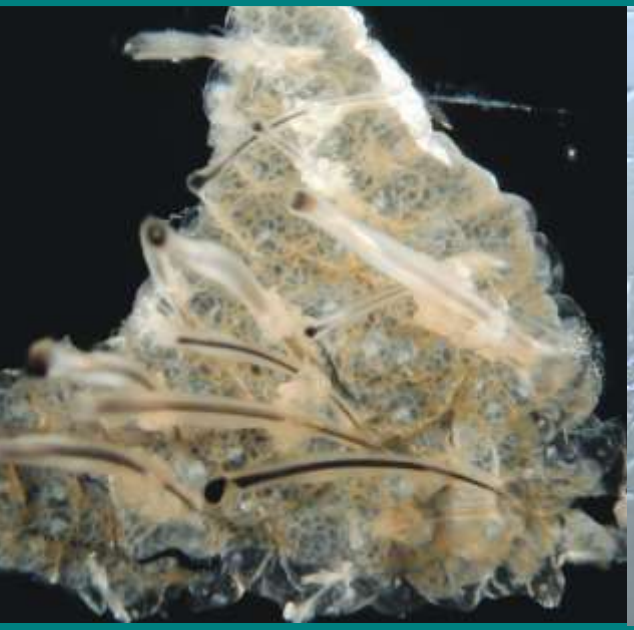
Neural crest stem cells in human hair follicles

Growth cycle of mammalian hair



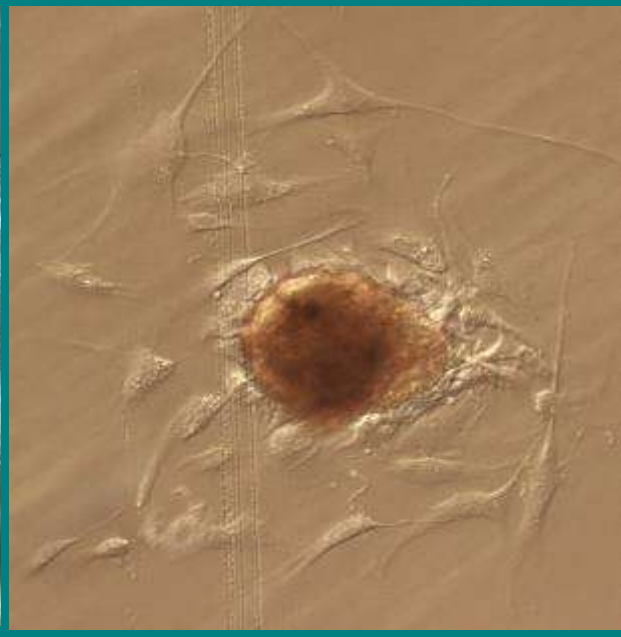
Isolation of human epidermal neural crest stem cells (hEPI- NCSCs) from hair follicles

Tissue source: skin biopsy from different body locations



Epidermis with follicles after dispase treatment

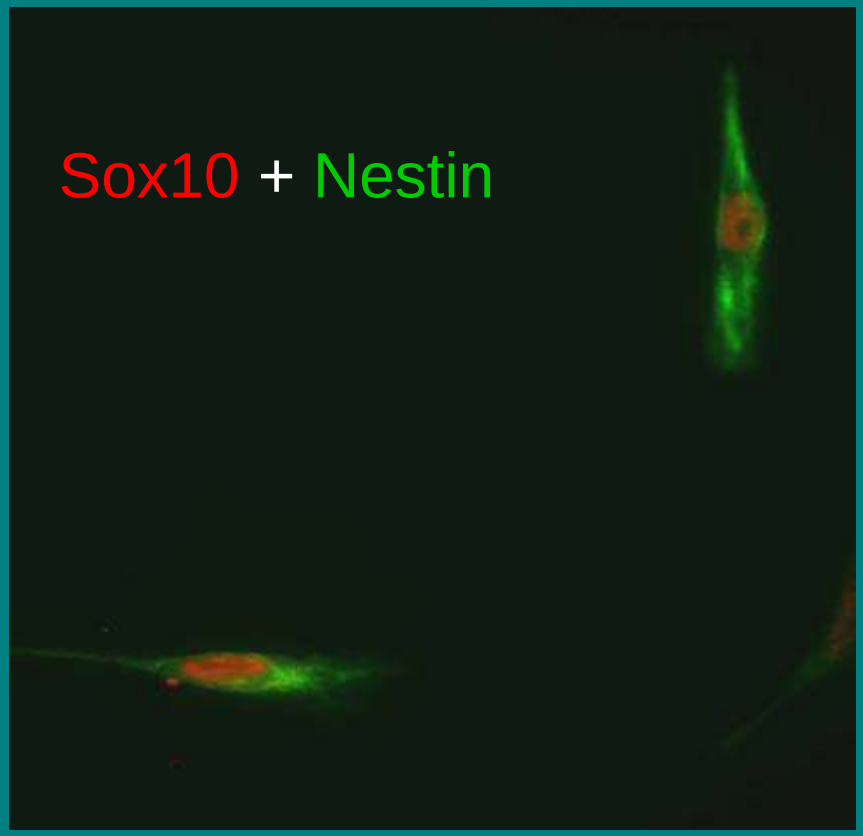
Primary culture cells after emigration from isolated follicle



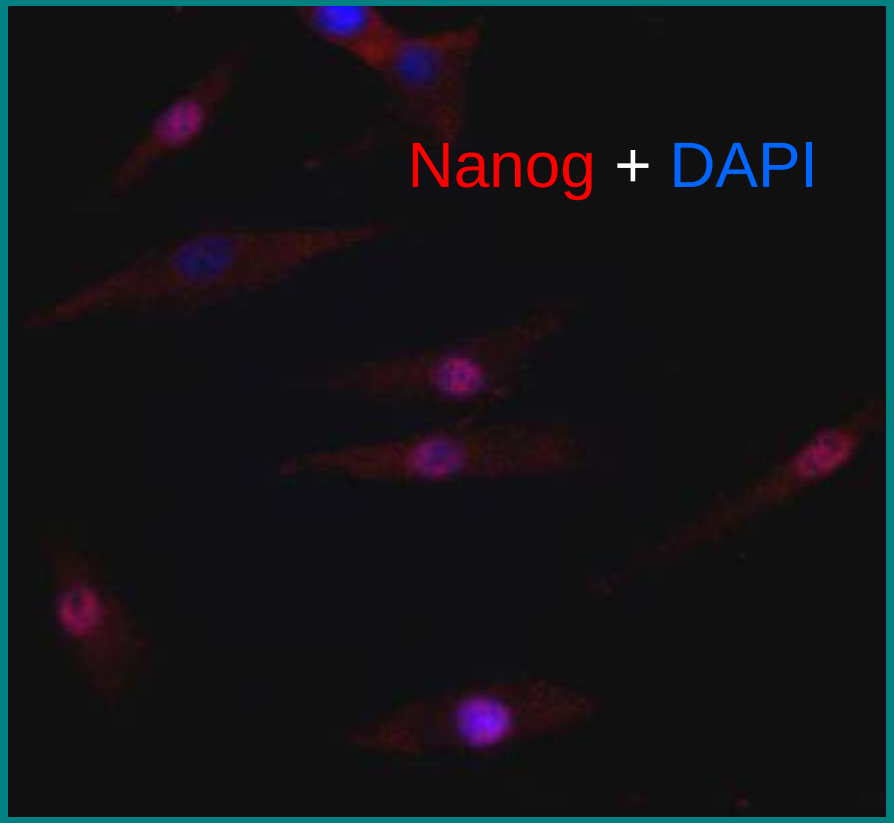
Sphere after readhesion

Folia Biologica (Praha) 56, (4): 149 – 157, 2010

Coexpression of Sox10 and Nestin,



Expression of Nanog

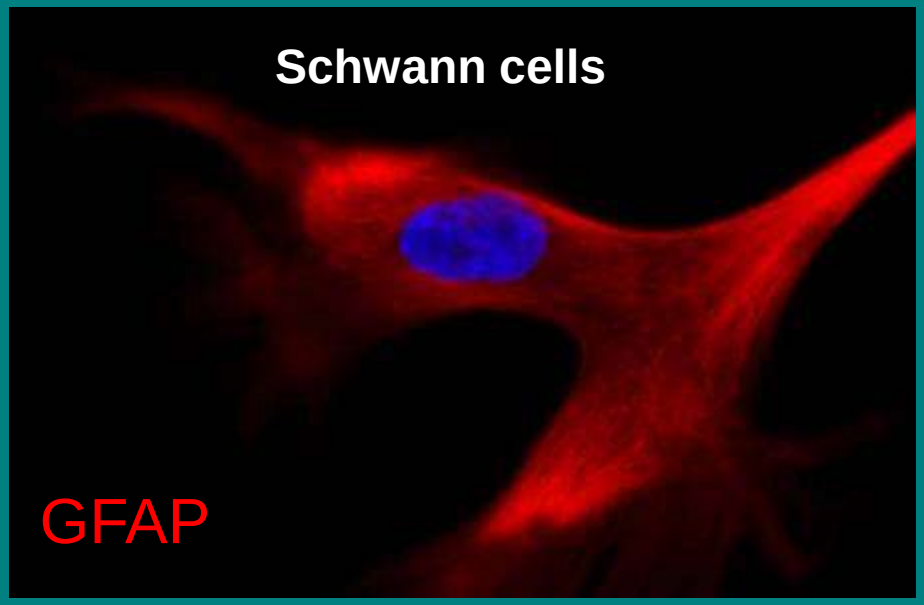


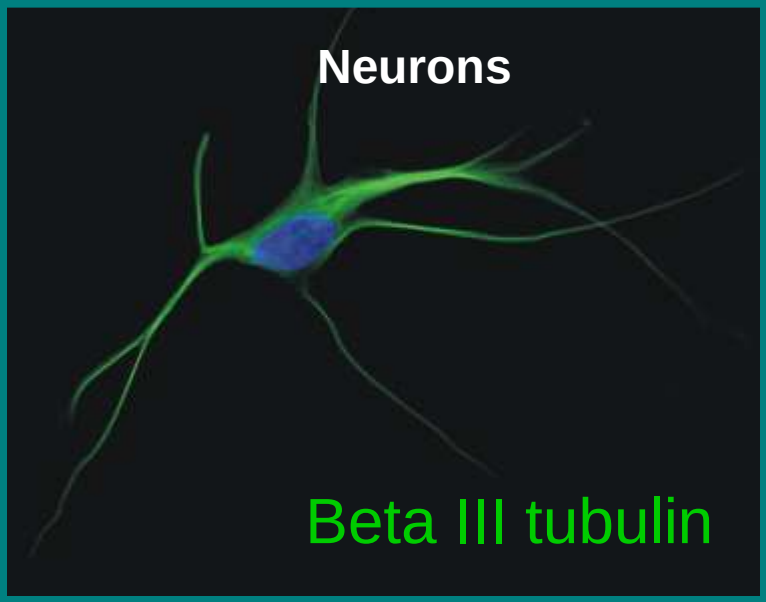
hEPI-NCSCs in tissue culture after emigration from back skin follicles

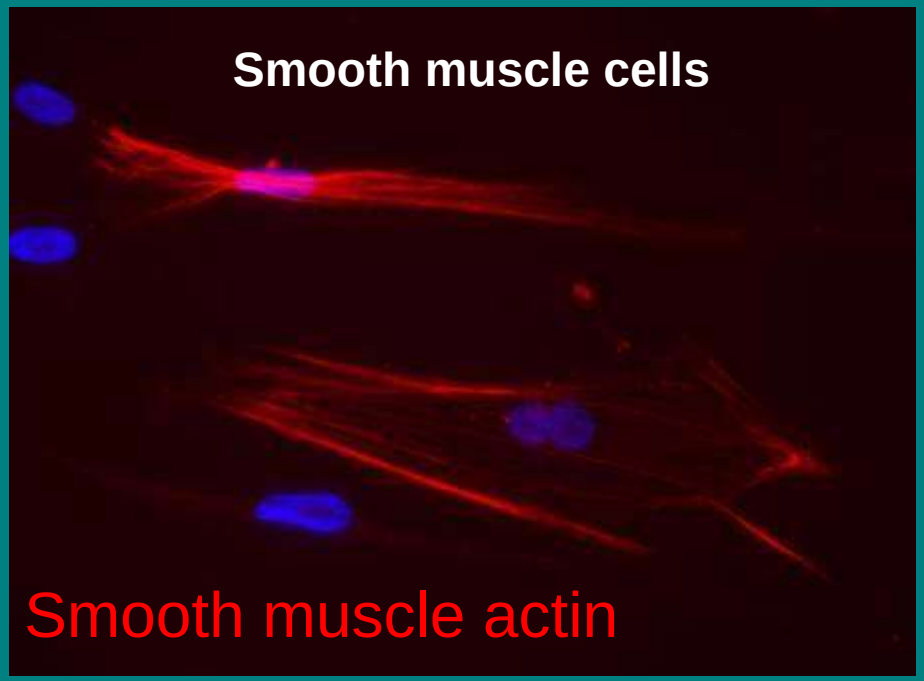
Folia Biologica (Praha) 56, (4): 149 – 157, 2010

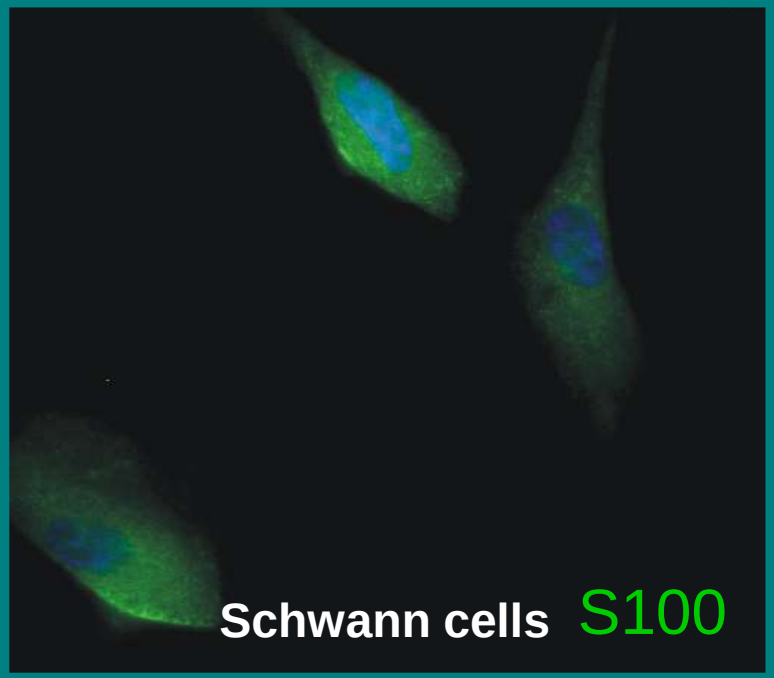
Differentiation of human epidermal neural crest stem cells fom hair follicles into neural crest progeny

Folia Biol. (Praha) 56, 149-157, 2010









Isolation and Characterization of Neural Crest Stem Cells from Adult Human Hair Follicles

(neural crest / stem cells / human hair follicle / expression profile)

E. KREJČÍ, M. GRIM

Folia Biologica (Praha) 56, 149-157 (2010)

Charles University in Prague, First Faculty of Medicine, Institute of Anatomy, Laboratory for Molecular Embryology, Prague, Czech Republic

Abstract. Neural crest (NC) is a transient embryonic tissue, whose cells are motile and multipotent until they reach their destination and differentiate according to microenvironmental cues into a variety of cell types. However, a subpopulation of these cells remains multipotent. They were found, among other locations, in a bulge of adult murine whisker follicle and were designated epidermal neural crest stem cells (EPI-NCSCs). The aim of this work is to ascertain whether the EPI-NCSCs could be isolated from human hair follicles as well. Due to their exceptional properties, they could represent potential candidates for stem cell therapy. The presented work focuses on the isolation and characterization of EPI-NCSCs from human skin. We obtained a population of cells that expressed markers of NC, NC progeny and general stem cell markers. After prolonged cultivation, the subpopulation of cells spontaneously differentiated into some of NC derivatives, i.e. neurons, smooth muscle cells and Schwann cell progenitors. Targeted differentiation with neuregulin 1 highly increased the number of Schwann cells in the culture. Human EPI-NCSCs could also grow under non-adherent conditions and form 3-dimensional spheres. Microarray analysis was performed and gene profile of human EPI-NCSCs was compared with the list of key genes of murine EPI-NCSCs and the list of genes up-regulated in newly induced NC cells. This revealed 94% and 88% similarity, respectively. All presented results strongly support the NCSC identity and multipotency of isolated human cells. These cells could thus be used in regenerative medicine, especially because of the easy accessibility of donor tissue.

Introduction

Tissue-specific adult stem cells have long attracted attention, especially since they could be isolated from one individual, expanded and eventually differentiated *in vitro* and transplanted back into the same individual. Accessibility of donor tissue is of considerable importance in such case. Compared to embryonic stem cells, there is no need to use immunosupressive medication since they are the patient's own cells and there will therefore be no graft rejection and no problems of ethical nature. Compared to induced pluripotent stem cells (Takahashi and Yamanaka, 2006) or nuclear transfer, there is no need for genetic or mechanic manipulation.

Neural crest (NC) is a transient embryonic tissue that arises at the border between neural and non-neural ectoderm in early stages of development. Following neural tube closure, NC cells (NCCs) undergo epithelial-mesenchymal transition and migrate along defined pathways to populate various regions of the embryo (reviewed in Le Douarin and Kalcheim, 1999). NCCs contribute to a diverse array of cell types, including multiple skeletal

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Krejčí E, Grim M: Isolation and Characterization of Neural Crest Stem Cells from Adult Human Hair Follicles. Folia Biologica (Praha) 56, (4): 149 – 157, 2010

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Institute of Anatomy, First Faculty of Medicine,
Charles University Prague, Czech Republic