Neural Crest Stem Cell Specification - Roles of Neurotrophin-3 and Stem Cell Factor

Maya Sieber-Blum
Dept. of Cell Biology, Neurobiology and Anatomy
Medical College of Wisconsin

Overview

Neurotrophin-3

- NT-3 is essential for the maintenance of cardiac neural crest stem cells

Stem cell factor

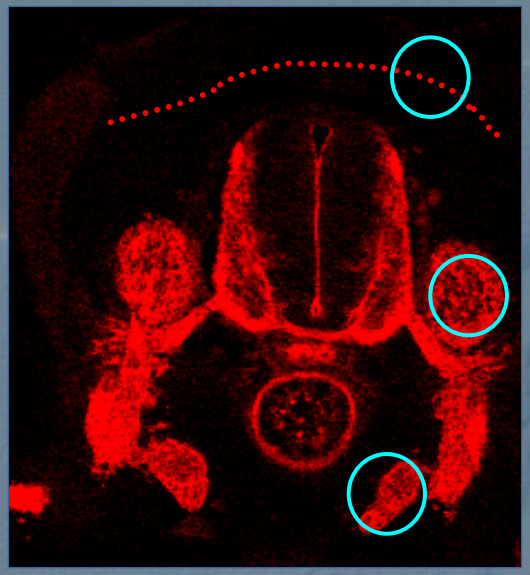
- SCF is essential for the differentiation of a subset of small diameter and medium diameter sensory neurons

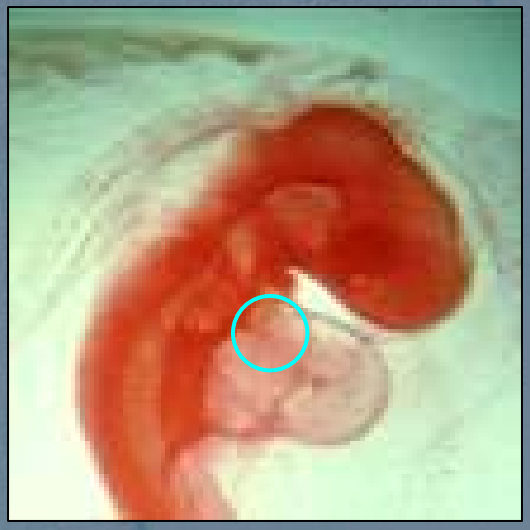
Adult neural crest stem cells

The migrating neural crest consists of stem cells, fate-restricted cells and lineage committed cells.

Neural crest stem cells are present in target tissues:

- Ectoderm
- Sympathetic ganglion
- Dorsal root ganglion
- Cardiac outflow tract



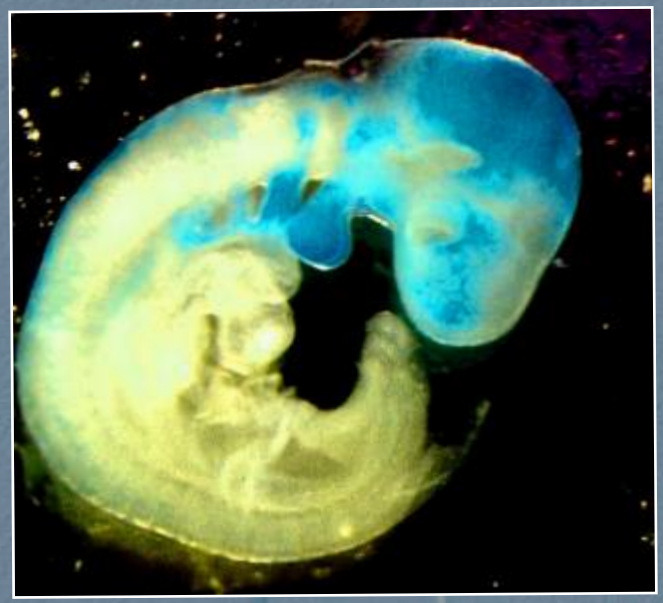


Duff et al., 1991
Richardson and Sieber-Blum, 1993
Ito and Sieber-Blum, 1993

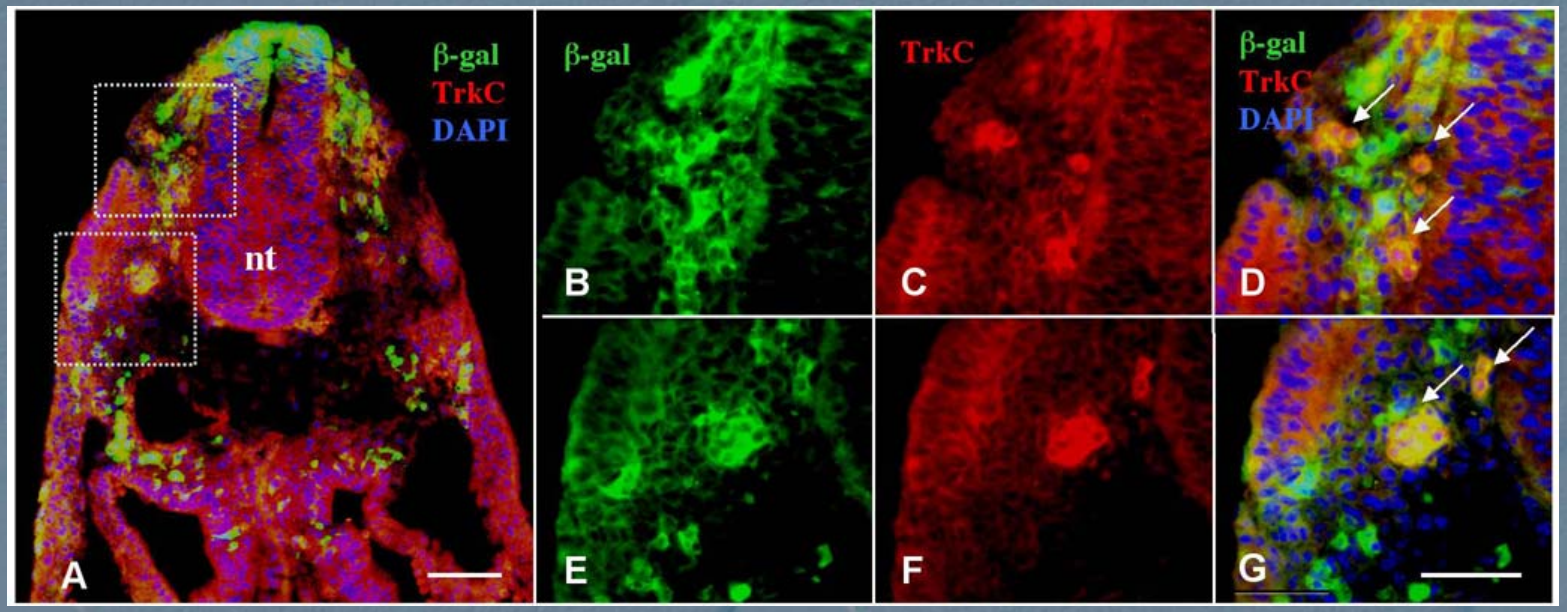
NT-3, which binds TrkC, is essential for the maintenance of cardiac neural crest cells

and for the organization of the endothelial tube of the outflow tract

‘Vagal’ neural crest cells populate the pre-umbilical gut, the lower jaw and the cardiac outflow tract

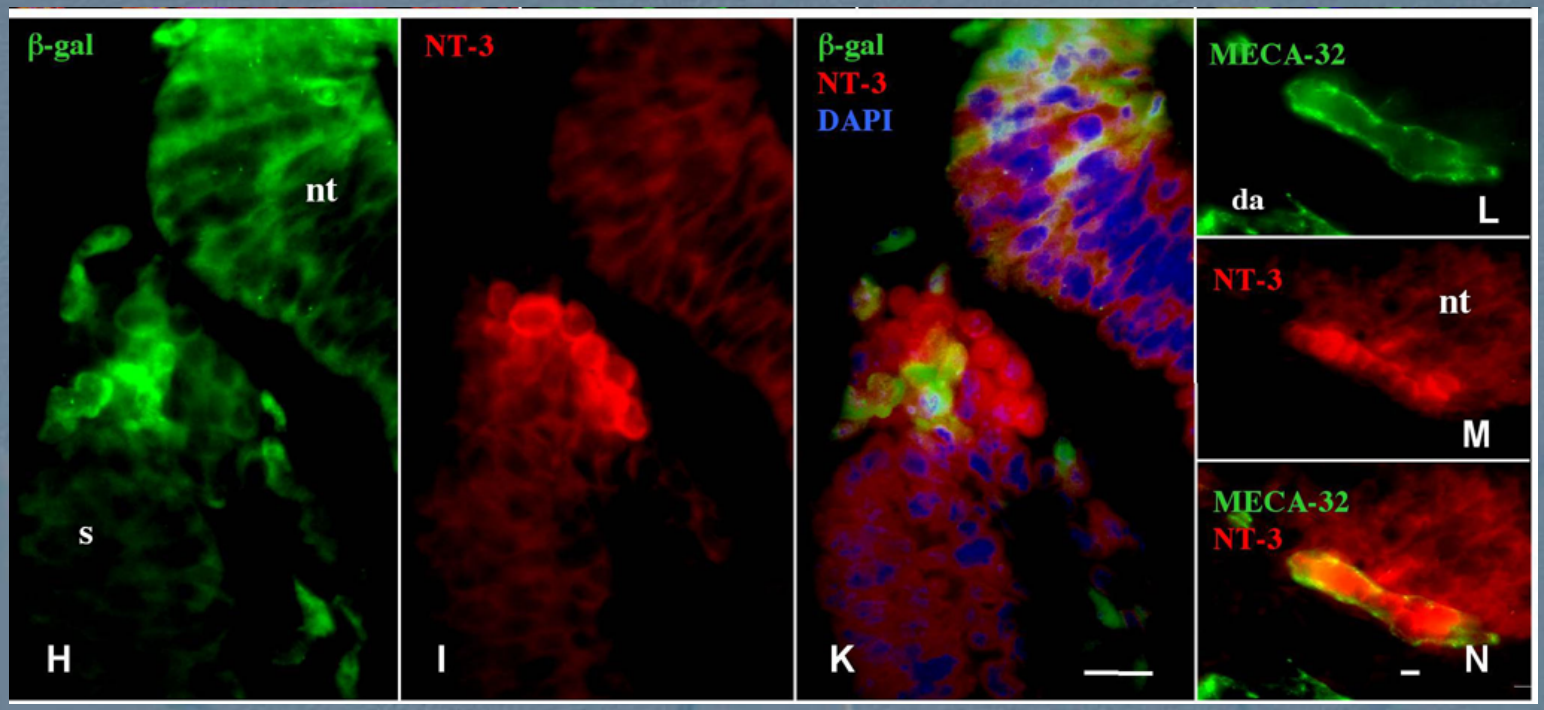


A subset of murine cardiac neural crest cells expresses TrkC



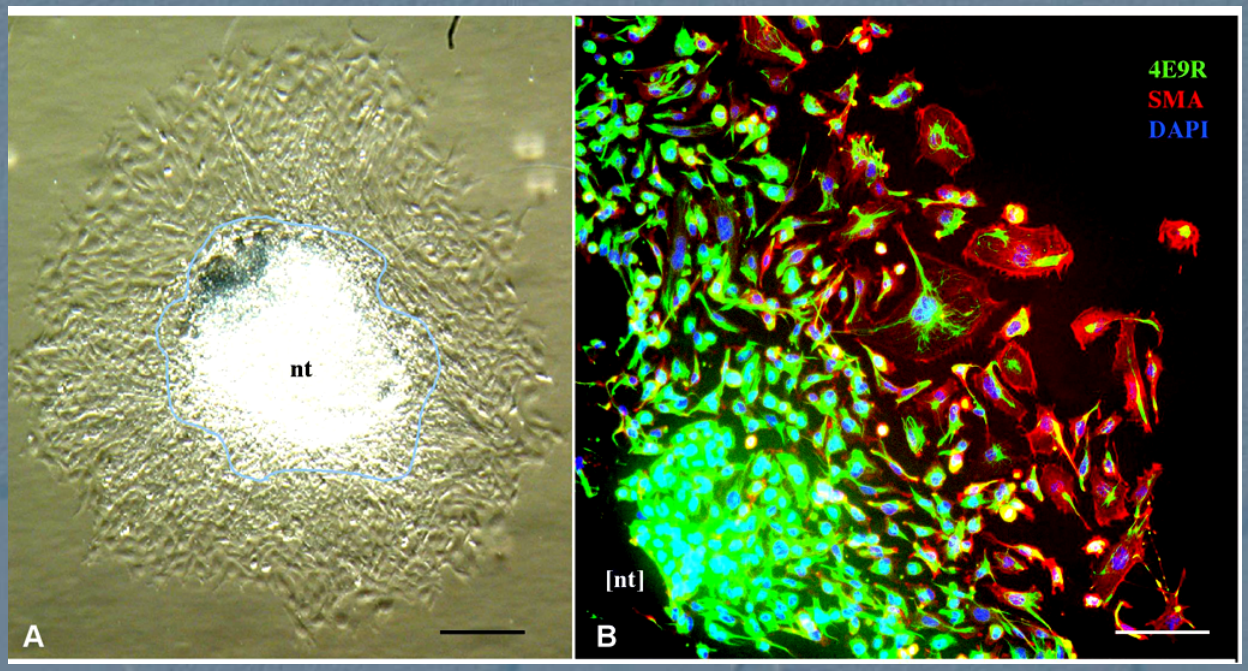
Wnt-lacZ mouse

Neighboring endothelial cells supply NT-3 to migrating neural crest cells

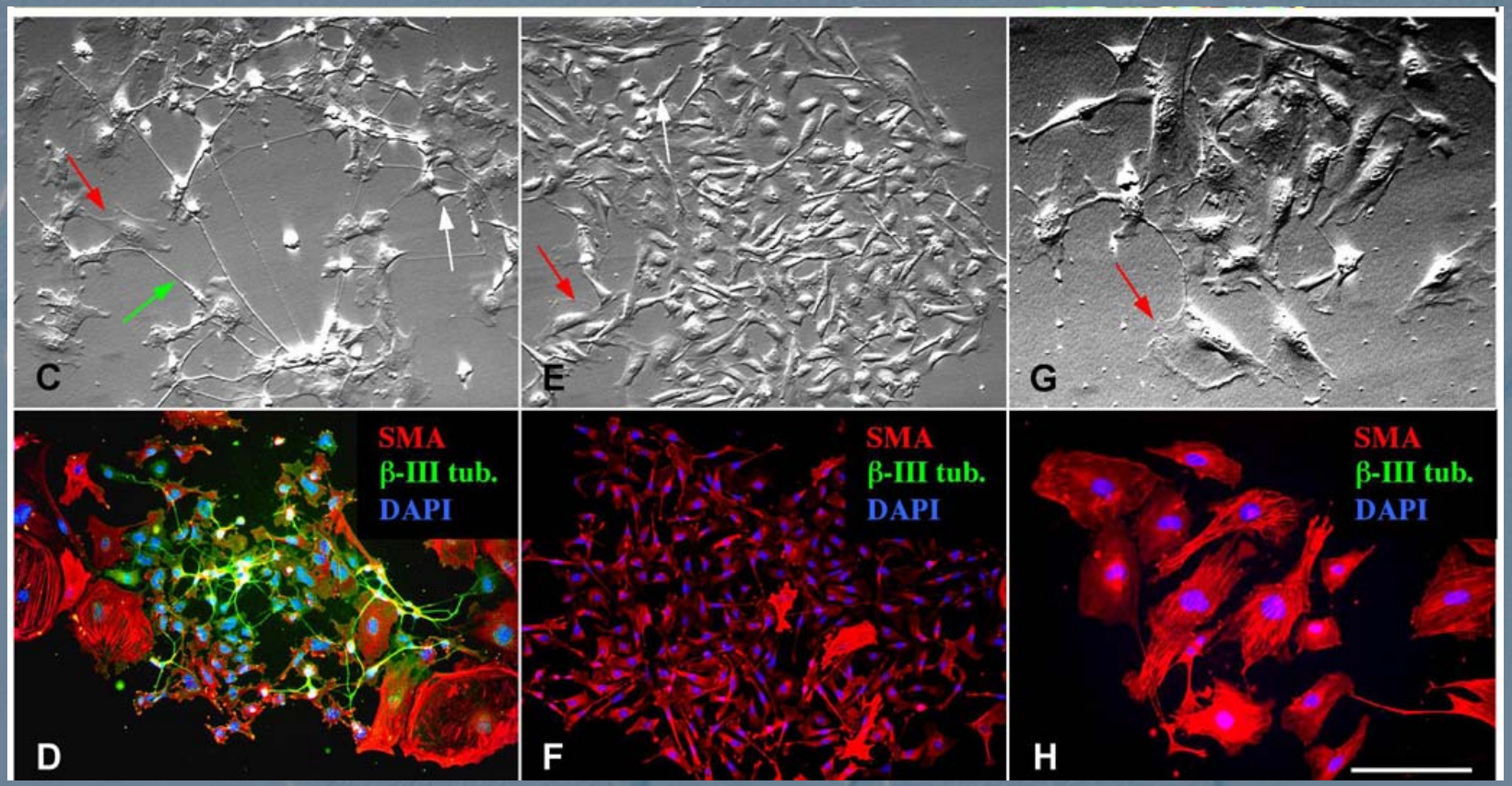


Wnt-lacZ mouse

Rate of migration and proliferation of wild type and TrkC null cardiac neural crest cells are indistinguishable



In colony assays we can distinguish 3 types of colony based on their morphology and content of smooth muscle cells and neurons



pluripotent

fate-restricted

Committed to the smooth muscle cell lineage

Cell types in cardiac neural crest colonies

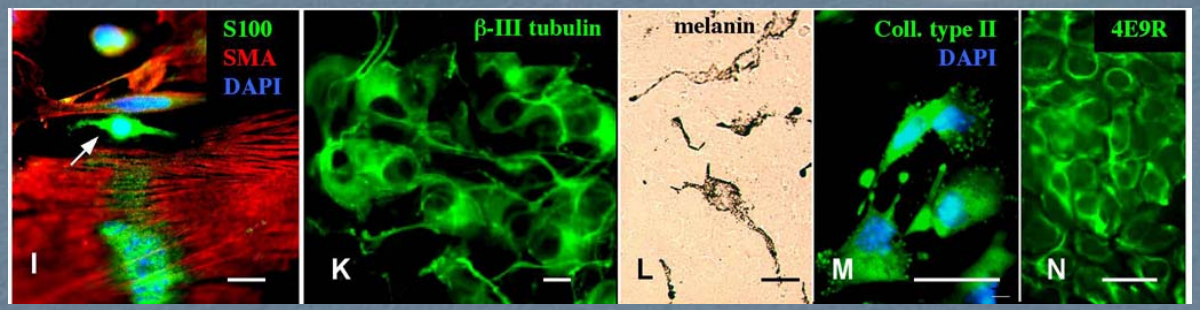


Table 1: **Phenotypes in the 3 Types of Colony**

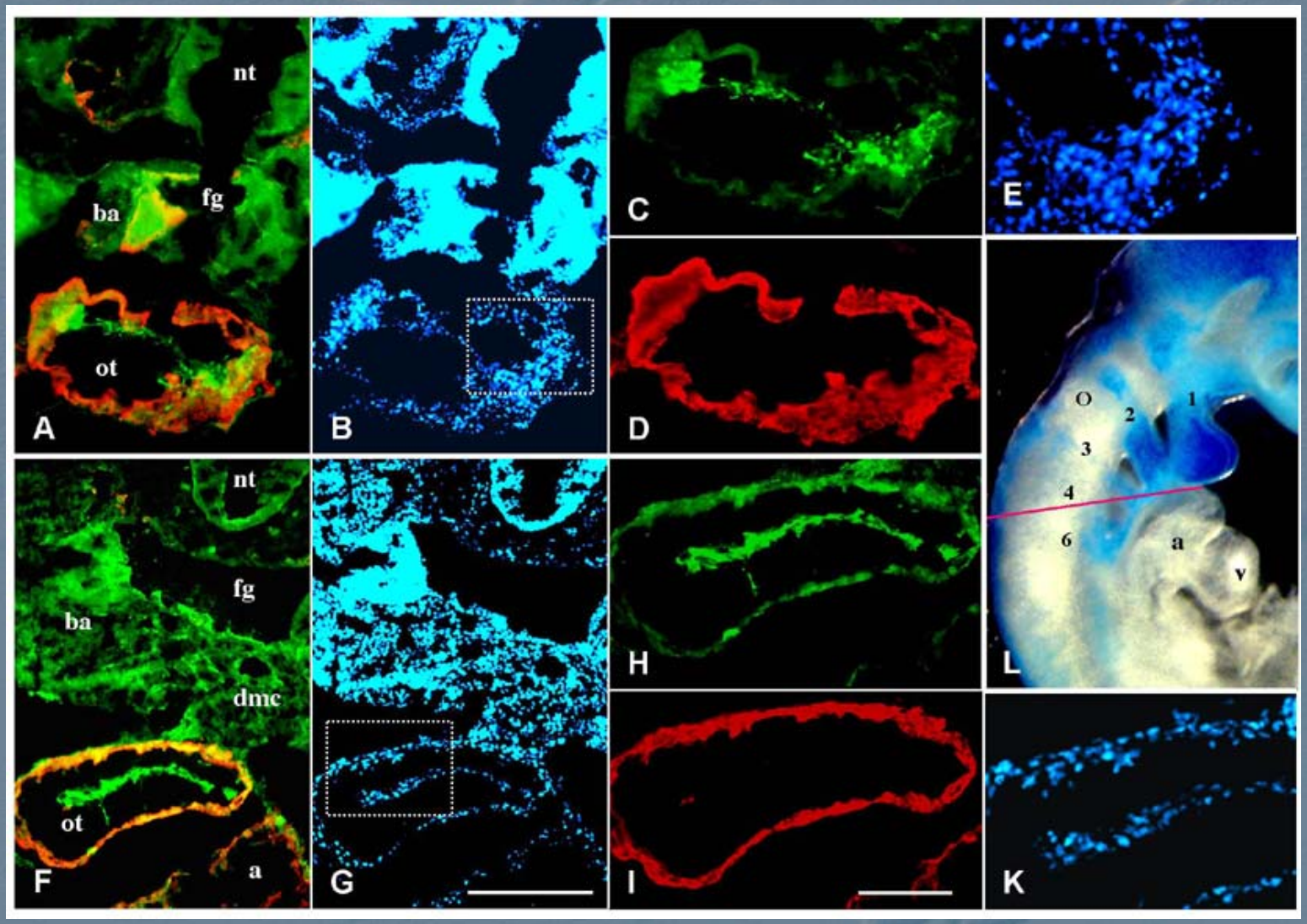
Colony Type	Cell Types Present						
	Smooth muscle cells (mature)	Smooth muscle cells (immature)	4E9R$^+$ progenitor cells	Schwann cells	Chondrocytes	Neurons	Pigment cells
CNC-SC	+	+	+	+	+	+	+
CNC-RC	+	+	+	(+)	(+)	0	0
CNC-smC	+	0	0	0	0	0	0

Pluripotent cardiac neural crest stem cells convert into fate-restricted progenitors prematurely

Progenitor Cell Composition

Genotype	Percent of total number colonies per plate ± S.E.M		
	CNC-SC	**CNC-RC**	**CNC-smC**
Wild type	**15.0±2.5**	**7.8±2.5**	**77.4±3.0**
TrkC null	**6.6±1.5**	**18.1±2.6**	**75.3±3.1**
	p=0.007	p=0.02	p=0.67

The endothelial tube of the cardiac outflow tract is disorganized in the Trkc null mouse



Youn Y.H. et al., 2003

Conclusion

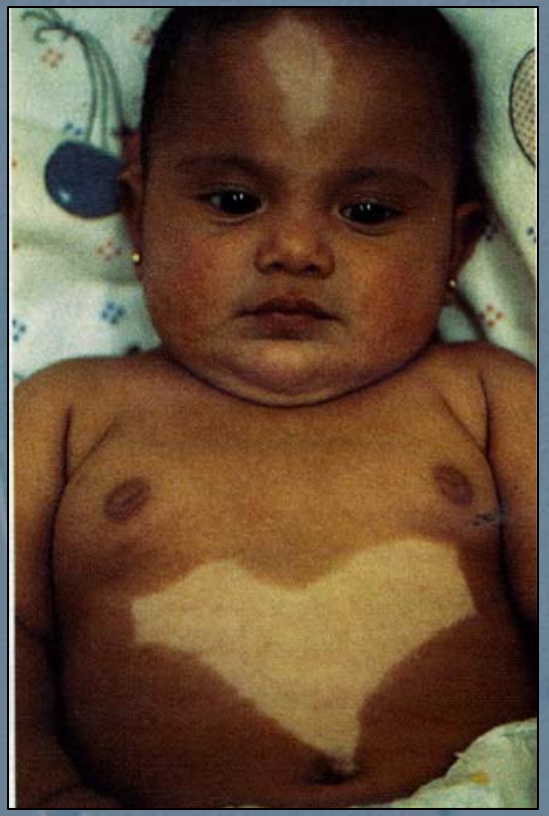
Role of NT-3 in the development of the cardiac outflow tract

1. NT-3 signaling through TrkC maintains the neural crest stem cell pool. Lack of NT-3 signaling leads to precocious fate-restriction.

2. NT-3 signaling through TrkC is important for the proper development of the endothelial tube of the cardiac outflow tract.

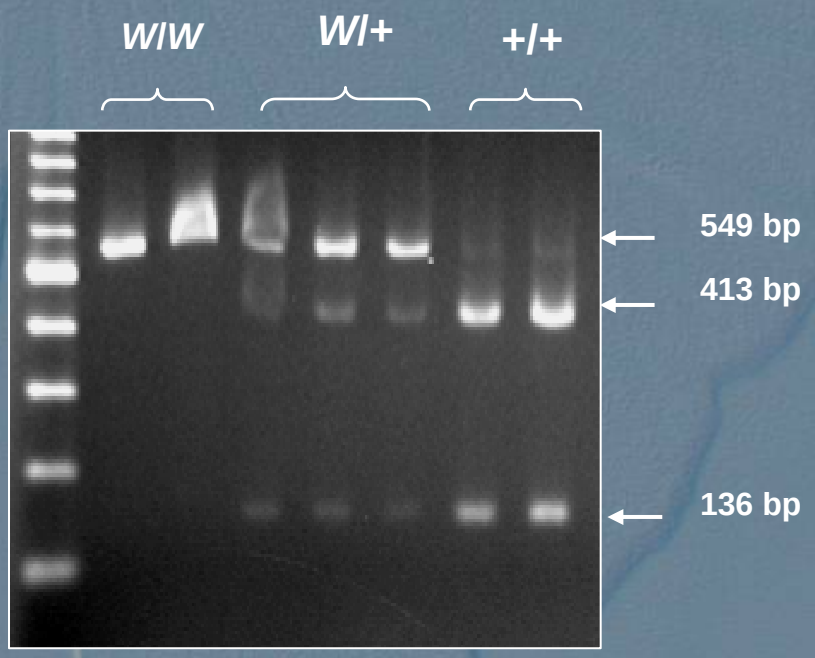
Role of stem cell factor (SCF) in sensory neuron development

Mutations in the receptor for SCF, c-kit, leads to piebaldism in mice and men

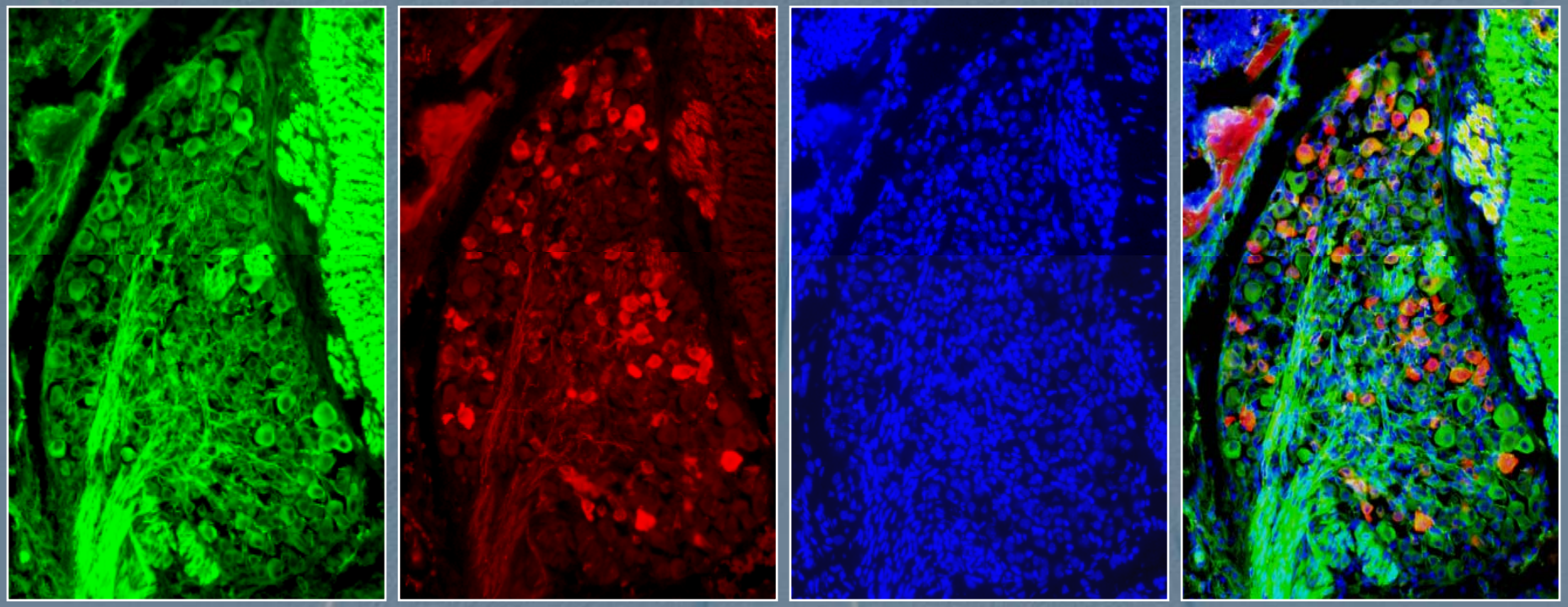


Taking advantage of a restriction site that is affected by the point mutation in the *W* mouse, we have developed a PCR-based genotyping protocol

W/W	*W/+*	*+/+*
549 bp	549 bp	
	413 bp	413 bp
	136 bp	136 bp



We scored the percentage of SP and CGRP immunoreactive neurons in the L6 dorsal root ganglion



ß-III tubulin CGRP DAPI merged

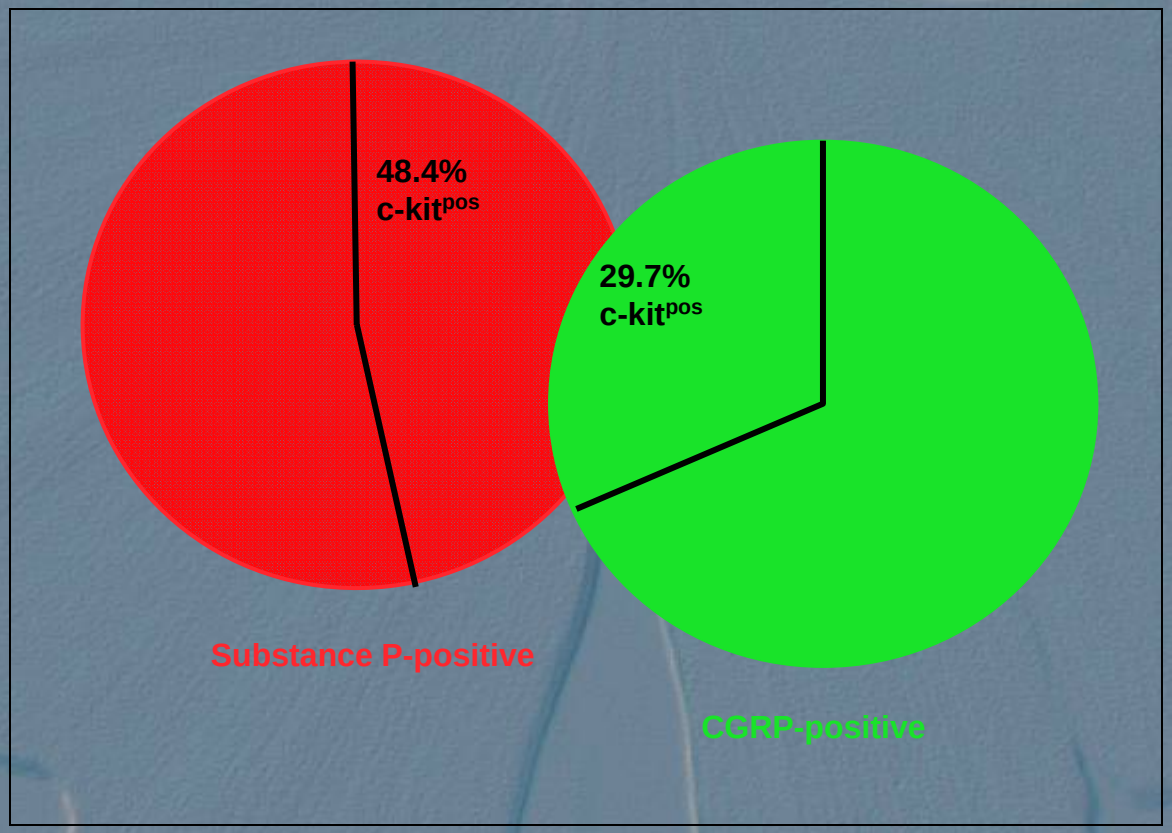
There is a significant decrease in the number of substance P and CGRP positive small and medium diameter sensory neurons

	Number of immunoreactive neurons per 10,000 μm² ± S.E.M.		
	E17.5	E18.5	Newborn
Substance P			
Wild type	1.6±0.1	3.6±0.2	6.1±0.2
W / W	1.0±0.1	3.2±0.1	2.9±1.2
CGRP			
Wild type	6.4±0.3	6.1±0.1	8.6±0.2
W / W	4.3±0.8	4.6±0.9	5.9±0.2

n = 93 - 126 sections from 3 mice per genotype and age group

p = 0.0001
p = 0.0001

Why are not all SP and CGRP immunoreactive cells lost?

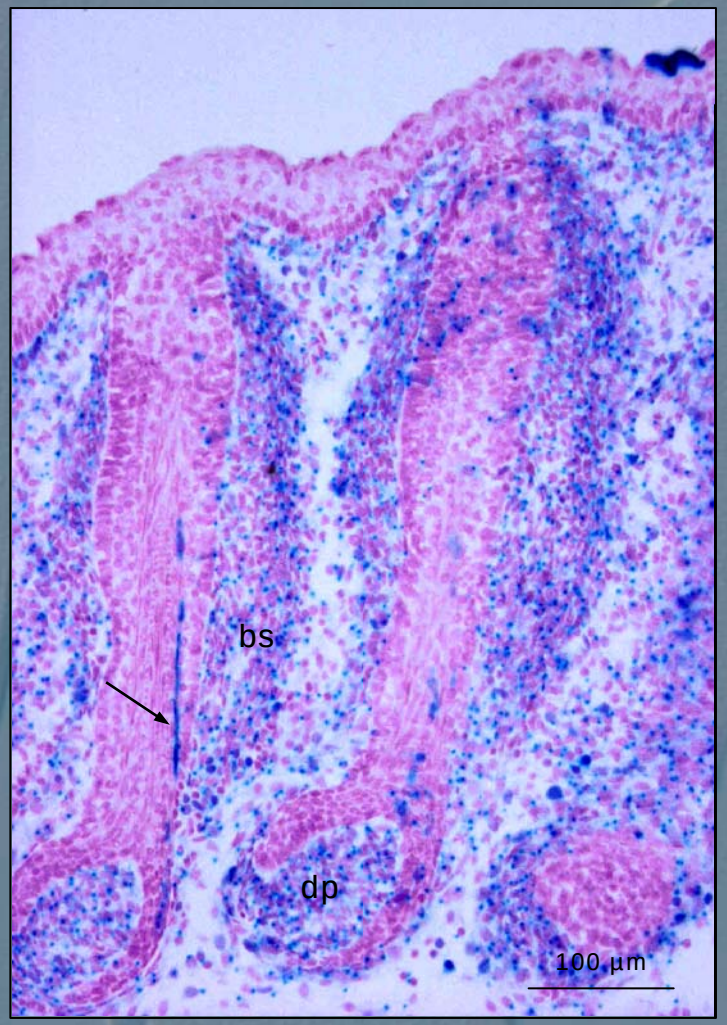


Conclusion

Role of SCF in sensory neuron development

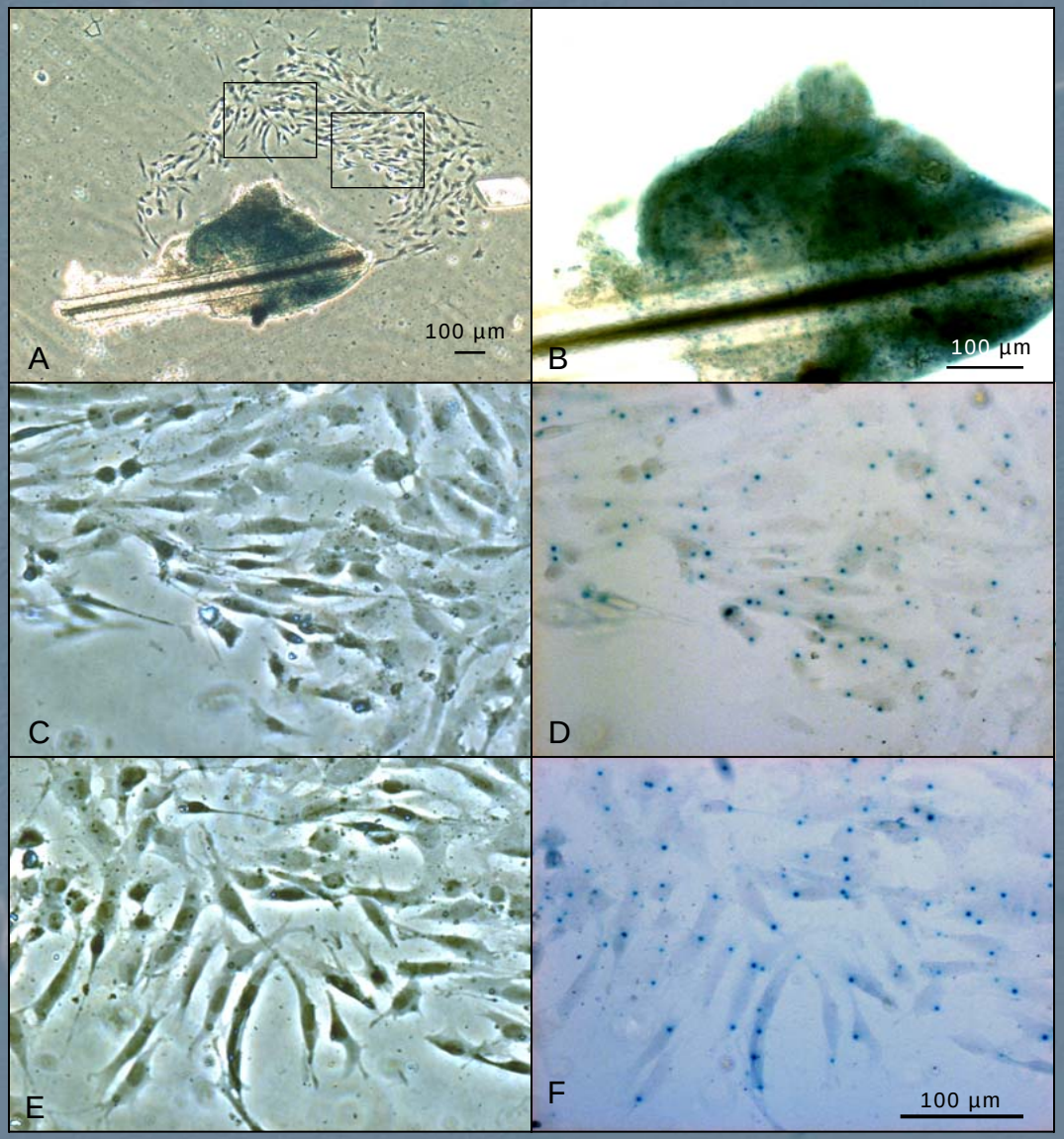
c-kit expressing sensory neurons (pain & visceral afferents) never develop in W/W mice.

Epidermal neural crest stem cells (eNCSC)

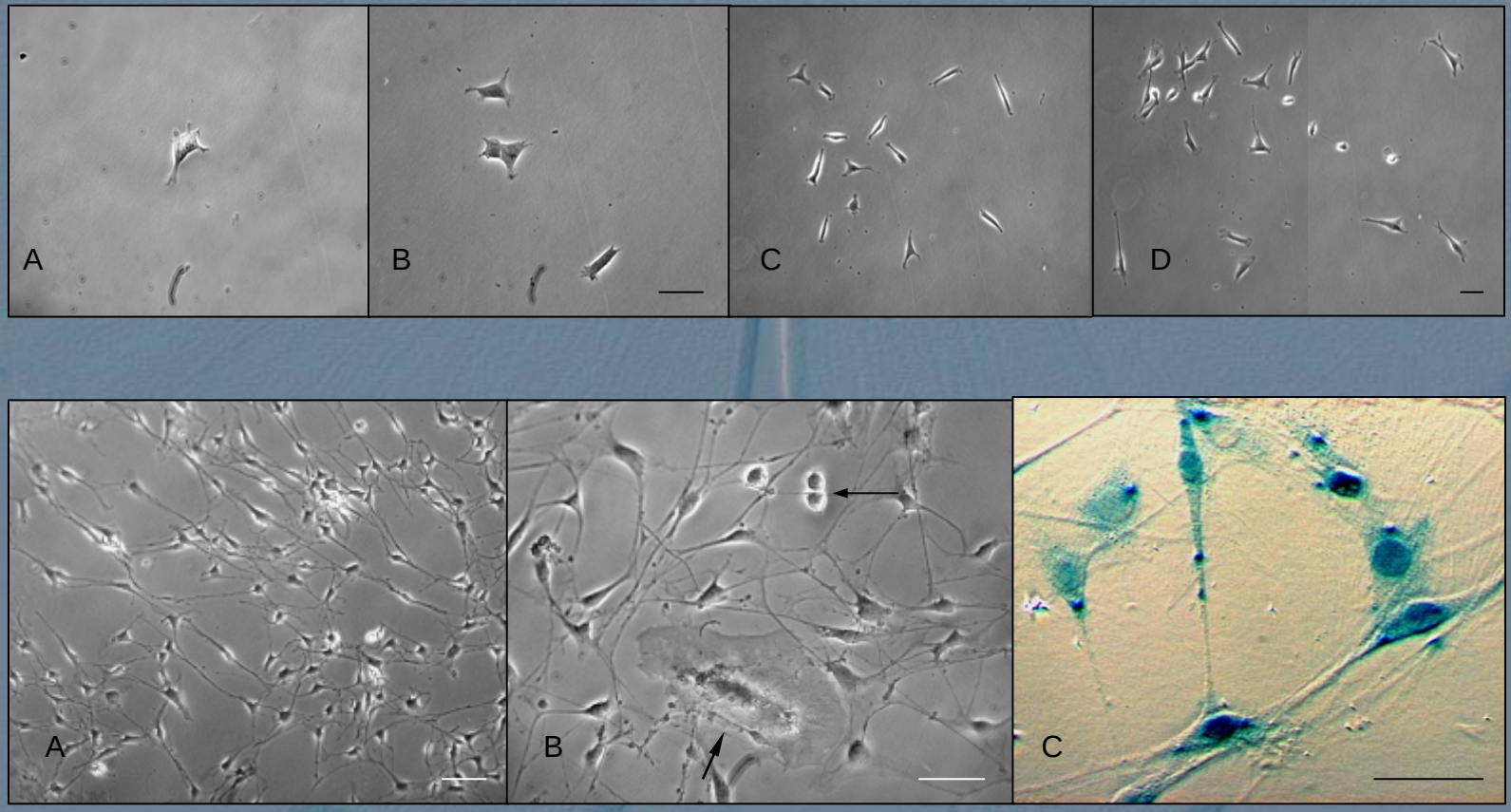


Xgal stain in Wnt1-cre/R26R facial skin

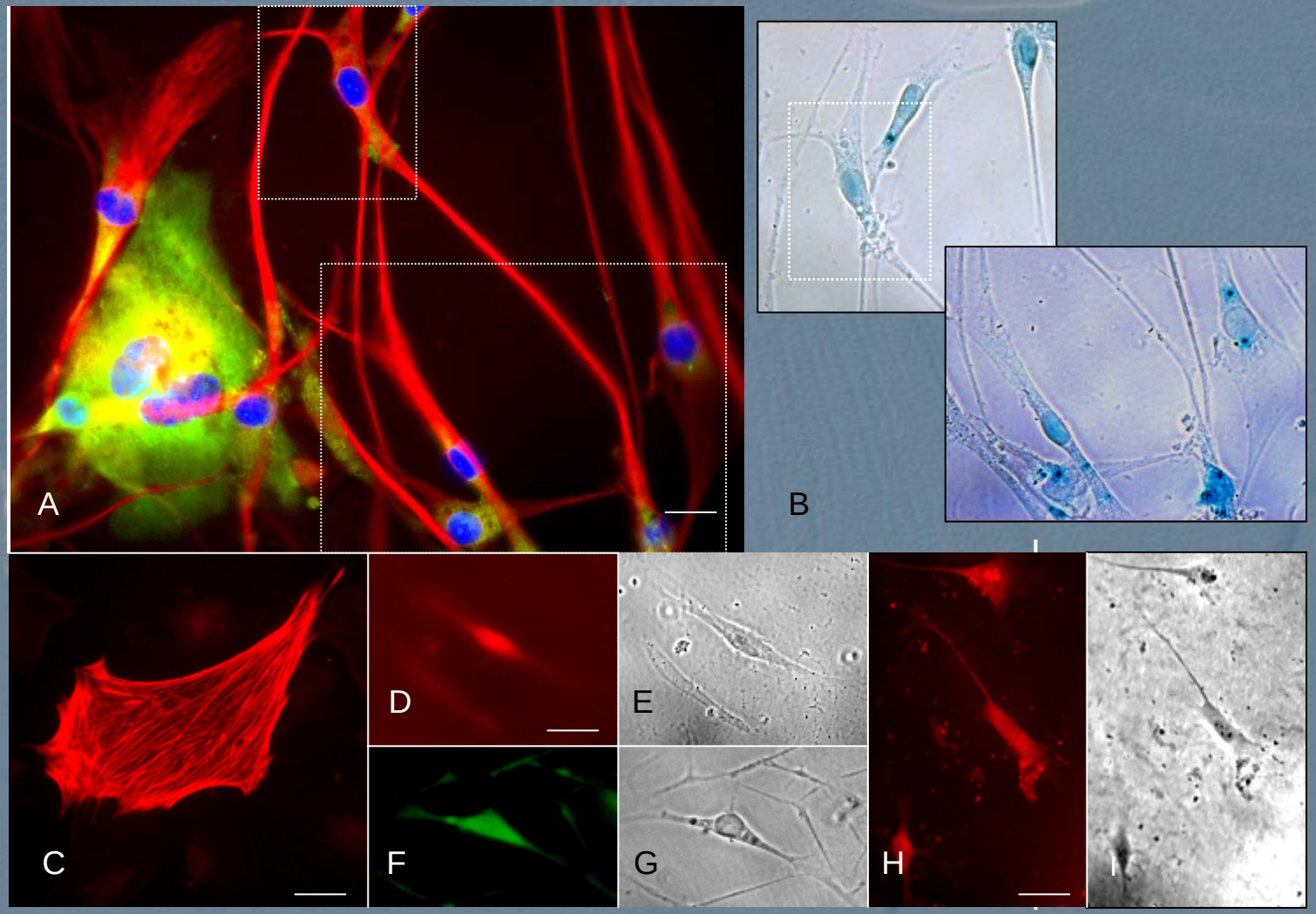
Neural crest-derived cells emigrate from bulge explants



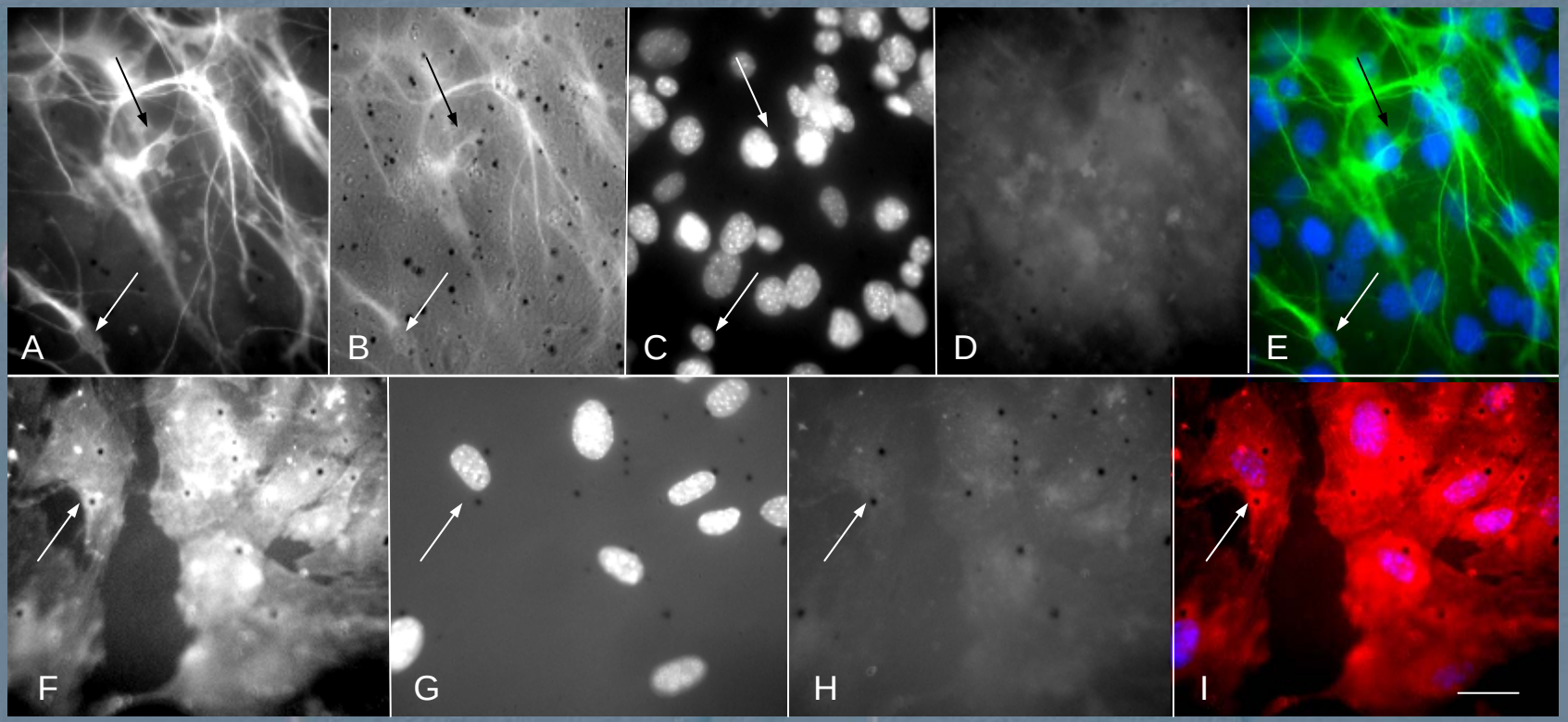
In clonal cultures eNCSC give rise to colonies that contain thousands of cells



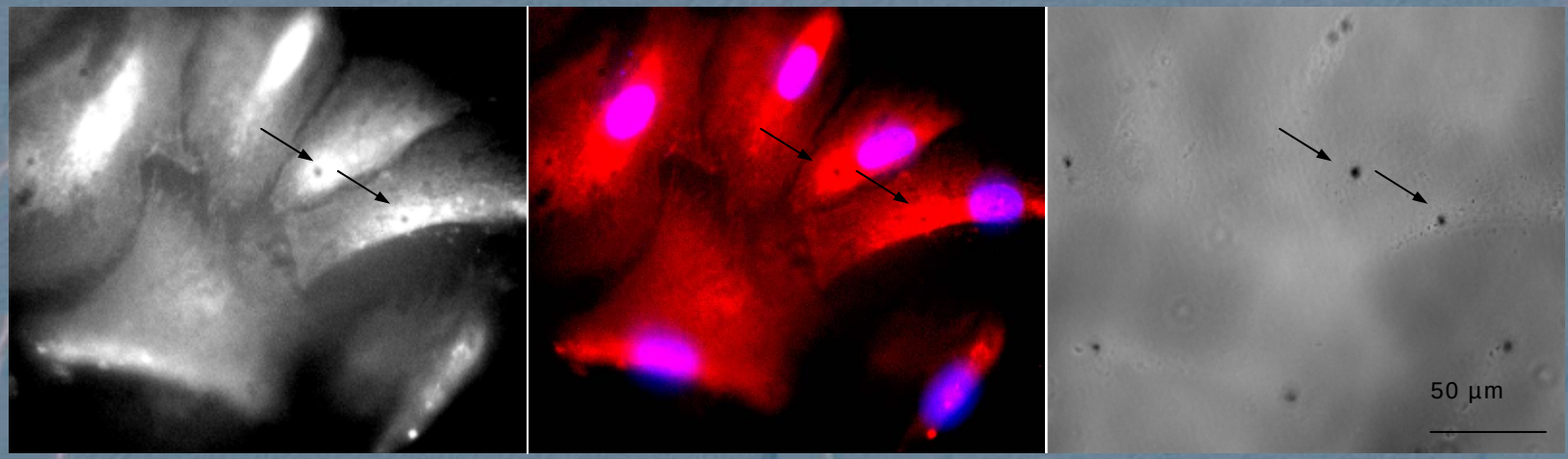
Clones contain neurons, smooth muscle cells, rare Schwann cell progenitors and melanocytes



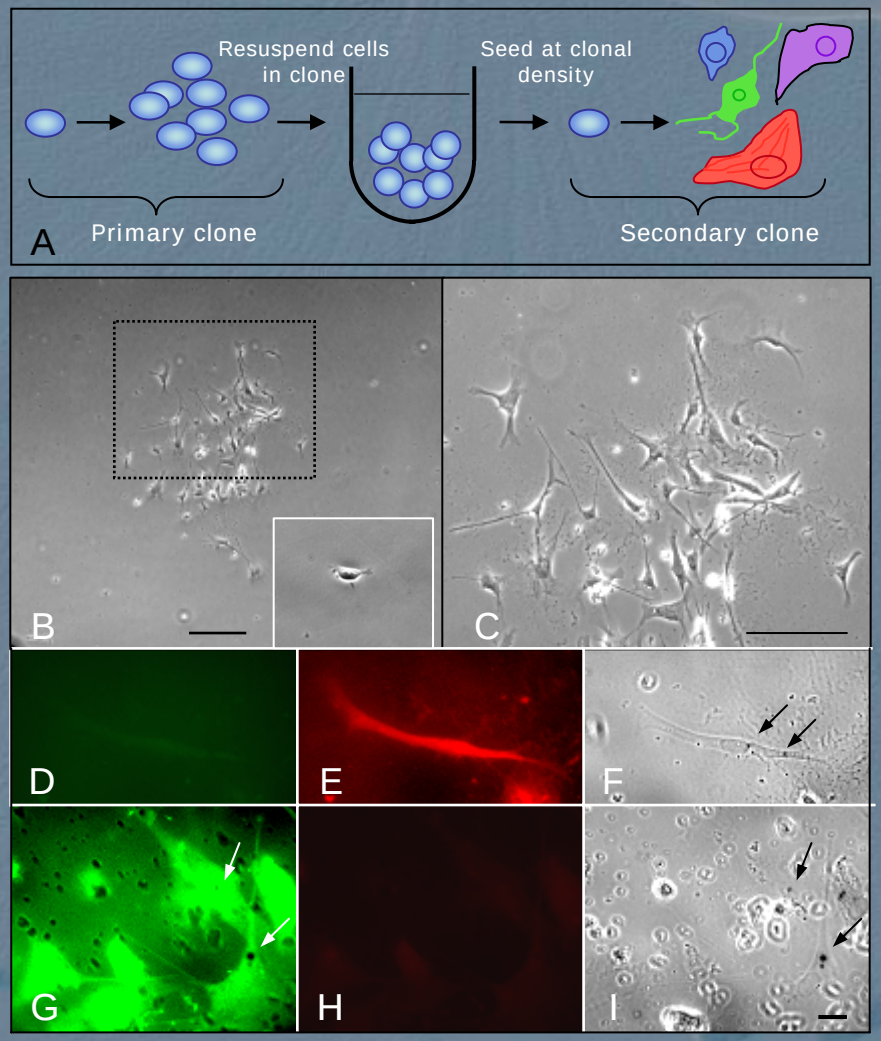
Targeted differentiation into Schwann cell progenitors



Targeted differentiation into chondrocytes



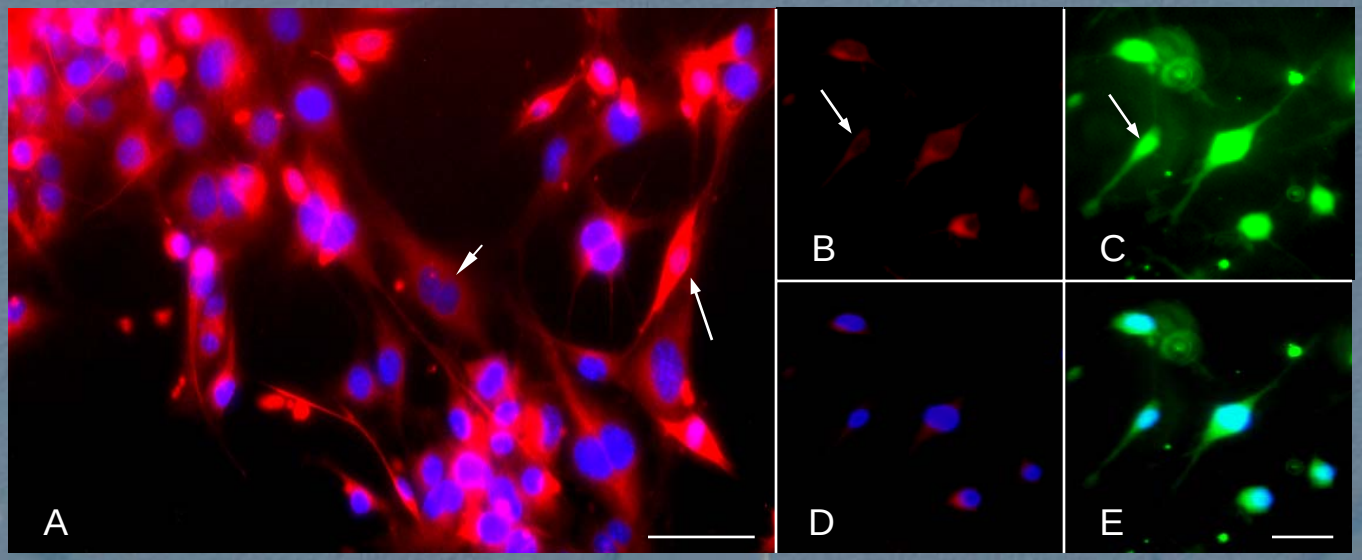
Serial cloning showed that eNCSC can undergo self-renewal



Stem cells are maintained at high levels

Type of clone	Clones formed by stem cells (% of total ± S.E.M.)
Primary clones (from day 4 primary explant)	83.0±2.7
Secondary clone (from day 3 primary clone)	73.5±6.7
Secondary clones (from day 5 primary clones)	66.2±4.4

eNCSC are distinctly different from Schwann cell progenitors of adult sciatic nerve



Conclusions

eNCSC

1. Neural crest cells from adult hair follicles are pluripotent stem cells.

2. Their inherent high degree of plasticity and their accessibility in the skin make them promising candidates for various cell therapy paradigms.

Collaborators

- Yong Ha Youn, Medical College of Wisconsin
- Zhi Jian Zhang, Medical College of Wisconsin
- Milos Grim, Charles University Prague
- Yao Fei Hu, Medical College of Wisconsin
- Viktor Szeder, Medical College of Wisconsin and Charles University Prague

Acknowledgements

- Henry Sucov
- Philippe Soriano
- Andrew McMahon
- Joshua Sanes
- Greg Lemke