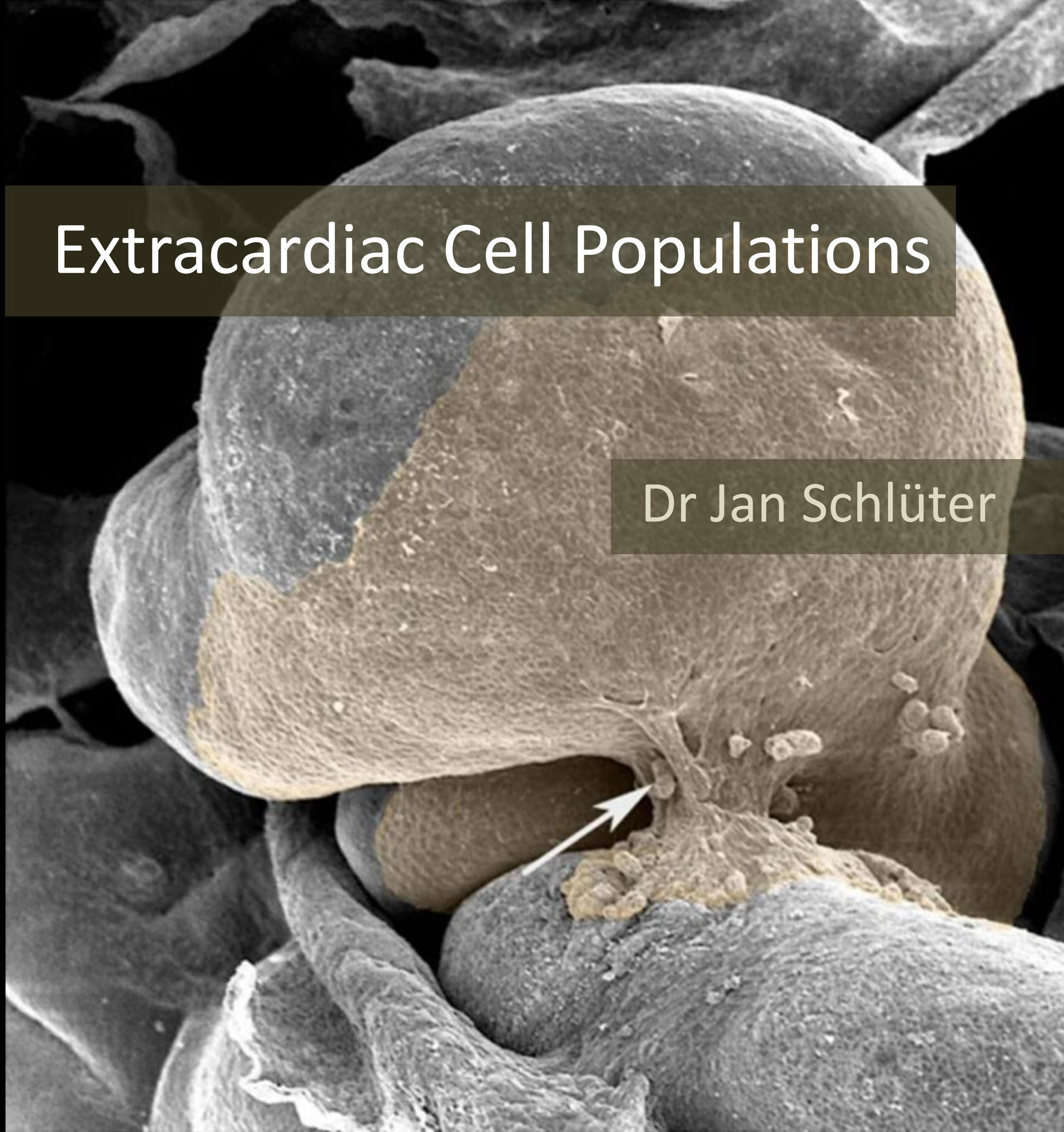
Extracardiac Cell Populations

Dr Jan Schlüter



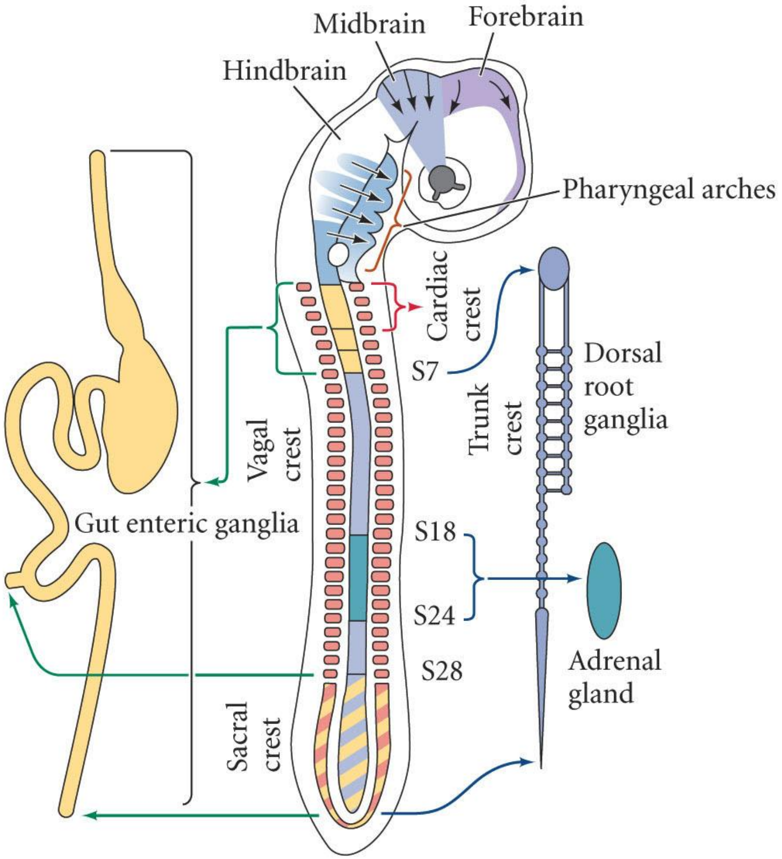
Extracardiac Cell Populations

I Neural Crest Cells

II Proepicardial Cells

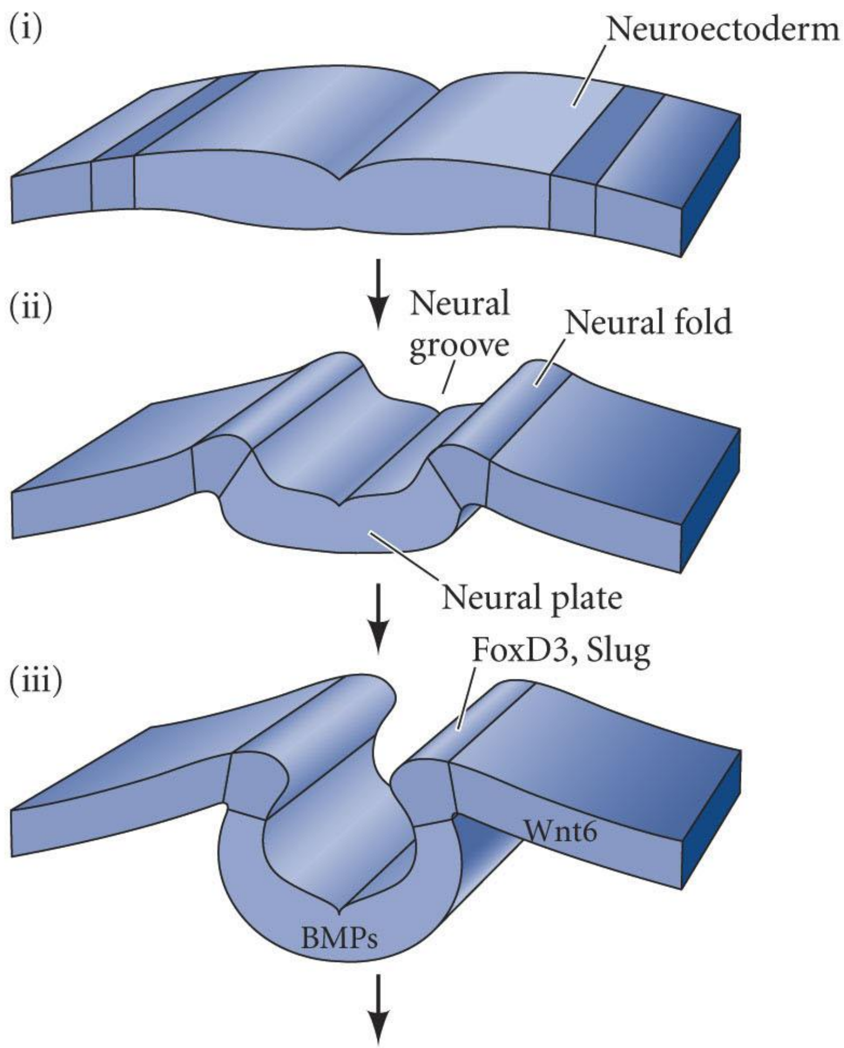
I Neural Crest Cells

- Neural crest cells are multipotent cells which delaminate from the neural tube and migrate throughout the body
- Cranial (cephalic) neural crest
- Trunk neural crest
- Vagal and sacral neural crest
- Cardiac neural crest

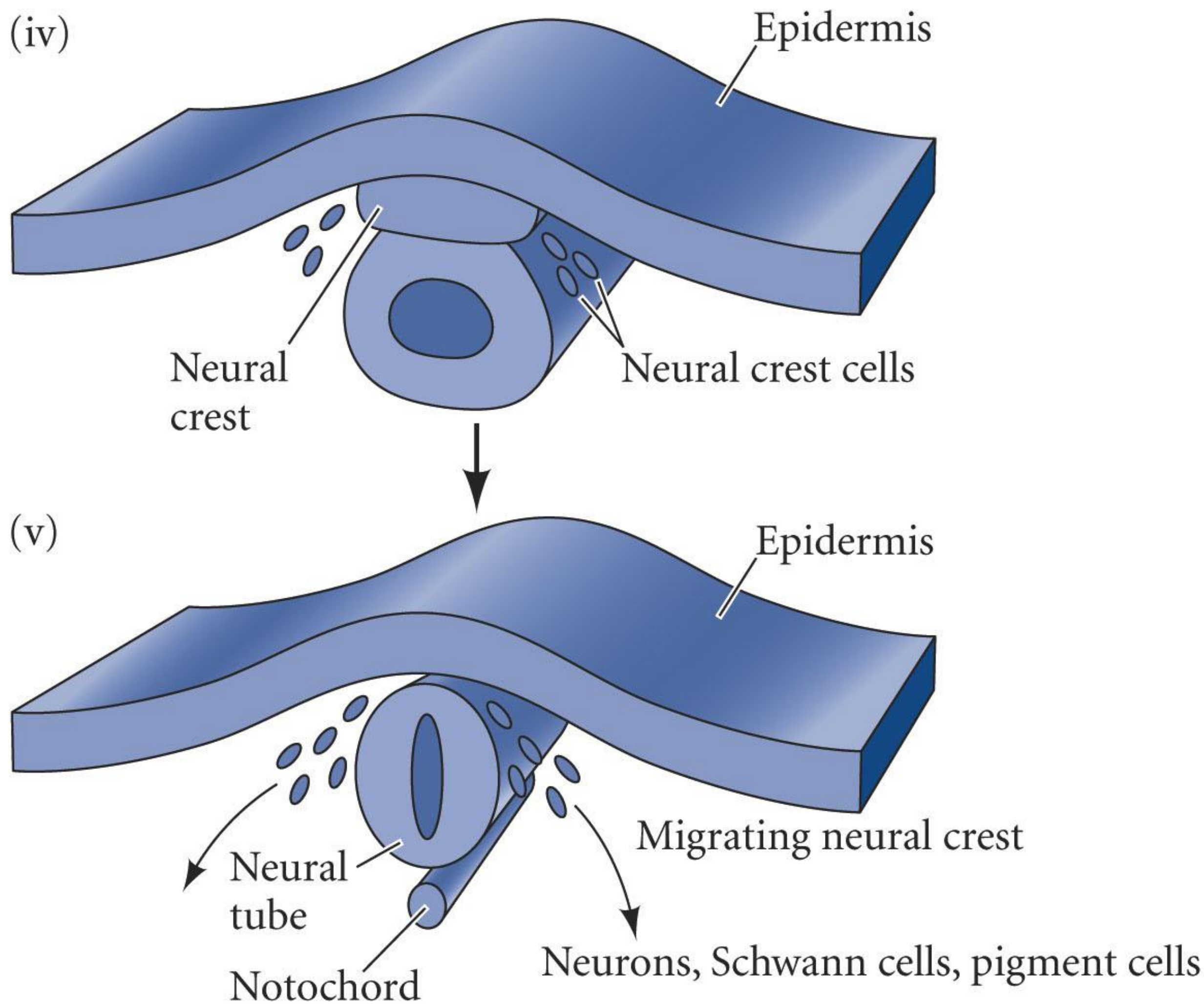


DEVELOPMENTAL BIOLOGY, Eighth Edition, Figure 13.2

I Neural Crest Cells

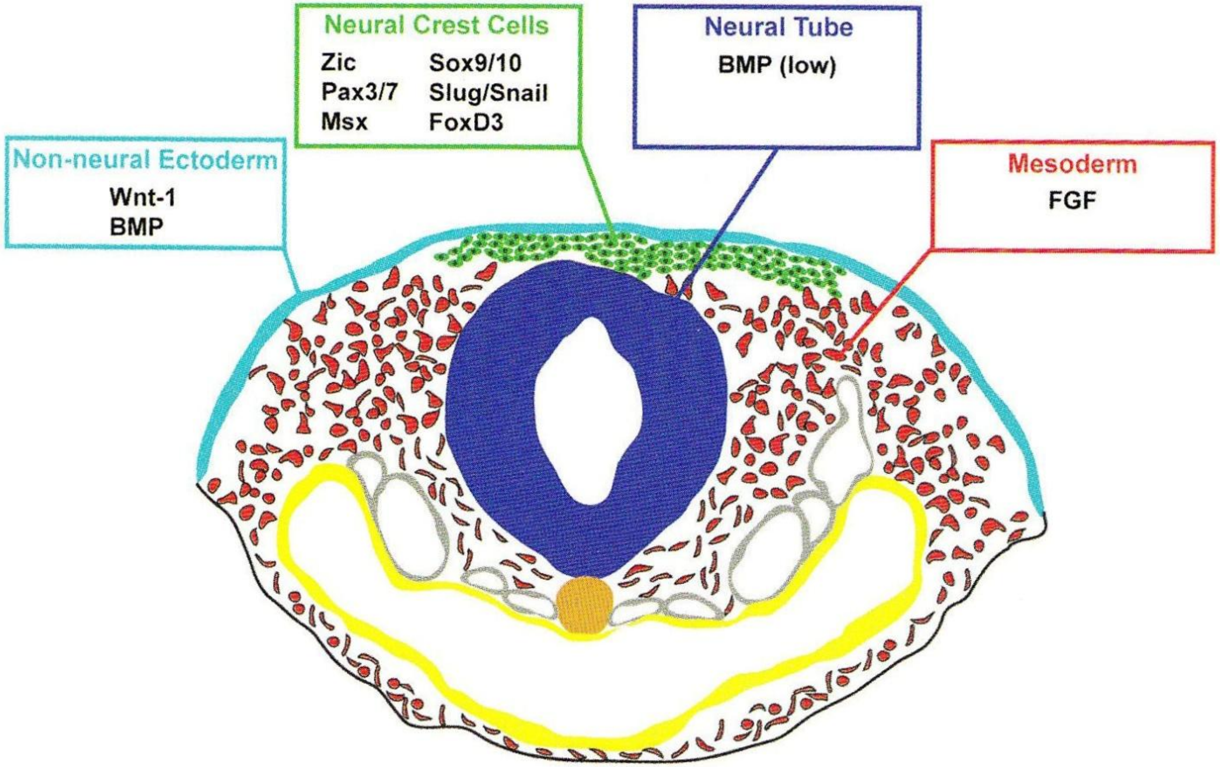


I Neural Crest Cells



DEVELOPMENTAL BIOLOGY, Eighth Edition, Figure 13.1 (Part 2)

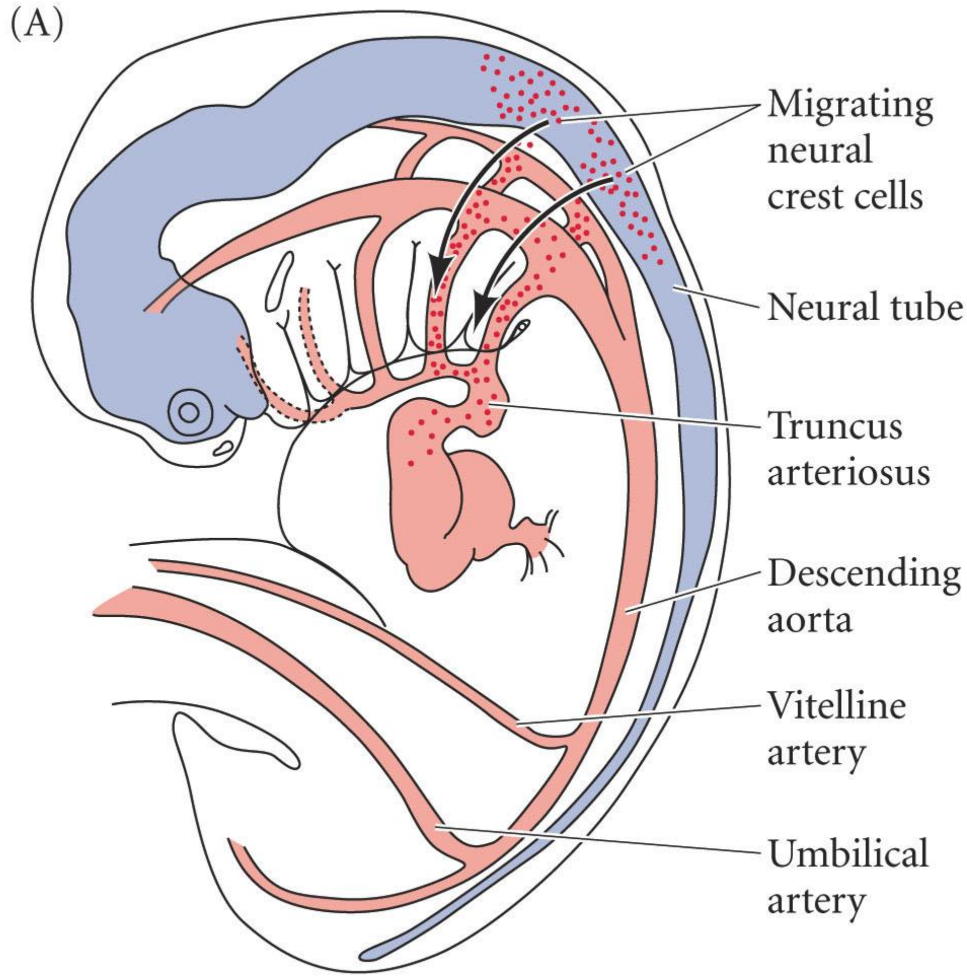
I Neural Crest Cells



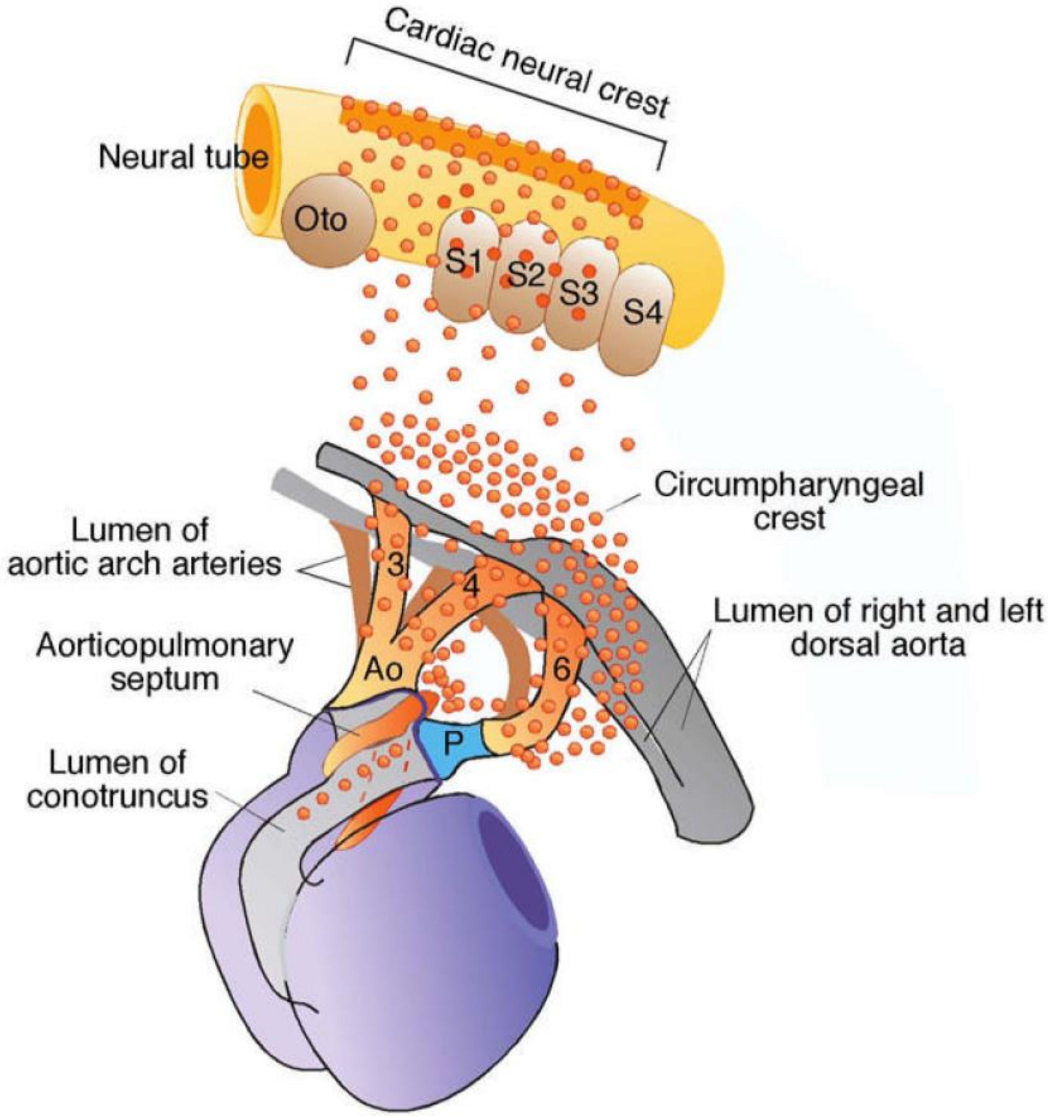
Cardiac neural crest cells

- Multipotent cardiac neural crest cells (CNCs) contribute to:
- Vascular smooth muscle
- Cushion mesenchyme
- Cardiac innervation

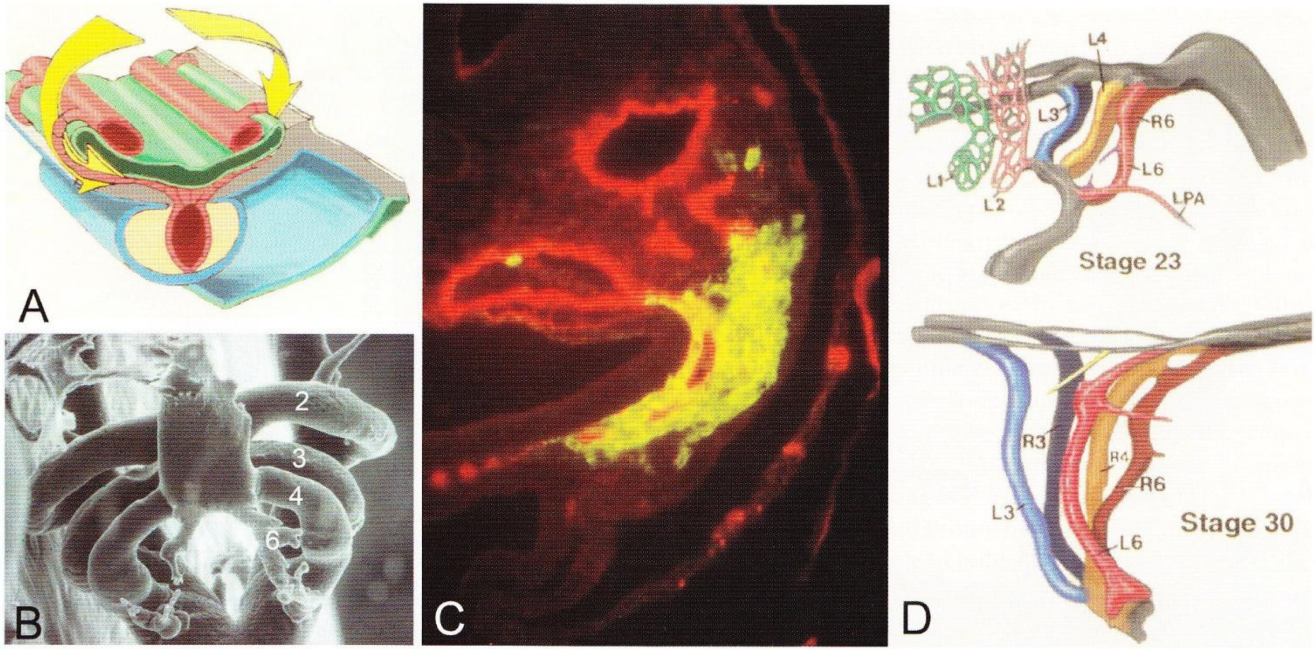
Cardiac neural crest cells



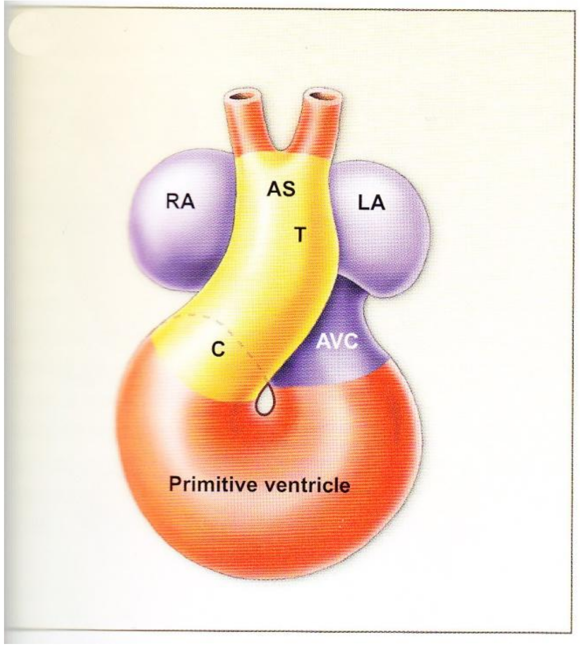
Cardiac neural crest cells

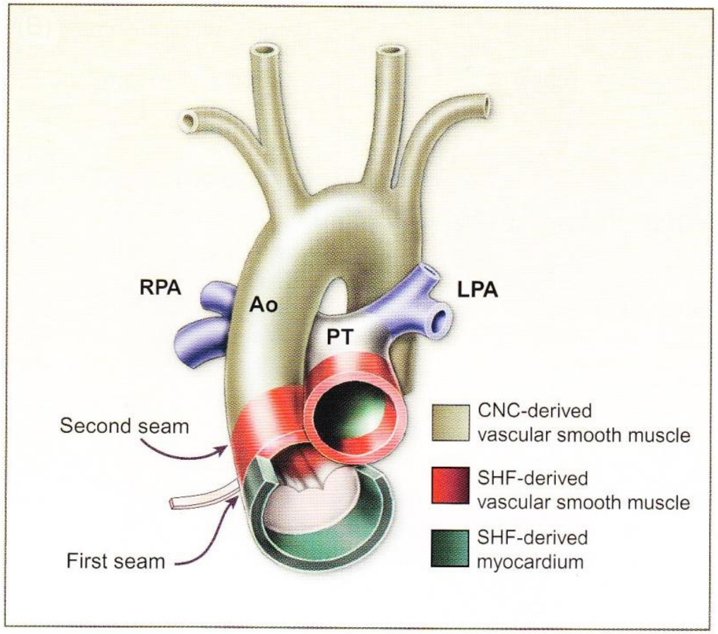


Cardiac neural crest cells

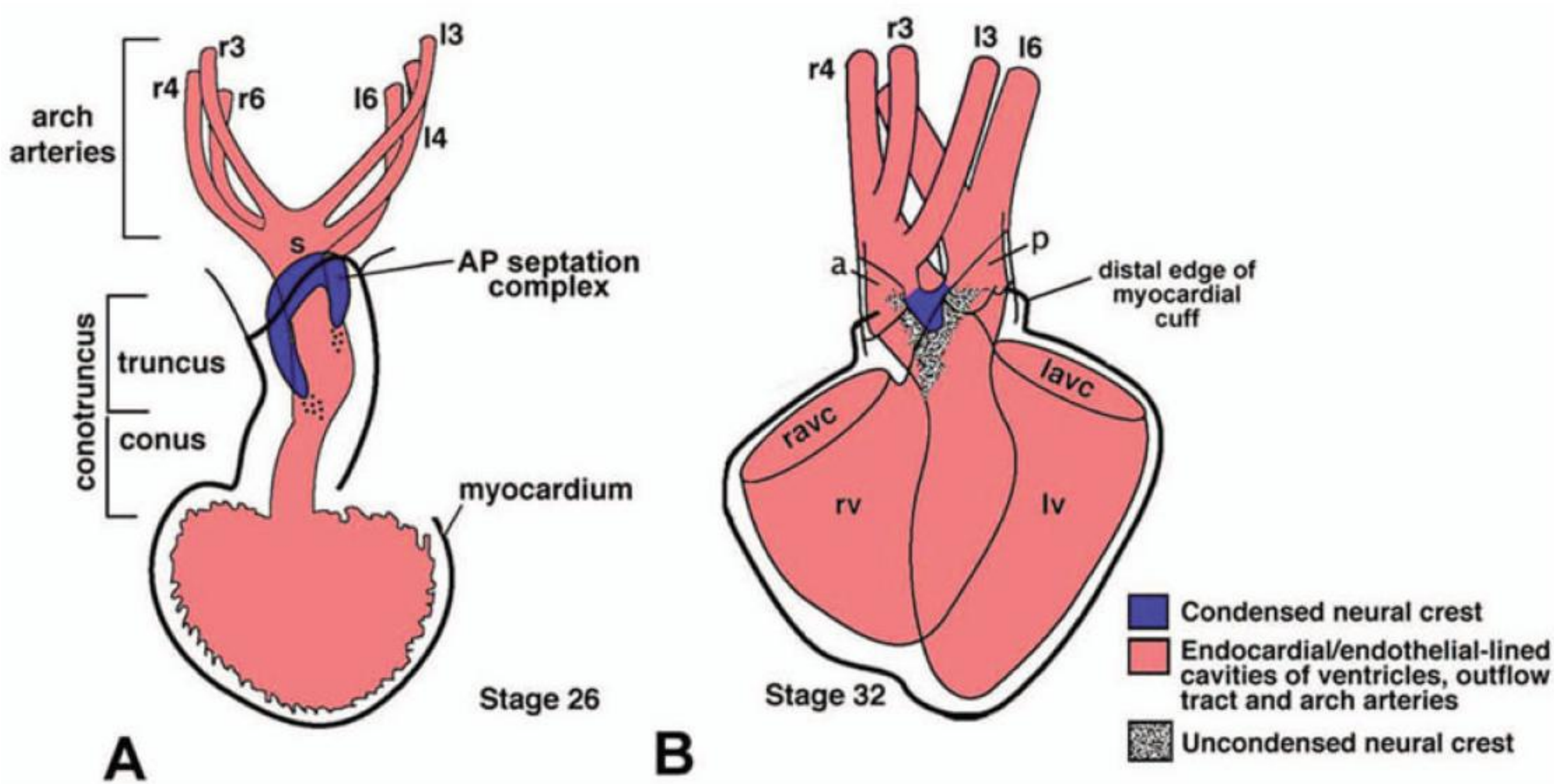


Cardiac neural crest cells

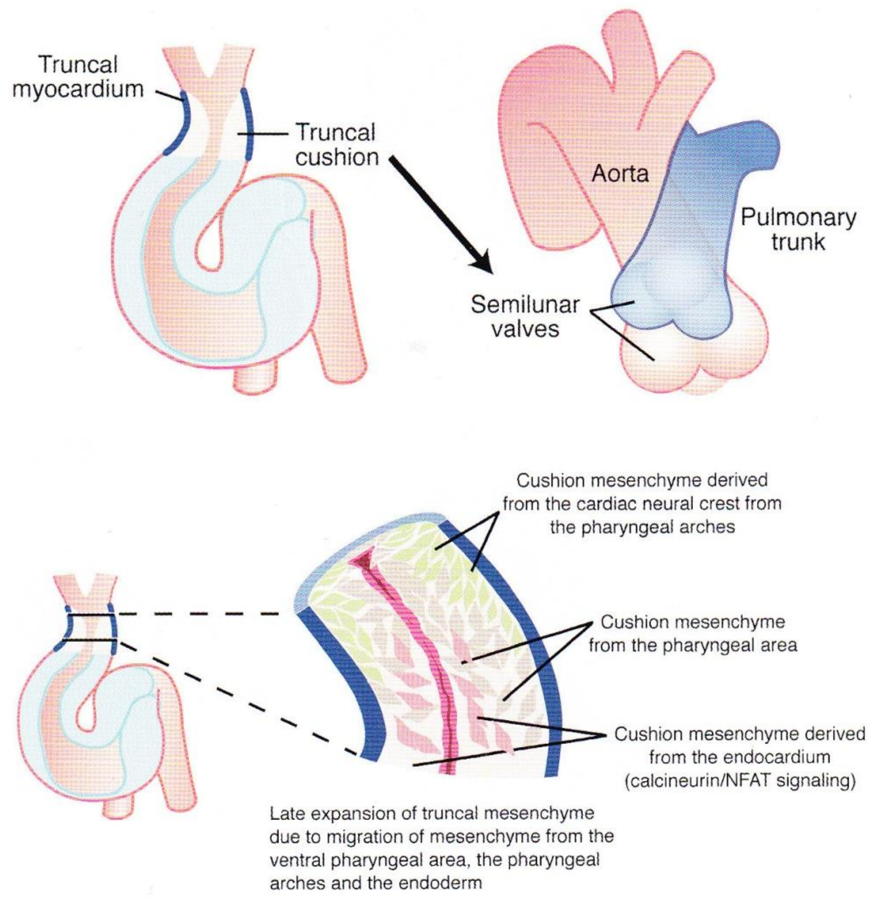




Cardiac neural crest cells

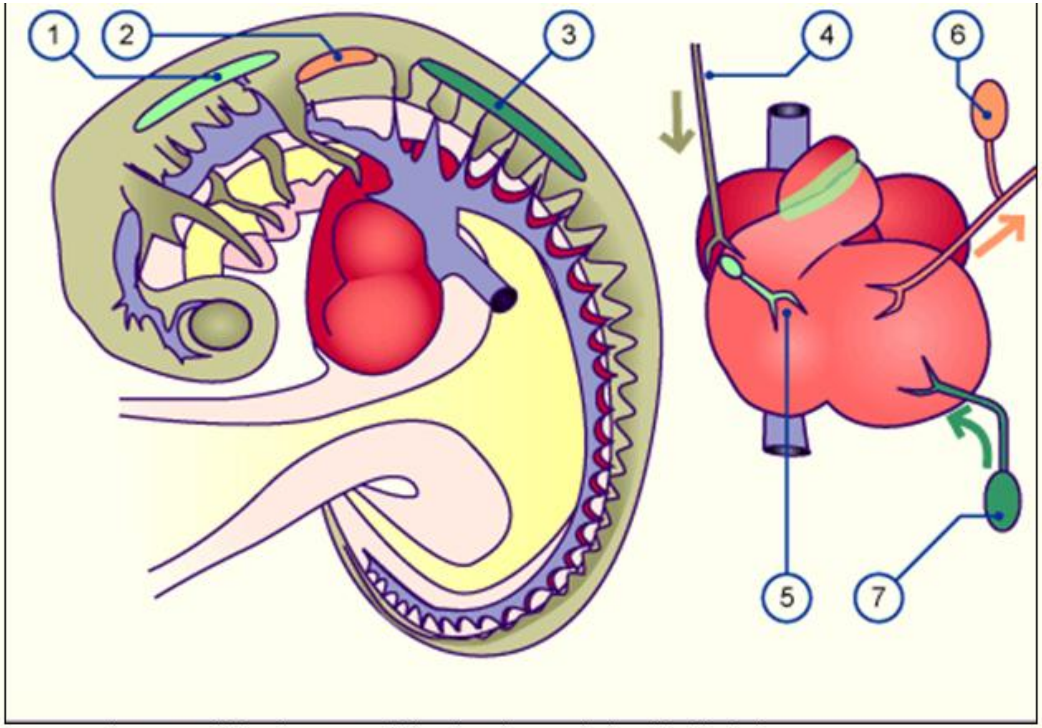


Cardiac neural crest cells



Cardiac neural crest cells

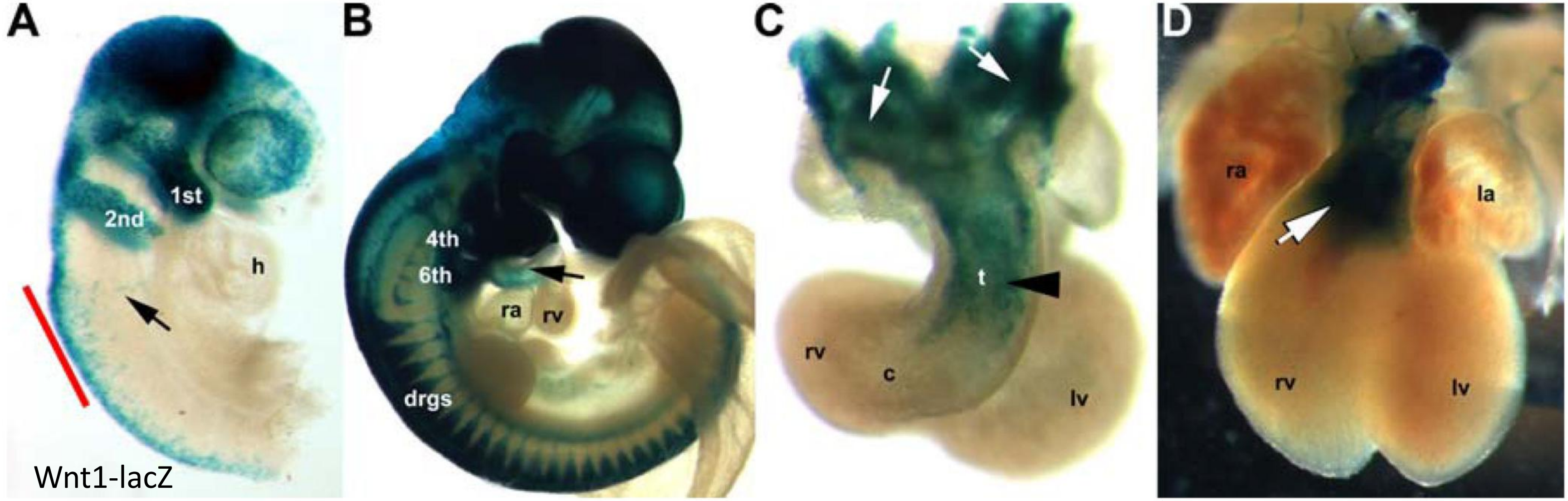
Cardiac innervation



1 Neural crest cells in the area of the rhombencephalon (hindbrain), parasympathetic, efferent
2 Ectodermal placode of the nodose ganglion (parasympathetic, afferent)
3 Neural crest cells of the trunk forming sympathetic ganglia (efferent)
4 First order parasympathetic neurons from cranial neural crest cells (1) contained in the vagus nerve (X)
5 Second order parasympathetic neurons from the cranial neural crest cells (1) that immigrate directly into the heart
6 Sensory ganglia that originate from the ectodermal placode of the nodose ganglion (2)
7 Sympathetic nerve fibers that originate from sympathetic ganglia of the trunk

Note also: cardiac outflow tract with neural crest cell material (1) migrated via the pharyngeal arches

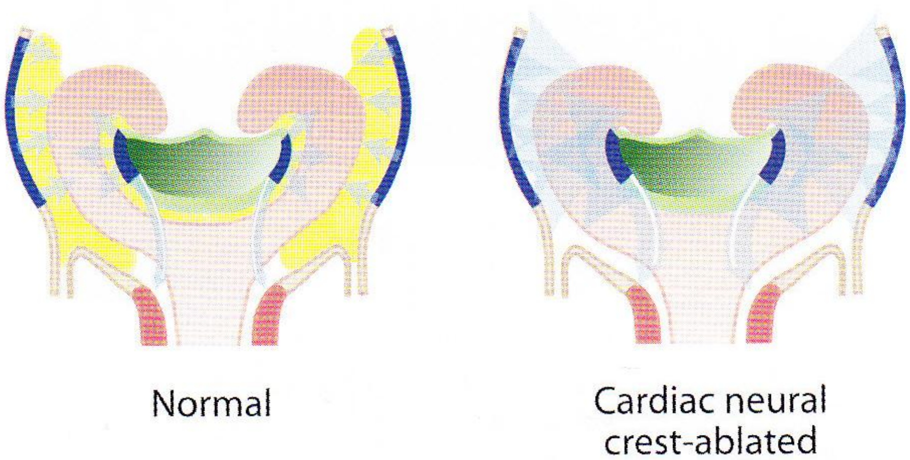
Cardiac neural crest cells



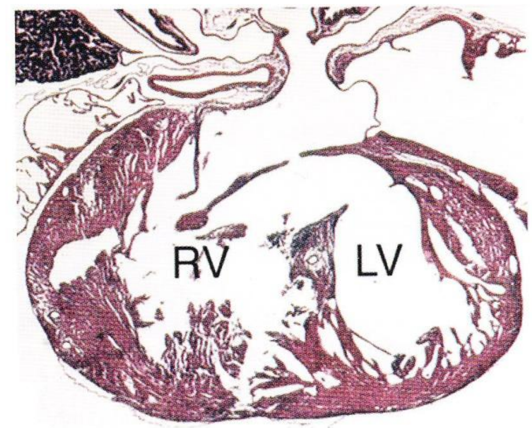
- CNC cells express Wnt1, Pax3, Cx43 during migration + colonization of the heart
- Wnt-signals: CNC induction, cell polarity
- Adhesion molecules (Cadherins) + Gap junction Cx43 provide „communication“ and interaction with their surrounding tissues during migration
- Pax3 is required for development of multiple neural crest lineages but is generally downregulated when NC cells leave the neural tube (maintains the undifferentiated state of mesenchymal NC cells)

Cardiac neural crest cells

CNC modulates endodermal FGF signals



Neural crest ablation



Ventricular septal defects

-Premigratory CNC ablation causes myocardial dysfunction
- suggestion: prolonged release of FGF signals by the pharyngeal endoderm
- these FGF signals are proposed to suppress myocardial development and alter myocardial proliferation/ differentiation
-Endocardium might have indirect negativ effects on myocardial maturation as a consequence of absent CNC colonization

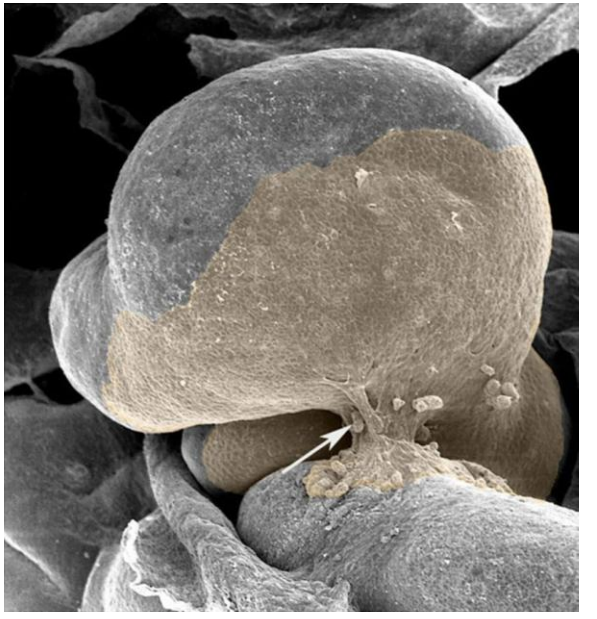
summary

- Cardiac neural crest cells delaminate from the anterior neural tube between the otocyst and the 4th somite
- CNCs colonize the pharyngeal arches and the cardiac outflow tract where they differentiate into vascular smooth muscle covering the arterial endothelium
- Condensed CNCs form the aortic-pulmonary septation complex which modulates the septation of the arterial truncus into the aortic and pulmonary trunc
- CNCs contribute to the formation of the semilunar valves
- CNCs colonize the venous pole and provide cardiac innervation
- Pax3 is important for specifying neural crest lineages, blocks differentiation and is downregulated upon neural crest migration into the heart
- Adhesion and gap junction molecules provide interaction of CNCs with their surrounding tissue during migration
- CNCs modulate endodermal FGF8 signalling, thereby facilitating myocardial proliferation and differentiation

literature

- Epstein, J.A., Li, J., Lang, D., Chen, F., Brown, C.B., Jin, F., Lu, M.m., Thomas, M., Liu, E., Wessels, A., Lo, C.W., 2000. Migration of cardiac neural crest cells in Splotch embryos. Development 127, 1869-1878
- Kirby, ML., Waldo, K.L., 1995. Neural Crest and cardiovascular patterning. Circ. Res. 77,211-215
- Snider, P., Olaopa, M., Firulli, A.B., Conway, S.J., 2007. Cardiovascular development and the colonizing neural crest lineage. TSW Dev + Emb. 2, 88-111

II Proepicardial Cells



1 Introduction

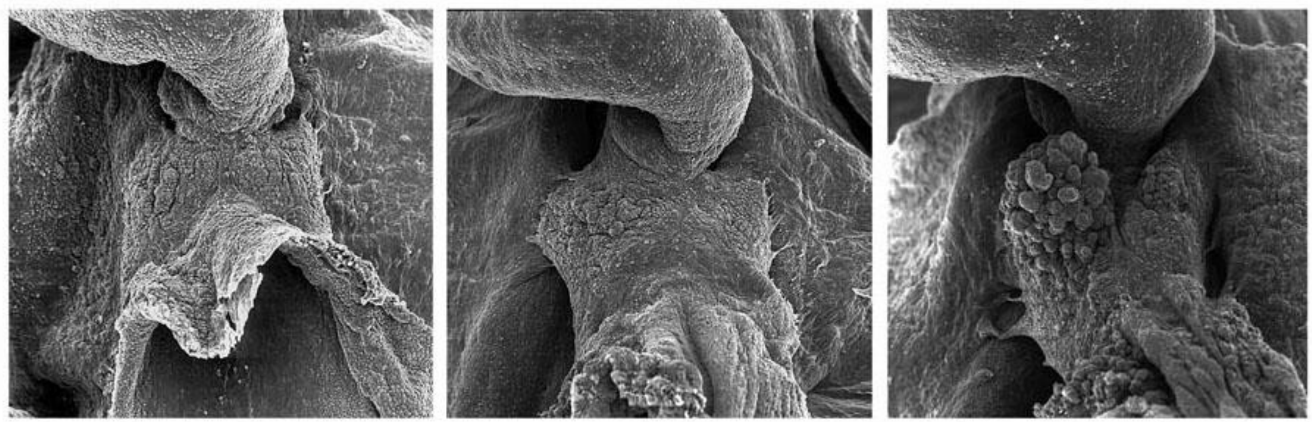
2 Asymmetrical PE development

3 The role of BMP and FGF

4 Neovascularization and regeneration

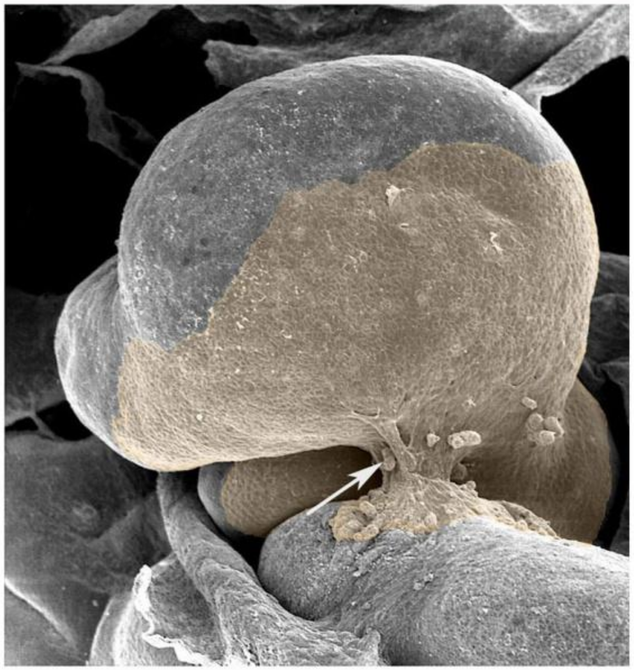
1. Introduction

- The proepicardium (PE) develops on the sinus venosus of the heart

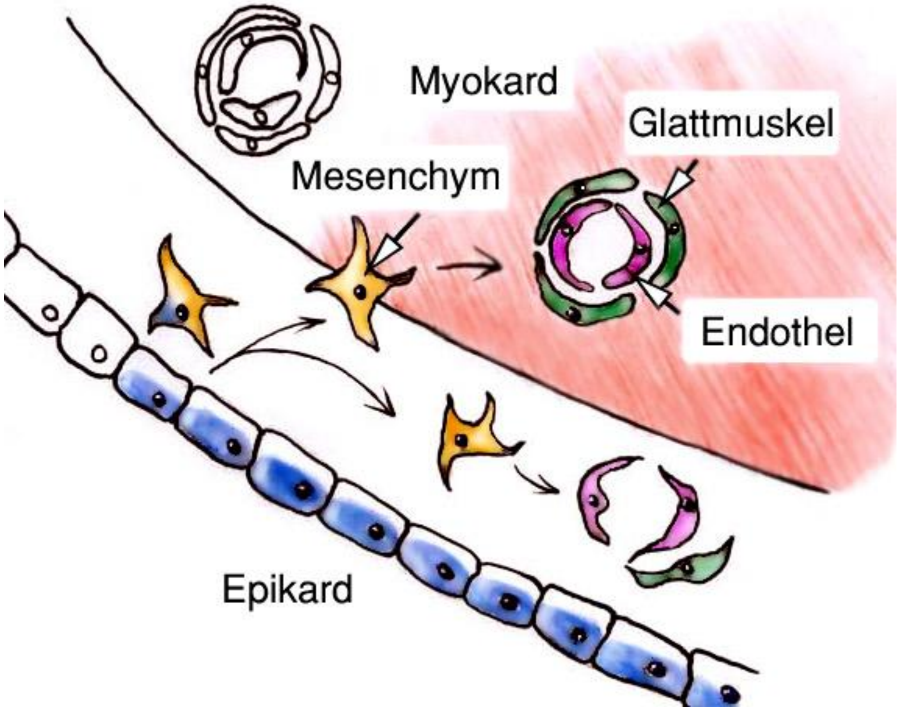


HH 14 HH 15 HH 16

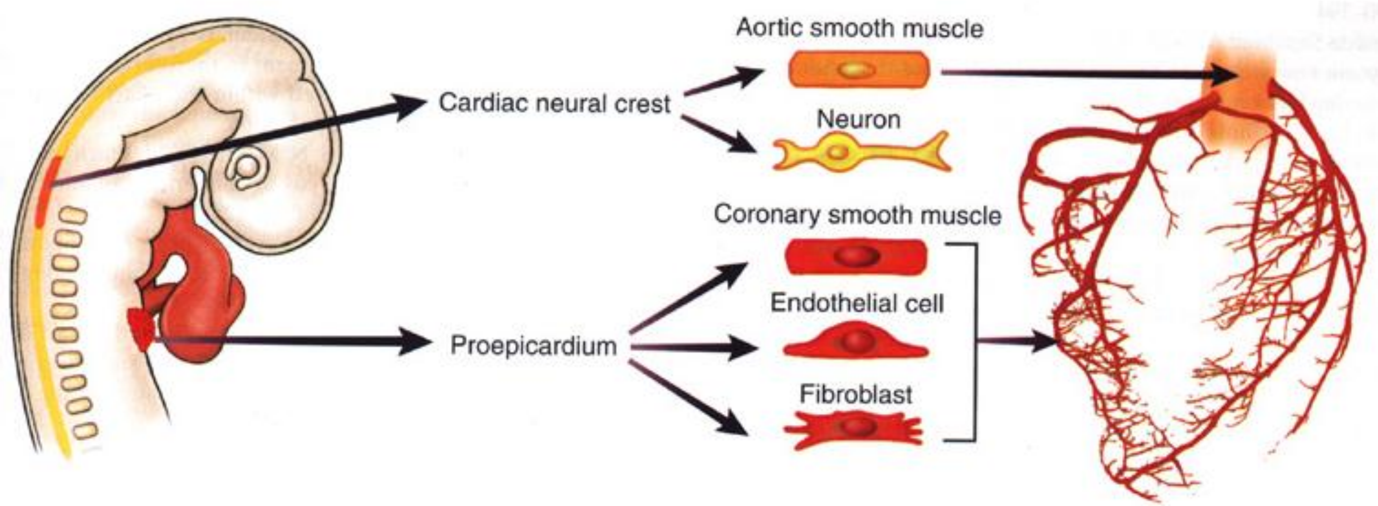
Epicardial cells invade the heart

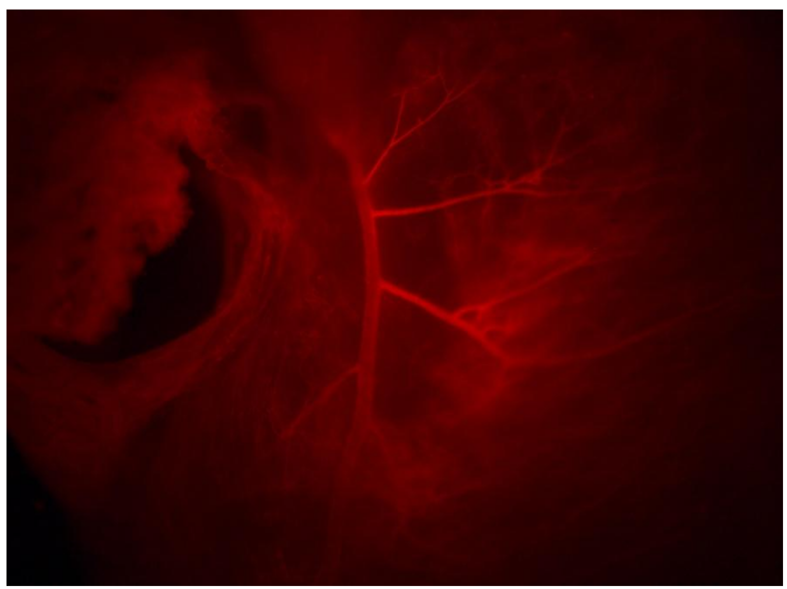


(Männer et al., 2000)

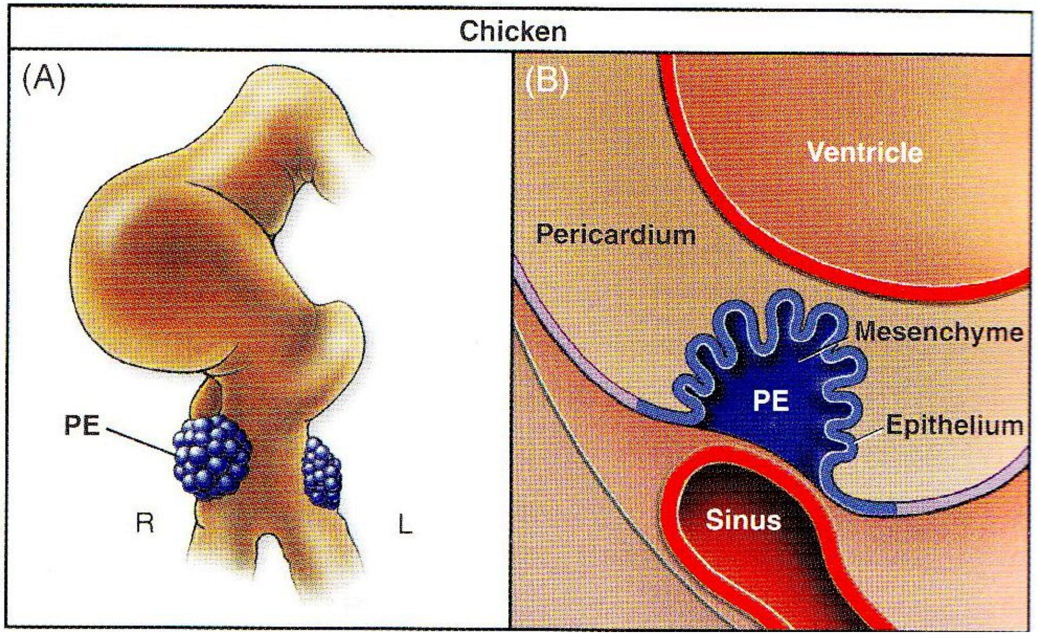


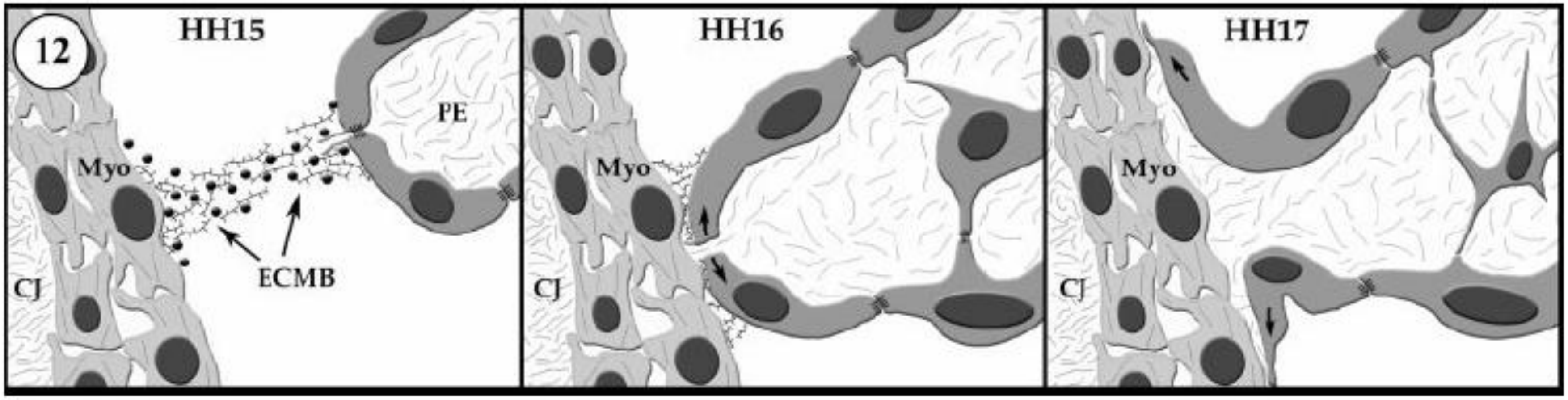
EPDCs (epicardial derived cells) differentiate into coronary blood vessels



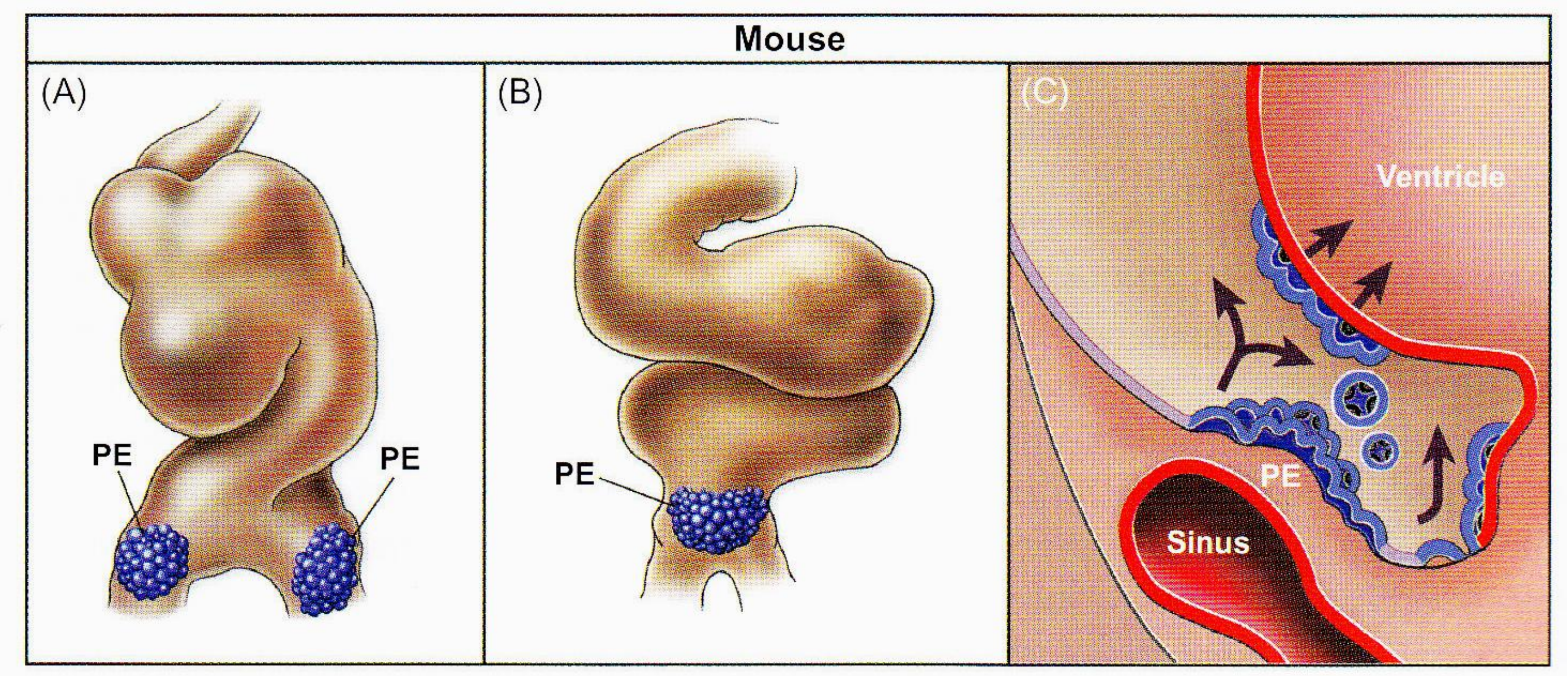


PE cells colonize the heart via a tissue bridge in the chicken embryo

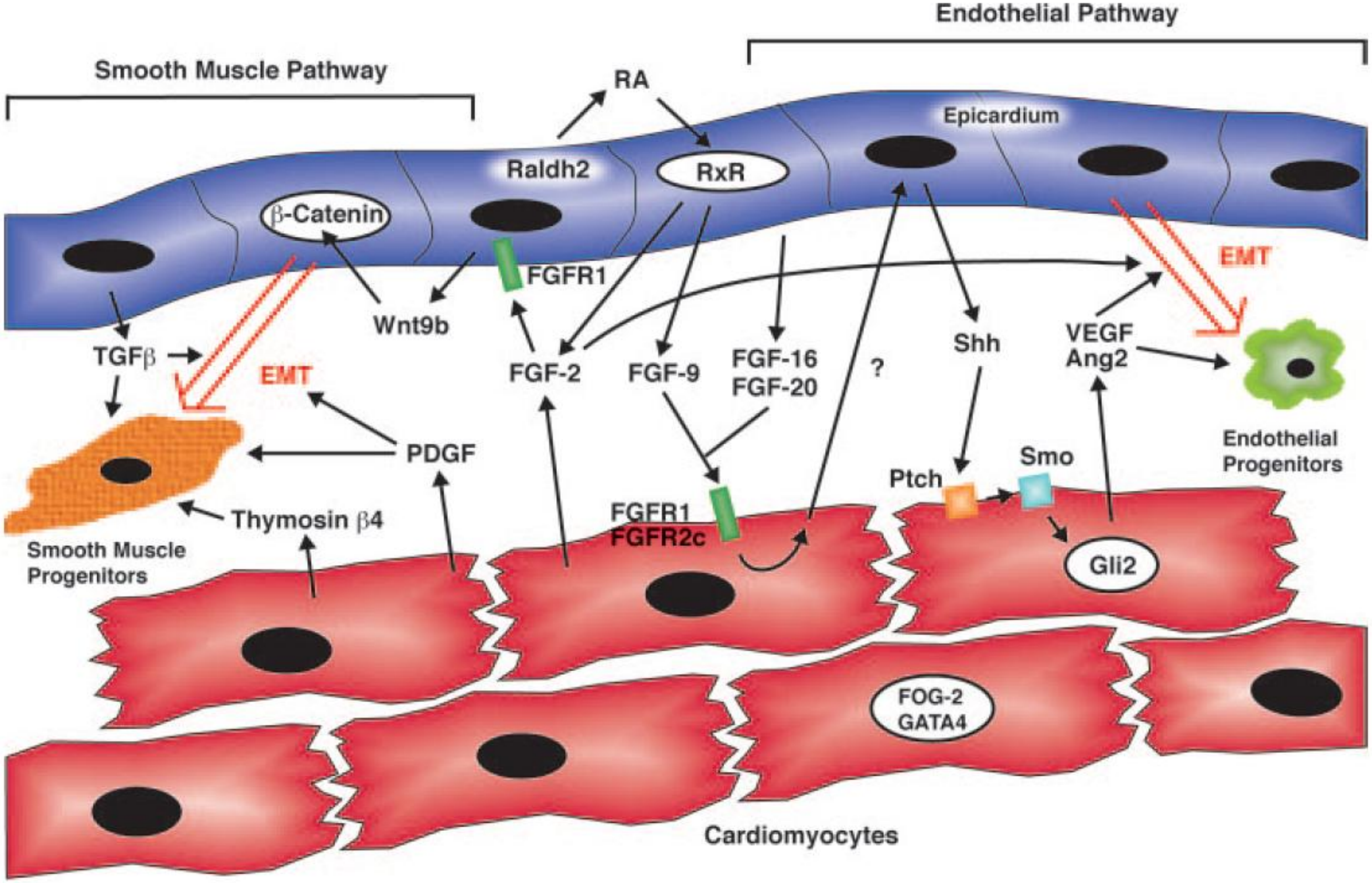




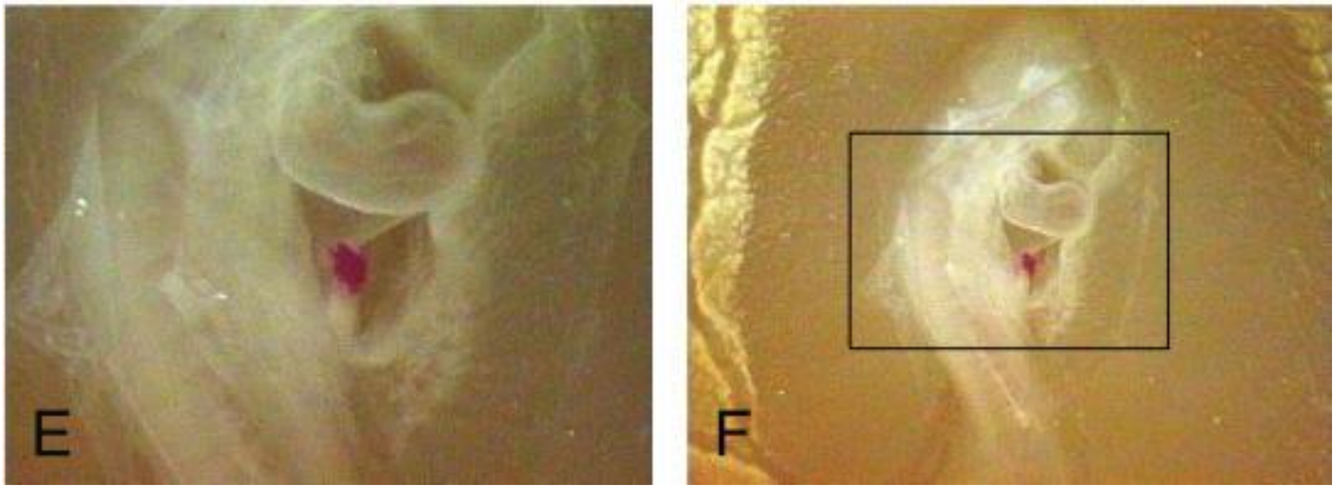
PE cells colonize the heart via proepicardial cysts in the mouse embryo

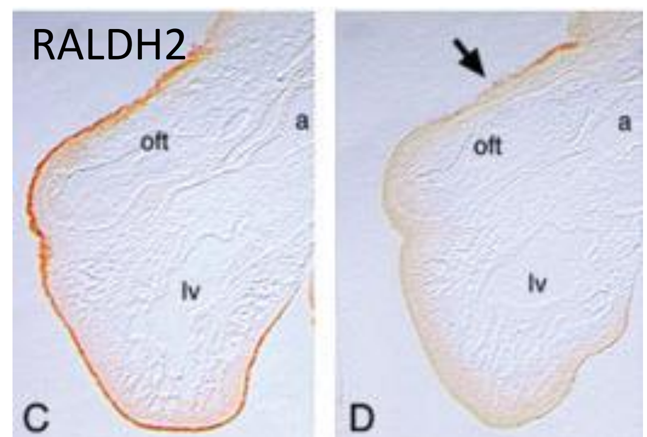


Epicardium-myocardium interactions

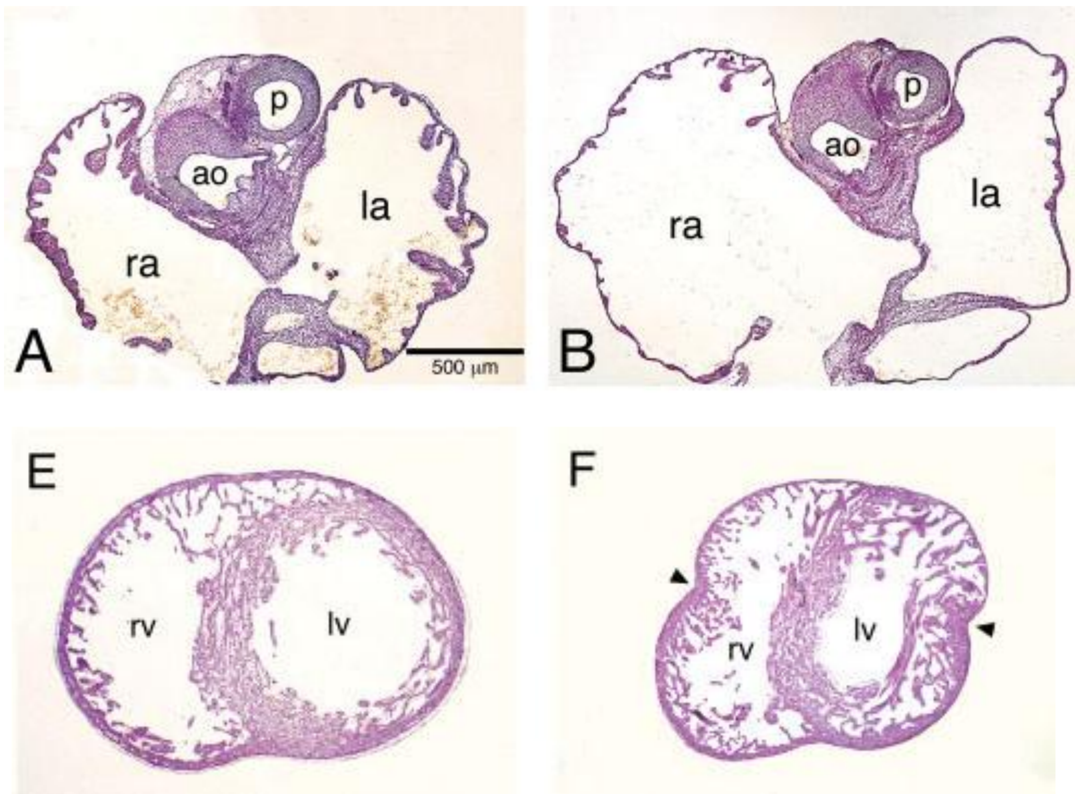


Loss of proepicardial cells leads to loss of the epicardium





Retarded myocardial growth without epicardium



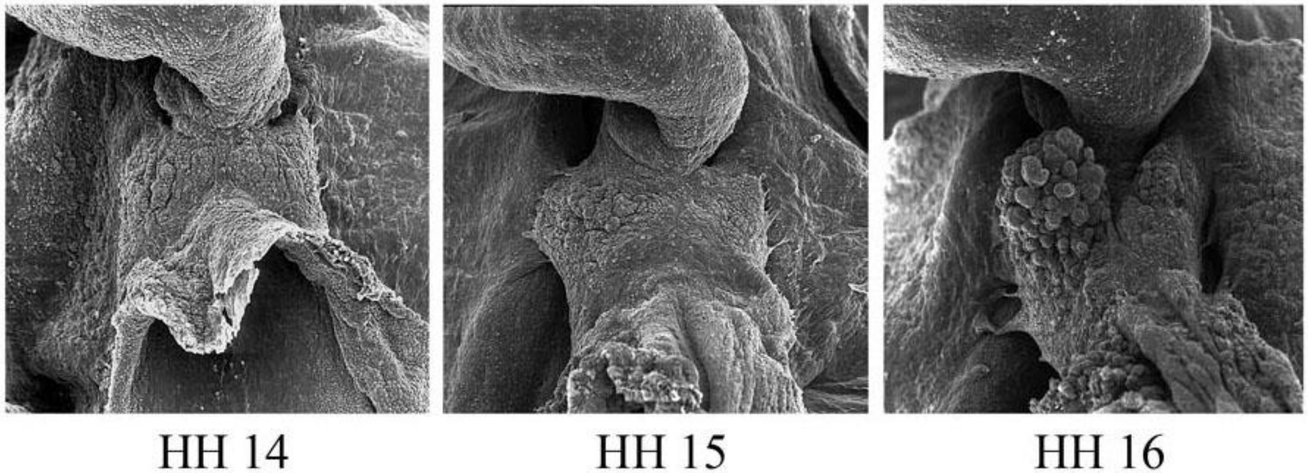
(Maenner et al., 2005)

Summary 1

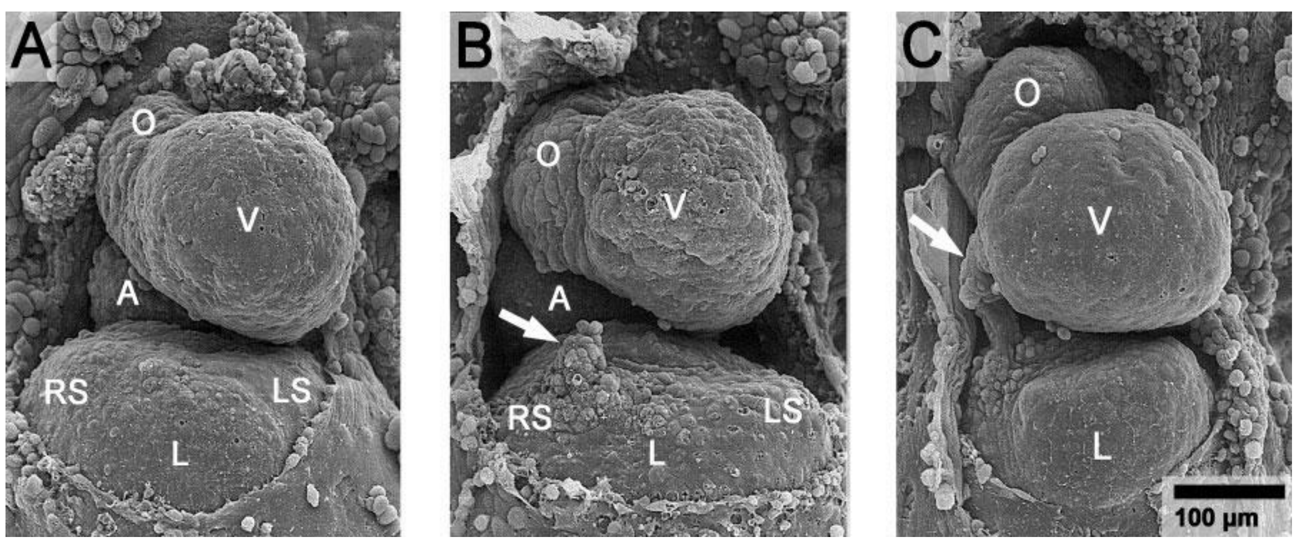
- The PE (proepicardium) develops on the venous pole of the heart and subsequentely colonizes the heart to form the epicardium
- the process of colonization differs in chick and mouse embryos
- Epicardial cells invade the heart and differentiate into the coronary vasculature
- The myocardium is strongly dependent on interaction with the epicardium which promotes myocardial growth

2. Asymmetrical PE development

chick



frog



The asymmetrical PE anlage

Vertebrata:

Agnatha

lamprey

Gnathostomata

Osteichthyes

zebrafish

Tetrapoda

Amphibia

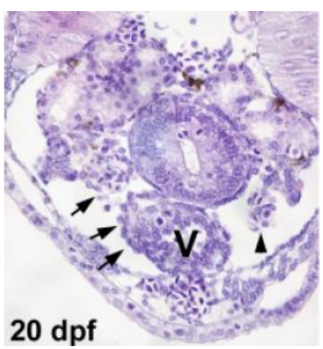
xenopus

Aves

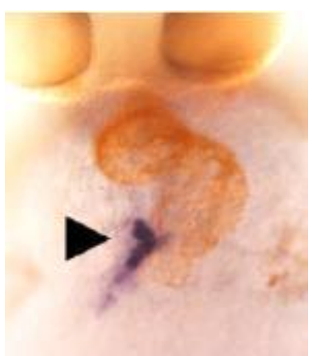
chick

Mammalia

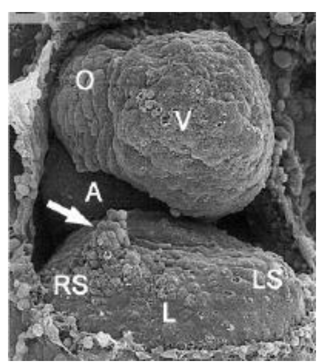
mouse



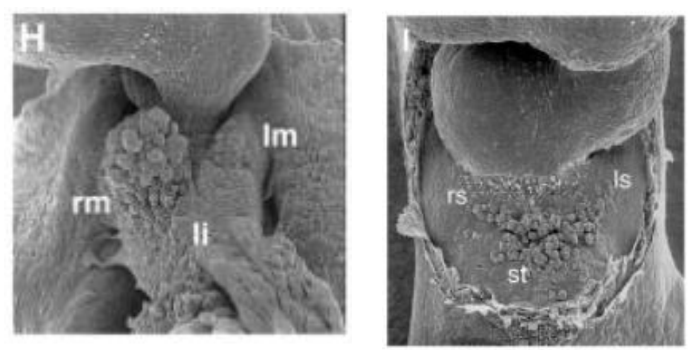
(Pombal et al., 2008)



(Serluca, 2007)



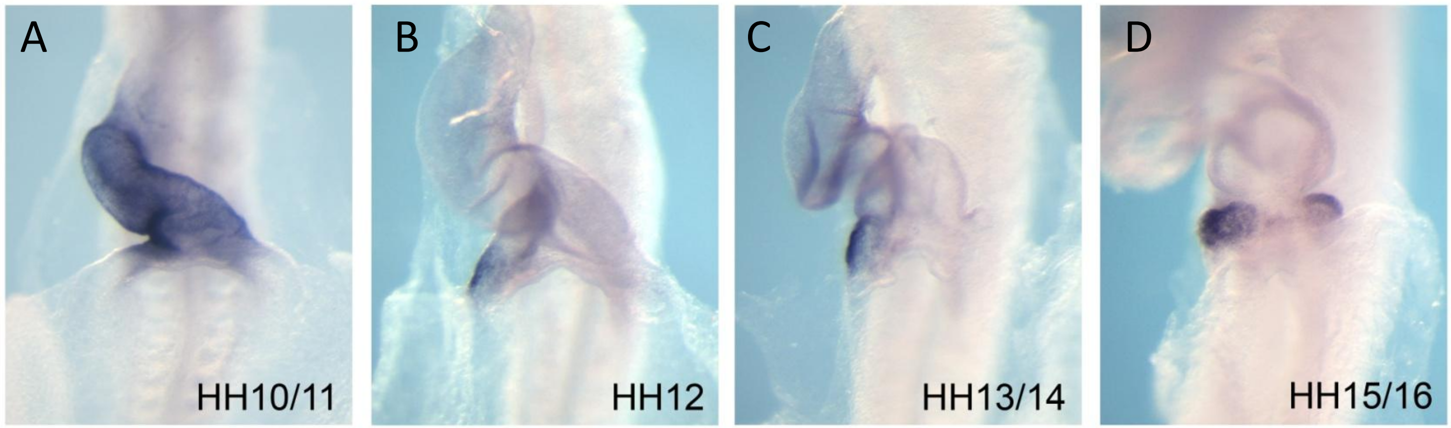
(Jahr et al., 2008)



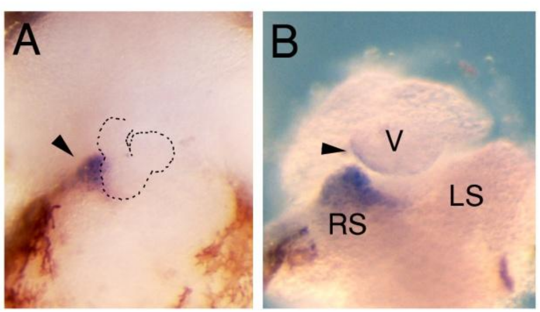
(Schulte et al., 2007)

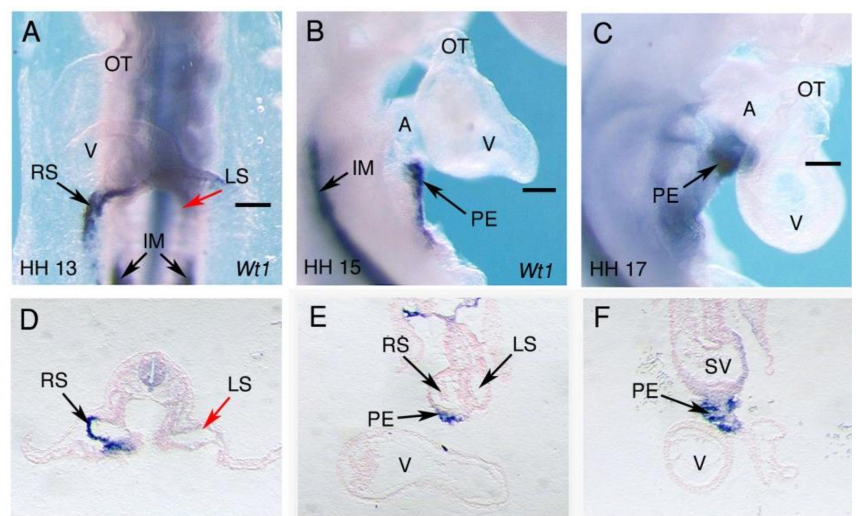
The expression of proepicardial marker genes

TBX18
chick



TBX18
Xenopus
(42h)

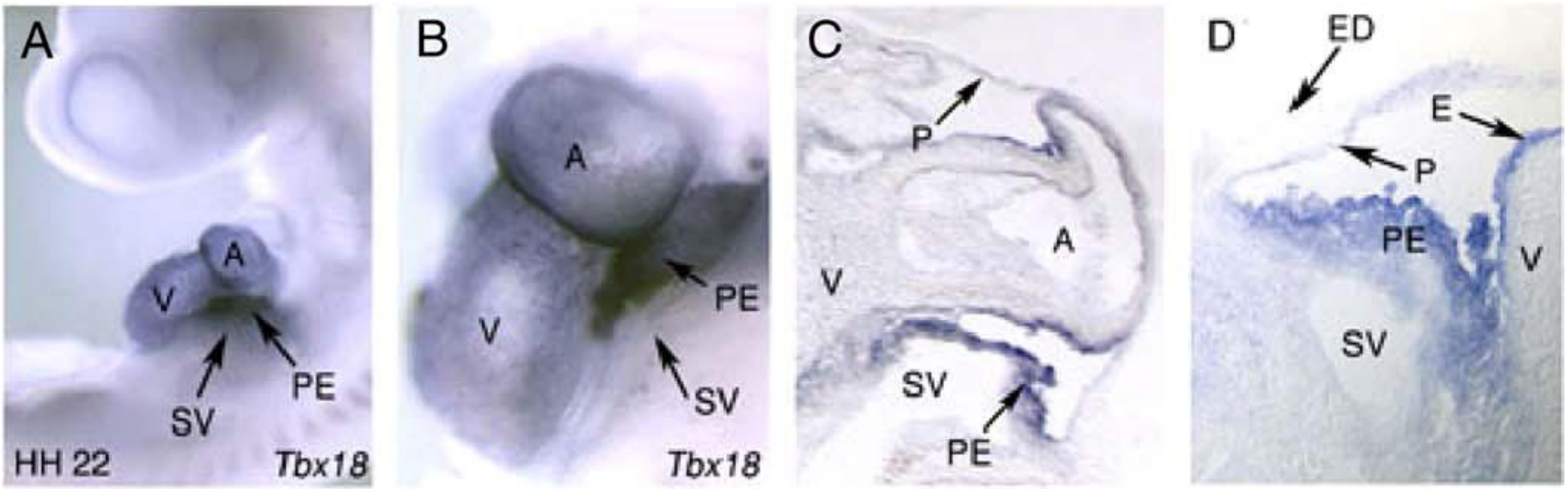


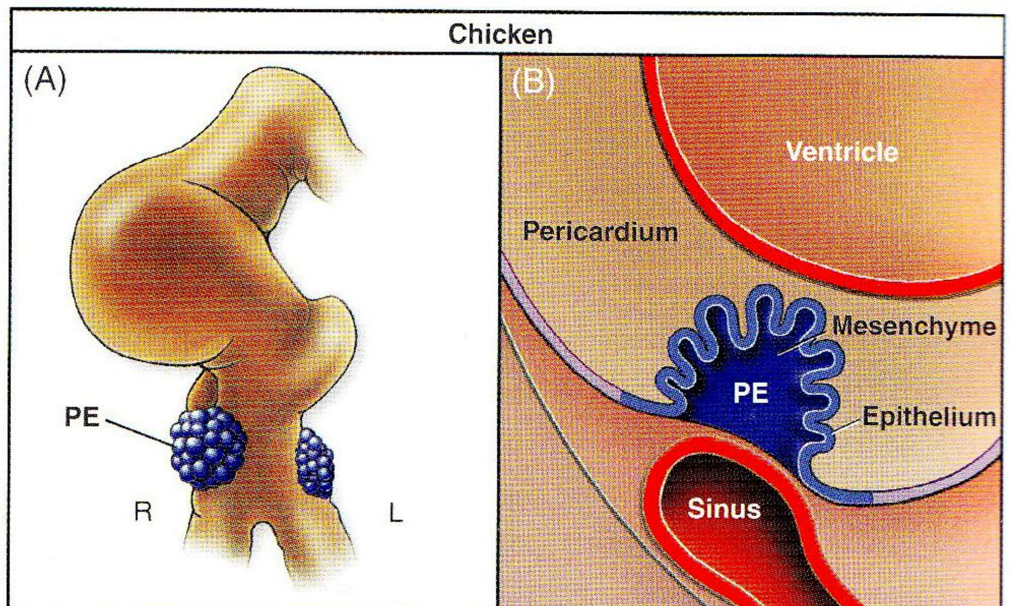


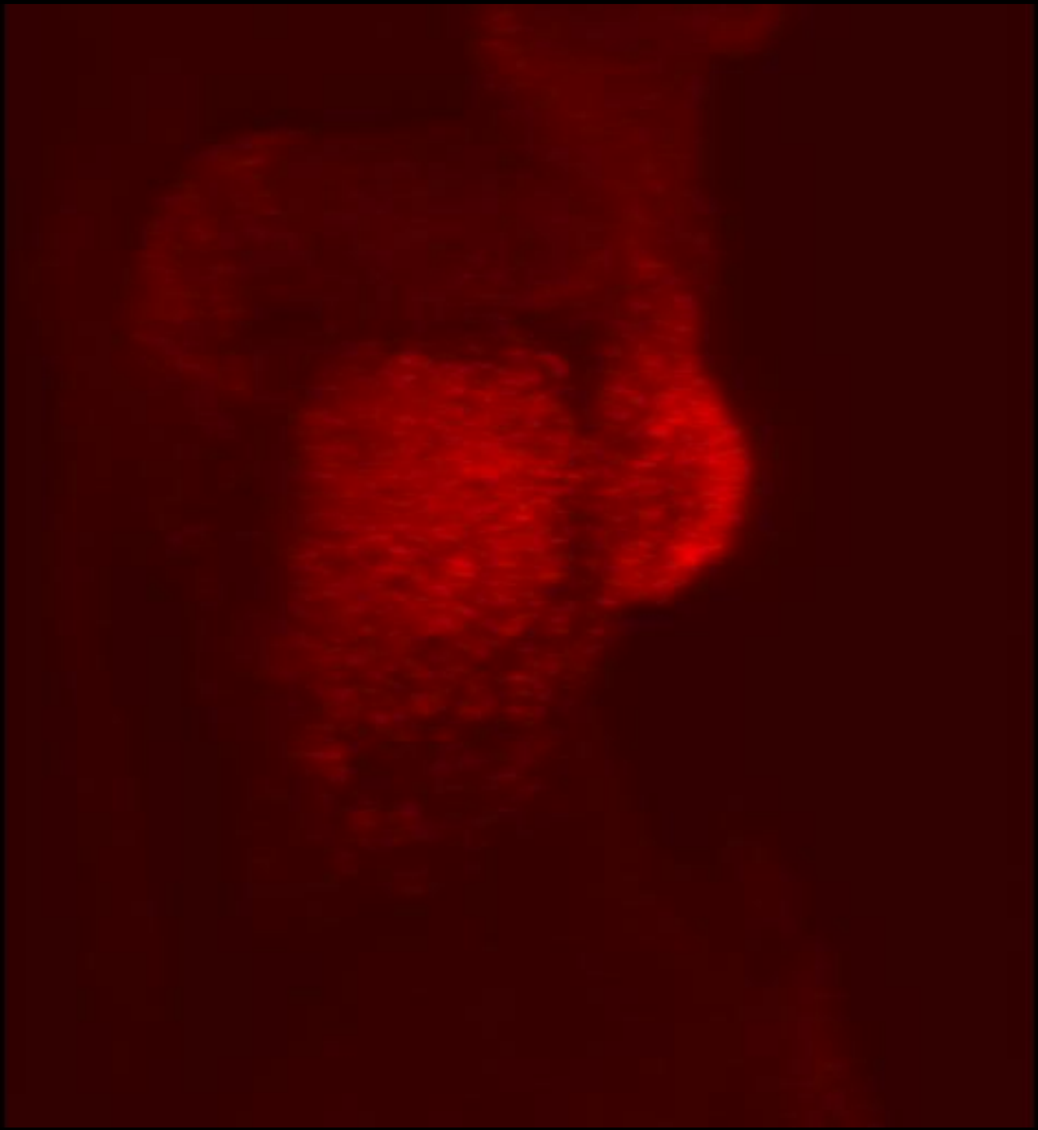
WT1 chick

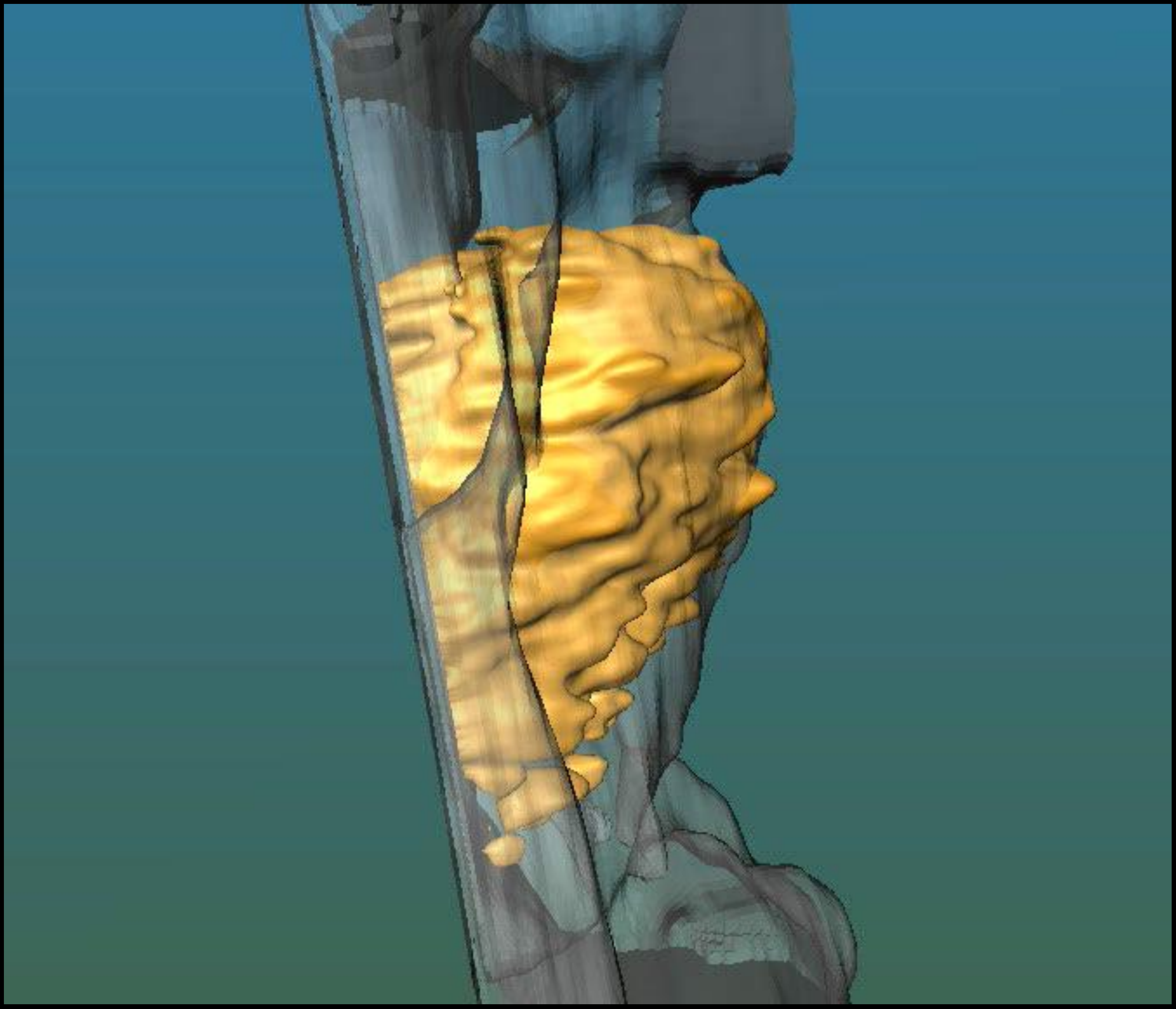
(Schlueter et al., 2006)

The expression of proepicardial marker genes

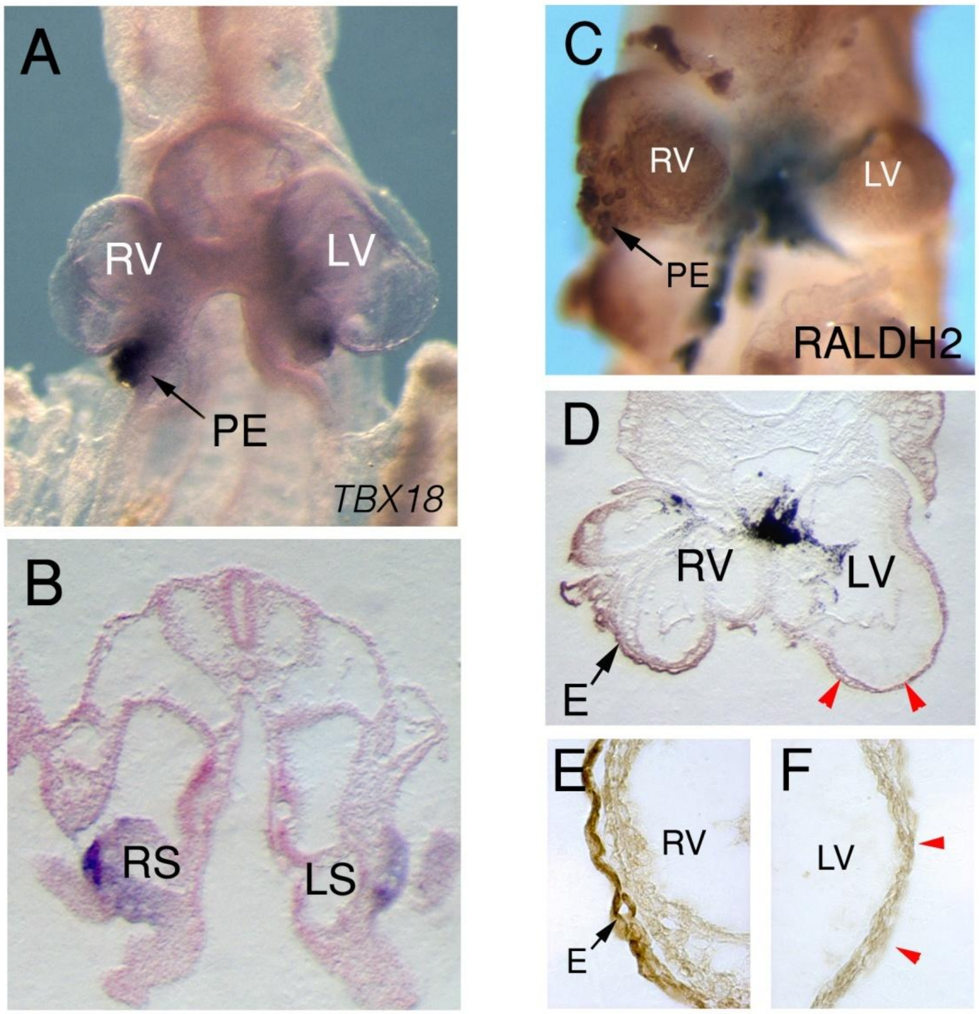








Cardia bifida embryos display only a right epicardium



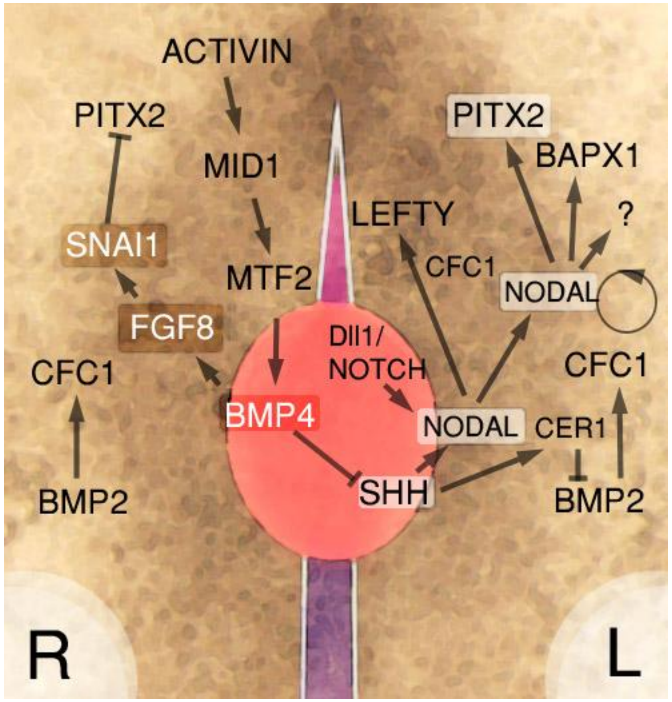
- The PE has a right identity

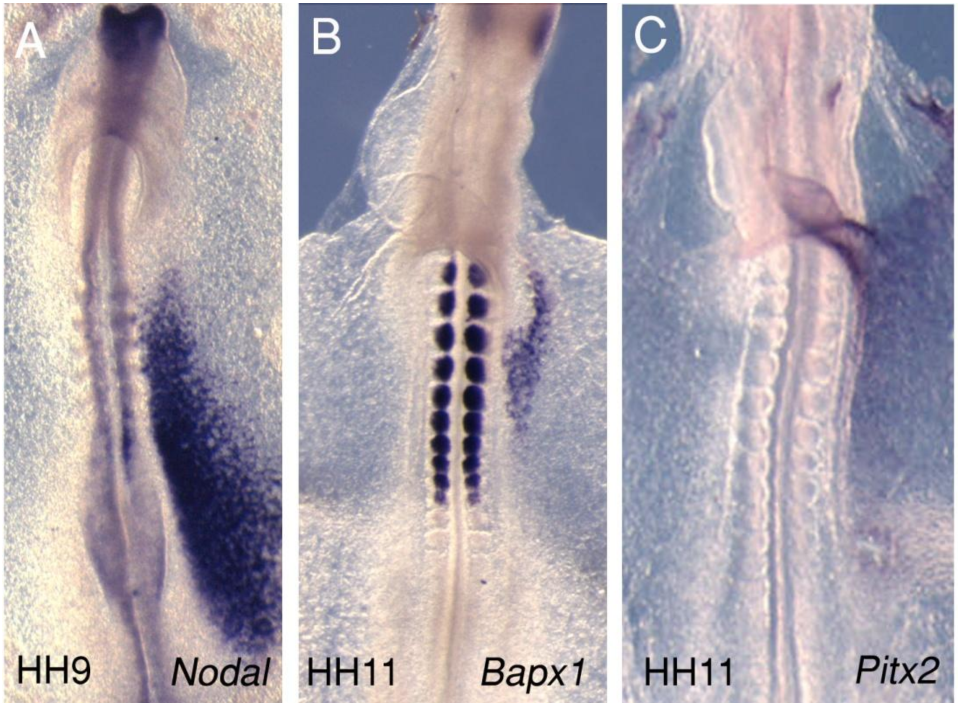
Question:

Is a leftsided repressive signal responsible for asymmetrical PE development?

The Nodal/PITX2 pathway

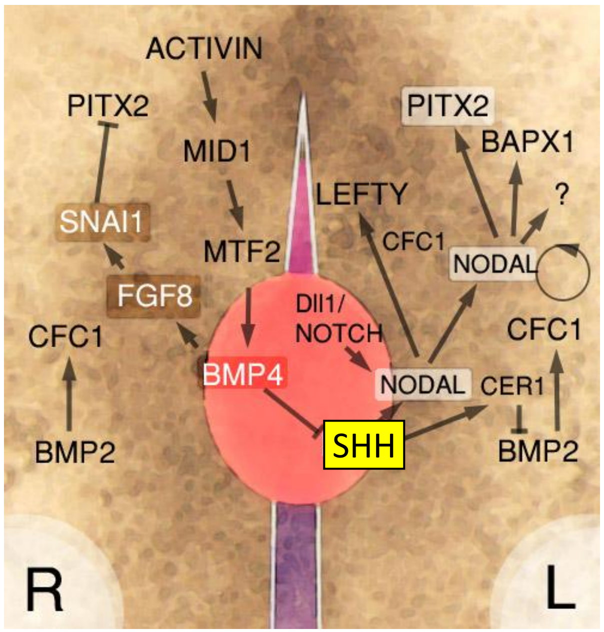
The conserved NODAL-PITX2 signaling pathway determines the left side

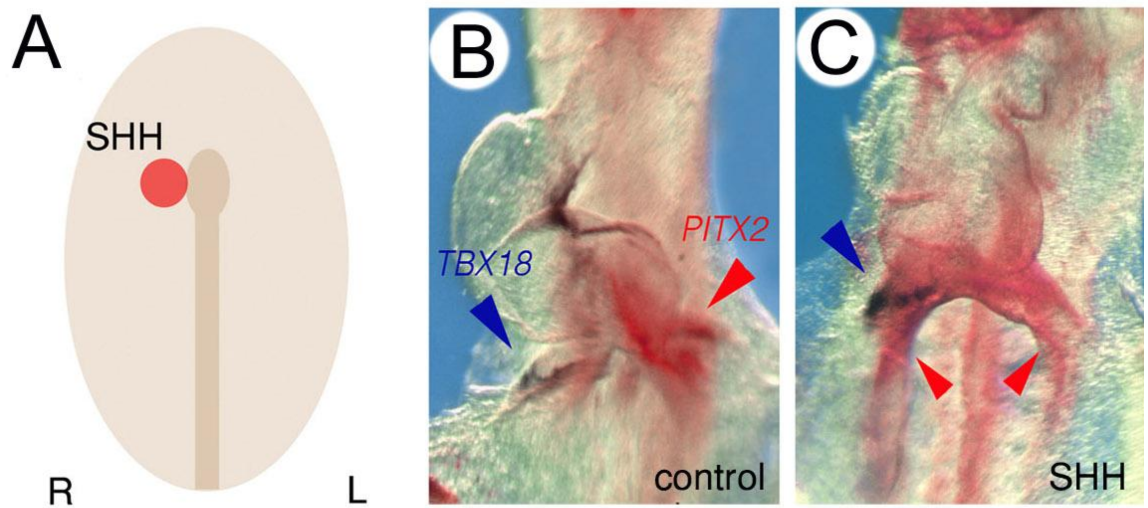


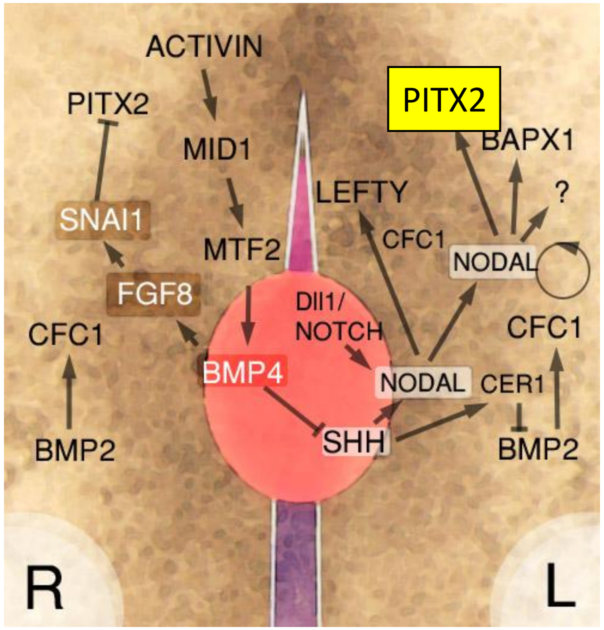


(Schlueter and Brand, 2007)

Gain-of-function of PITX2

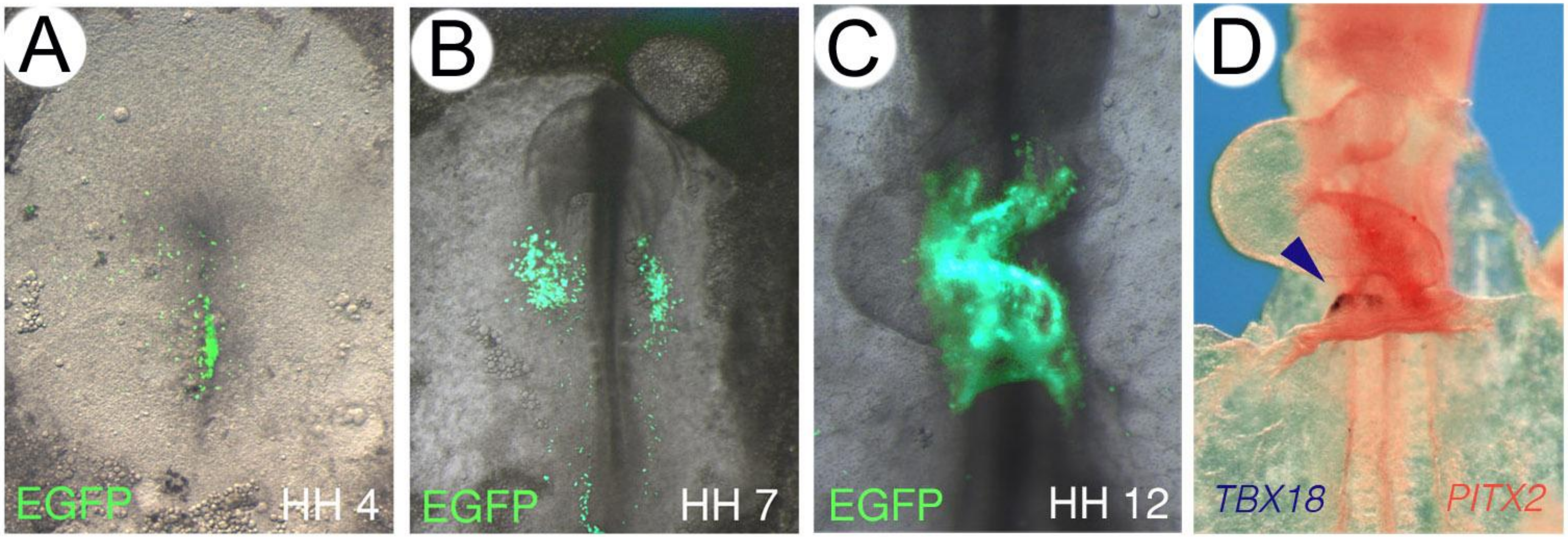




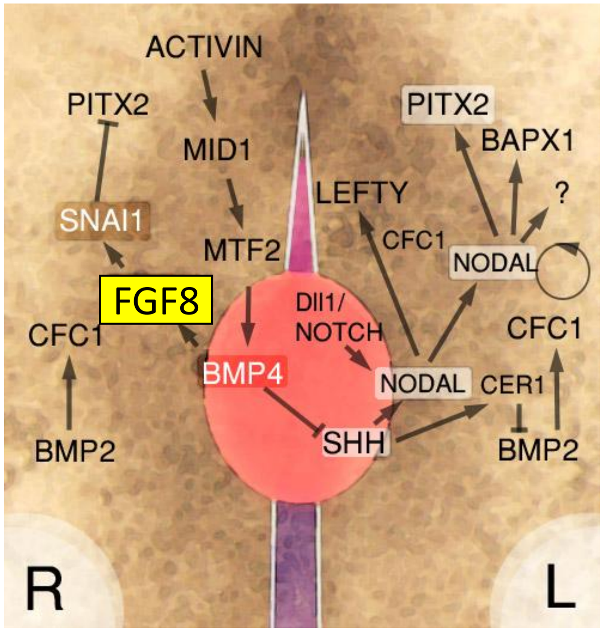


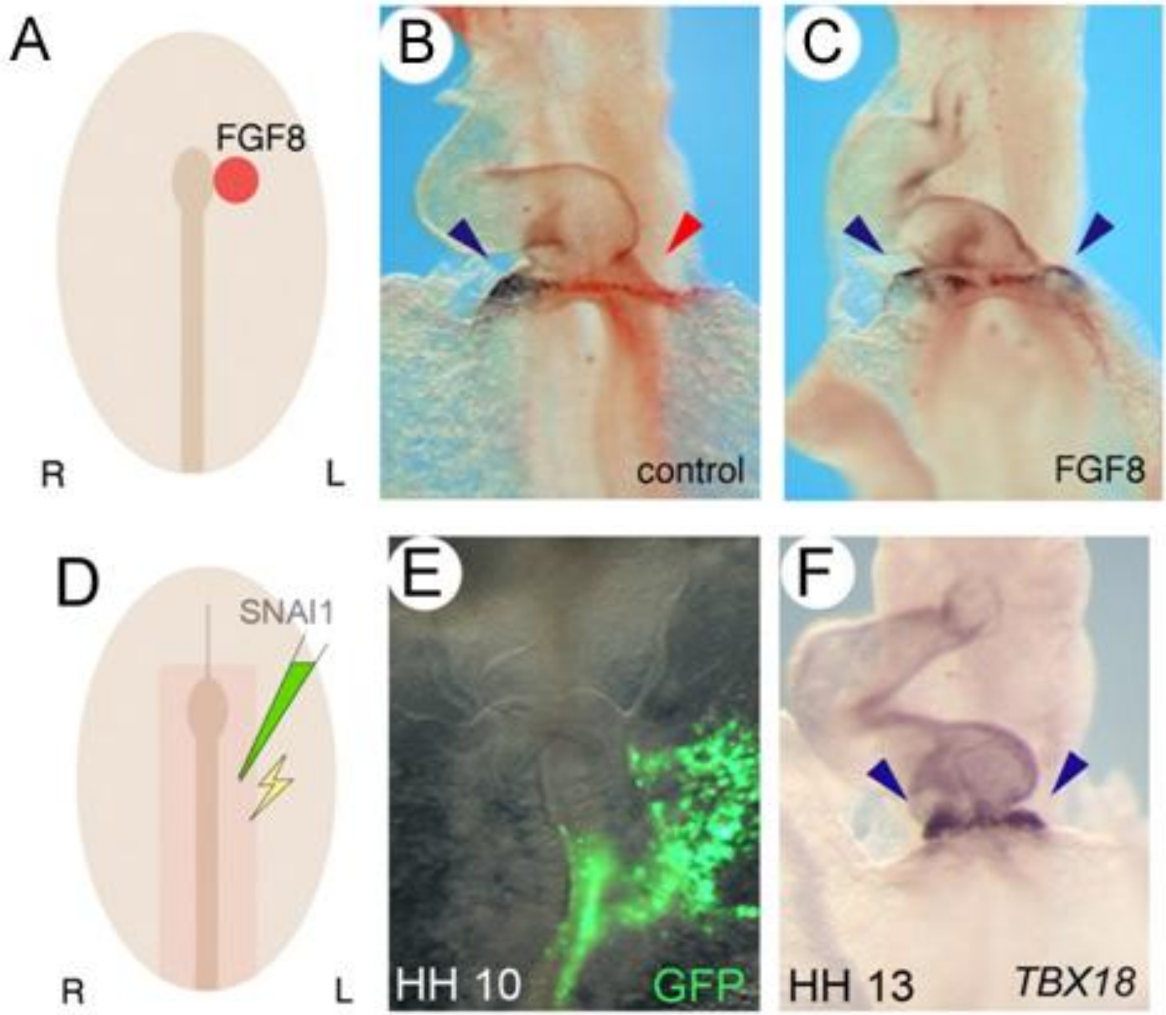
The overexpression of PITX2

- Electroporation of sinuatrial progenitors with PITX2/RCAS construct
- No effect on TBX18
- the PE is lateralized independently of the NODAL-PITX2 signaling pathway!

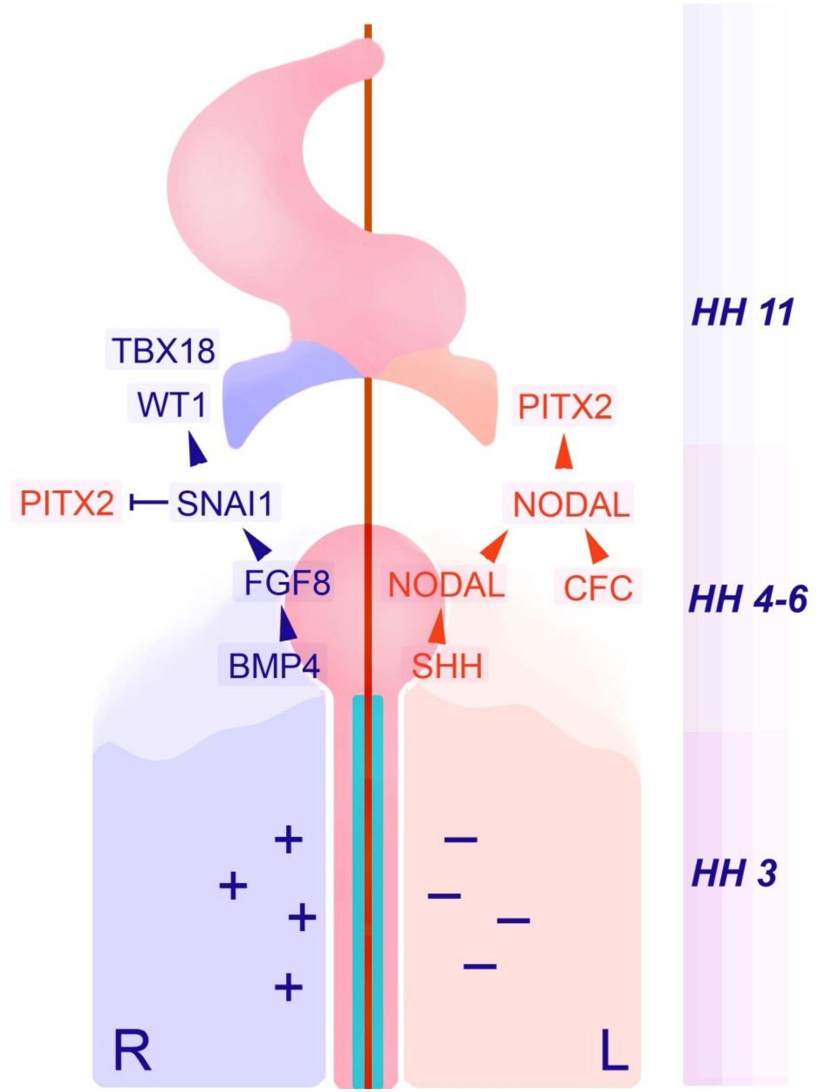


Gain-of-function of FGF8/ SNAI1 leads to bilateral PE anlagen



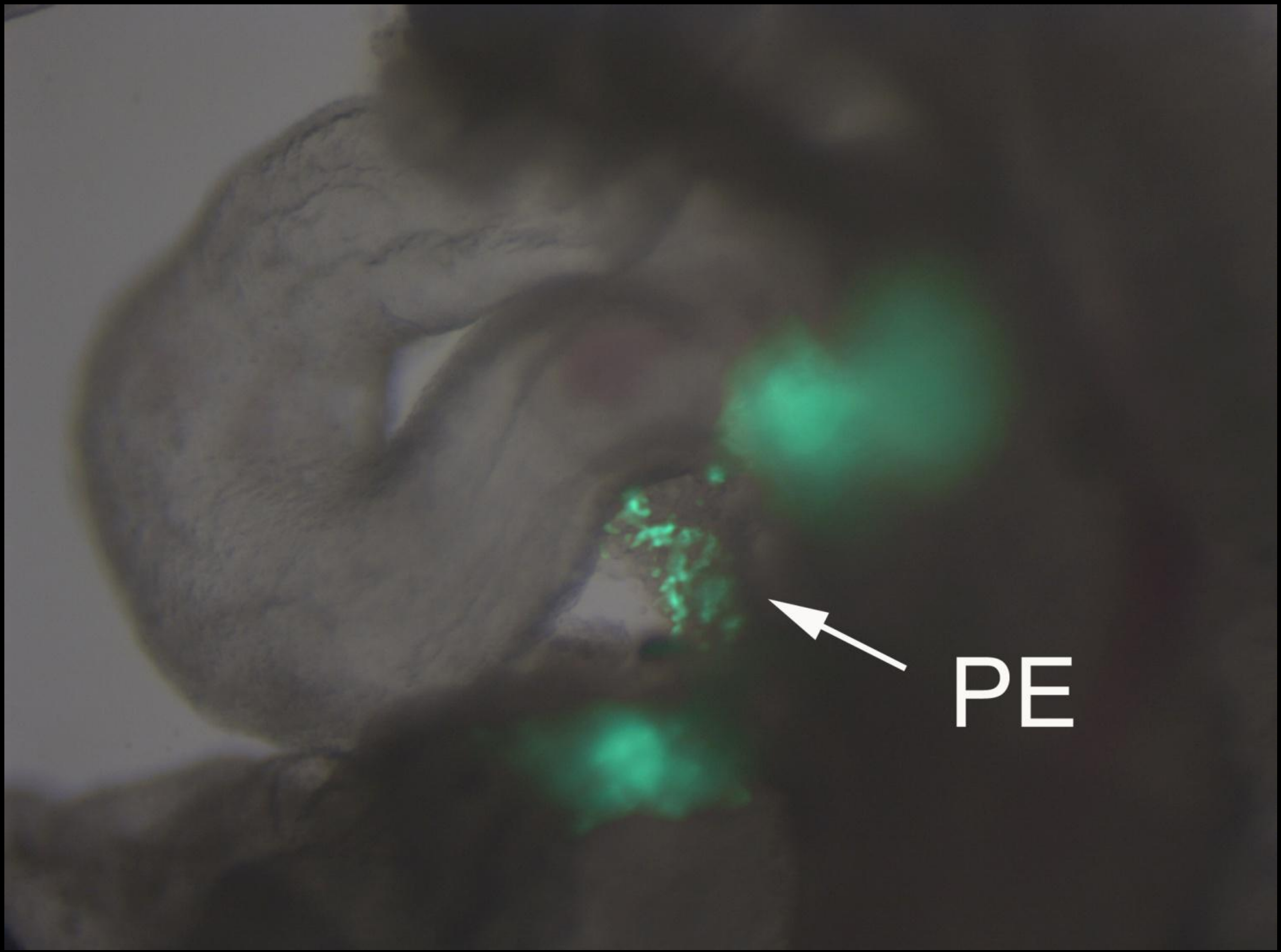


A rightsided FGF8/SNAI1 pathway induces proepicardial gene expression

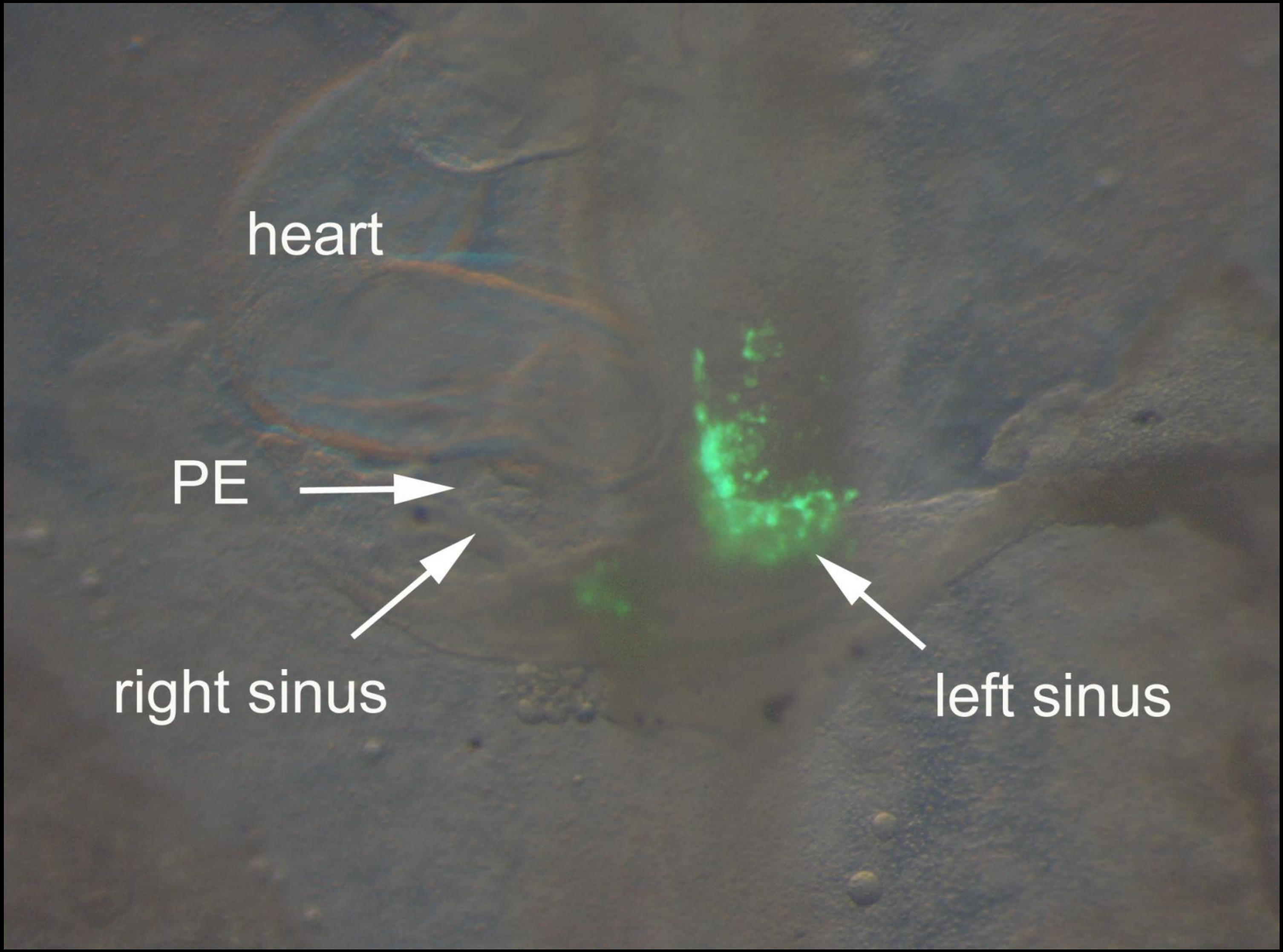


(Schlueter and Brand, 2009)

Transfection of proepicardial cells – gene delivery (live imaging)



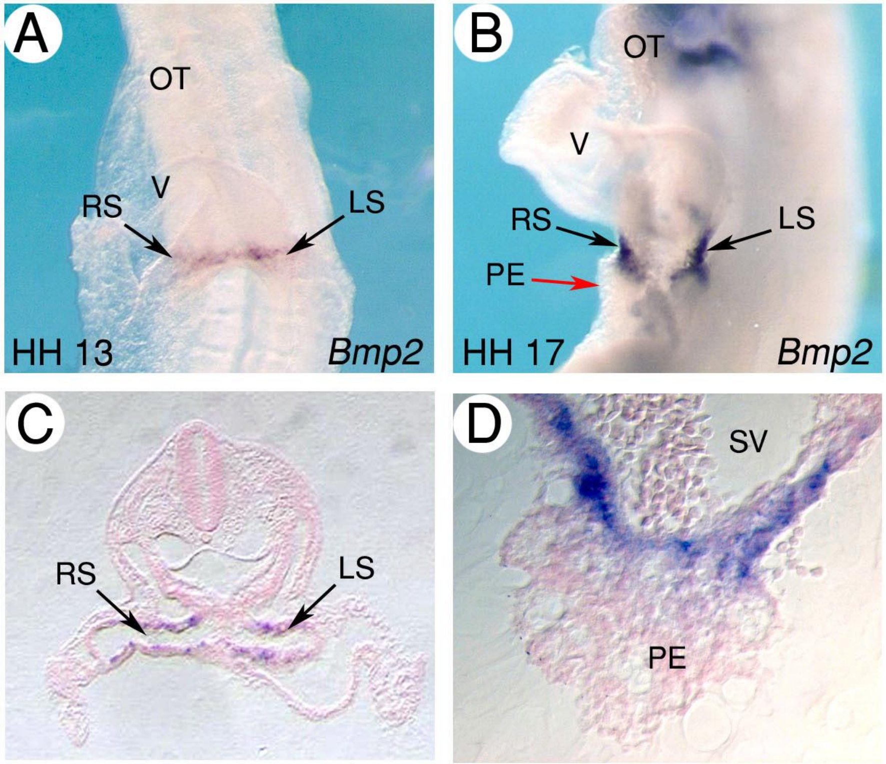
Induction of proepicardial gene expression programme on the left side

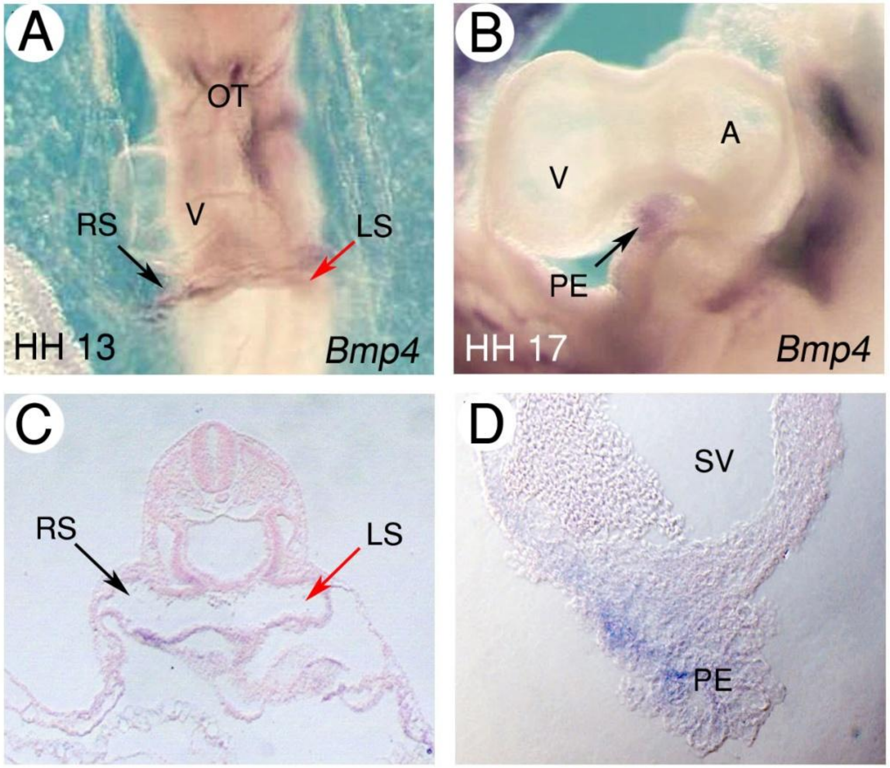


Summary 2

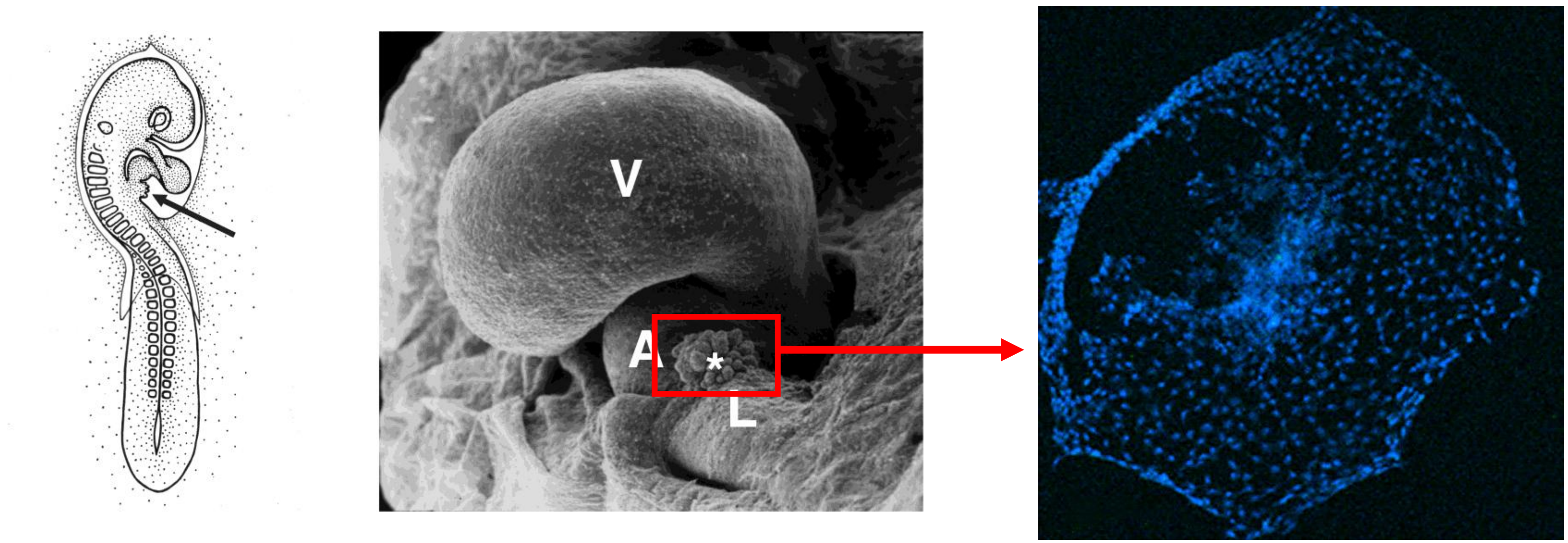
- The PE develops stronger on the right sinus venosus in lower vertebrates
- TBX18 and WT1 are expressed in the PE and the pericardium
- PE marker genes are not regulated by the NODAL/PITX2 pathway
- Instead, both marker genes are regulated by a rightsided FGF8/ SNAI1 pathway

3. The role of BMP and FGF during PE development

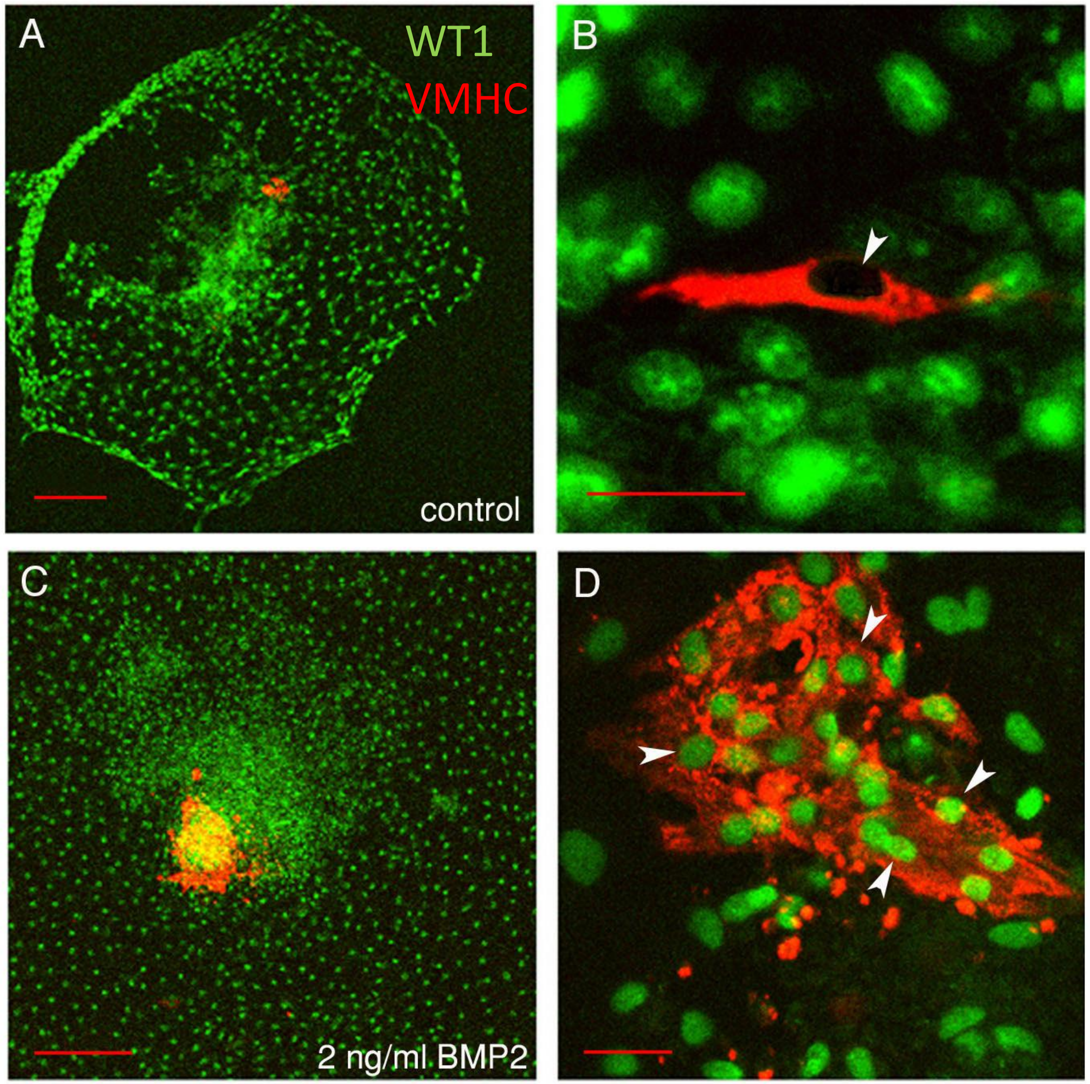




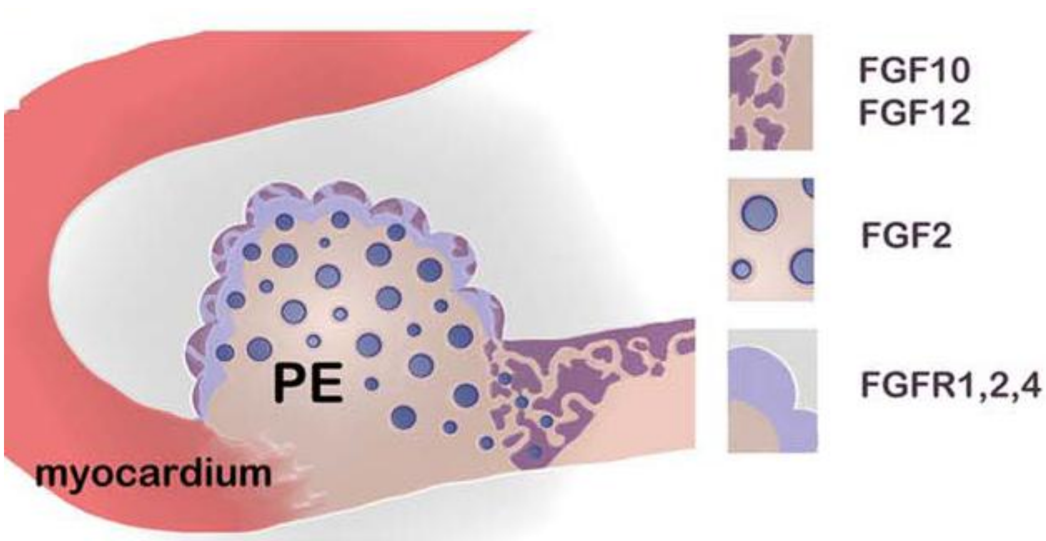
In vitro explant assay

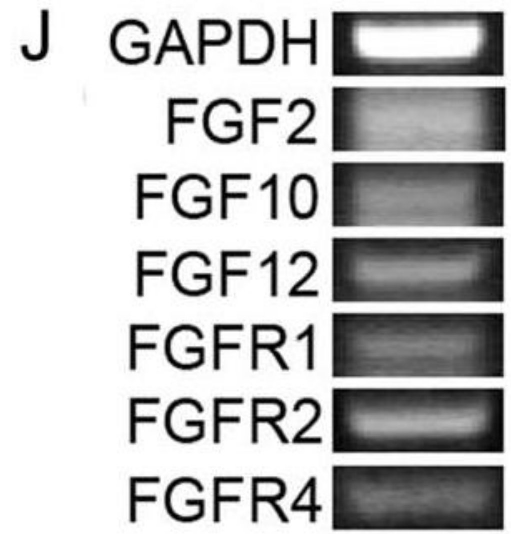


BMP promotes myocardial differentiation

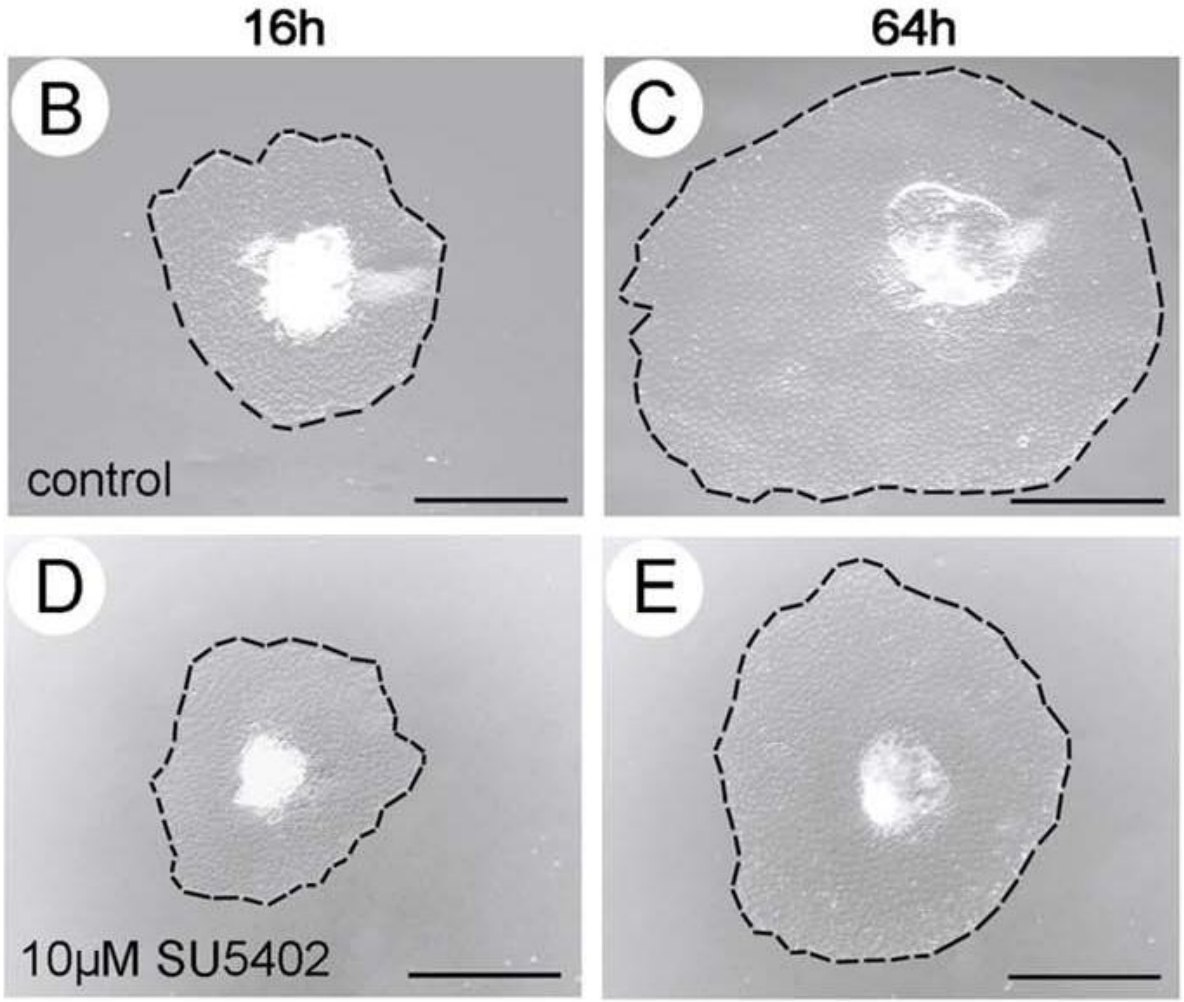


Proepicardial expression of FGF ligands and receptors

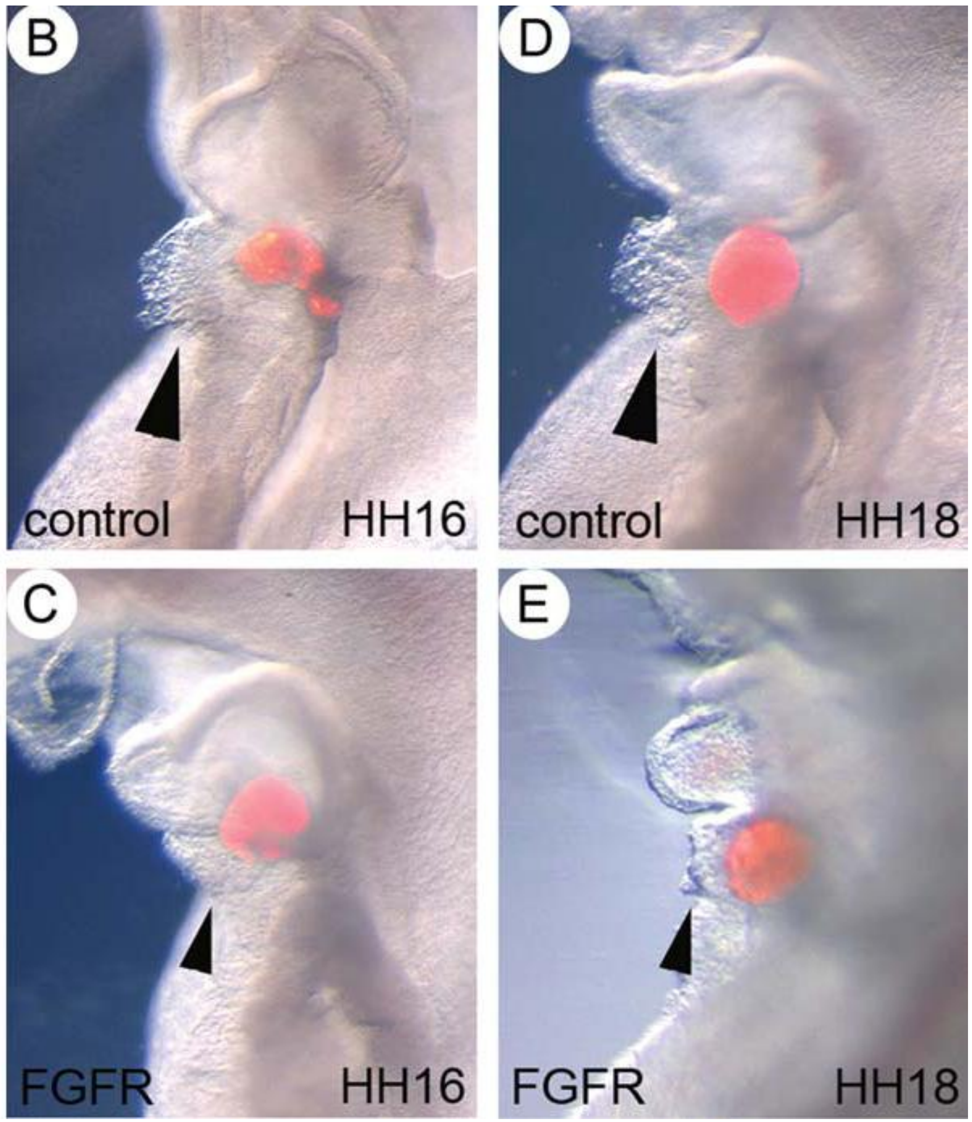




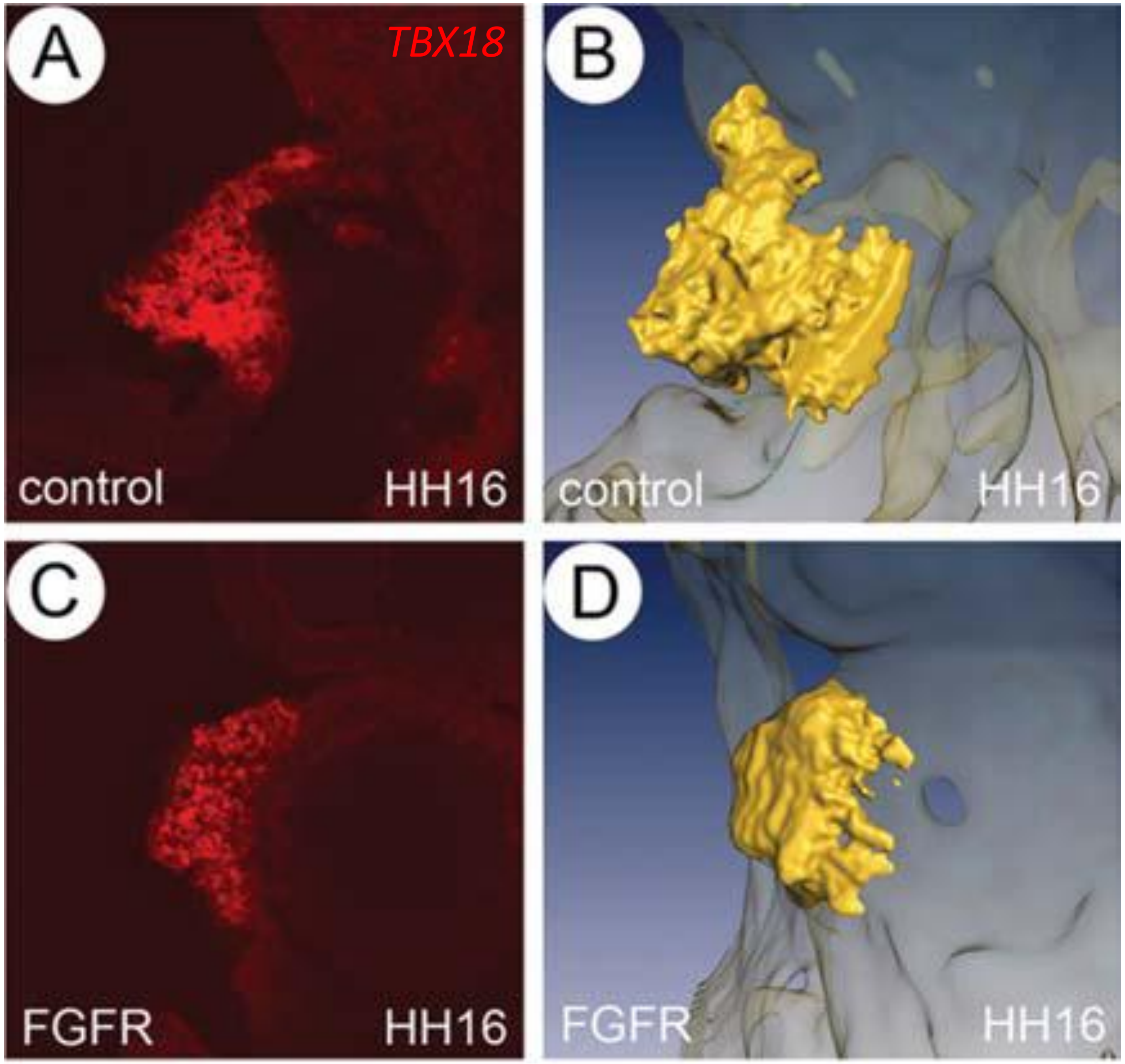
Loss of FGF signalling leads to retarded growth and cell death in PE explants



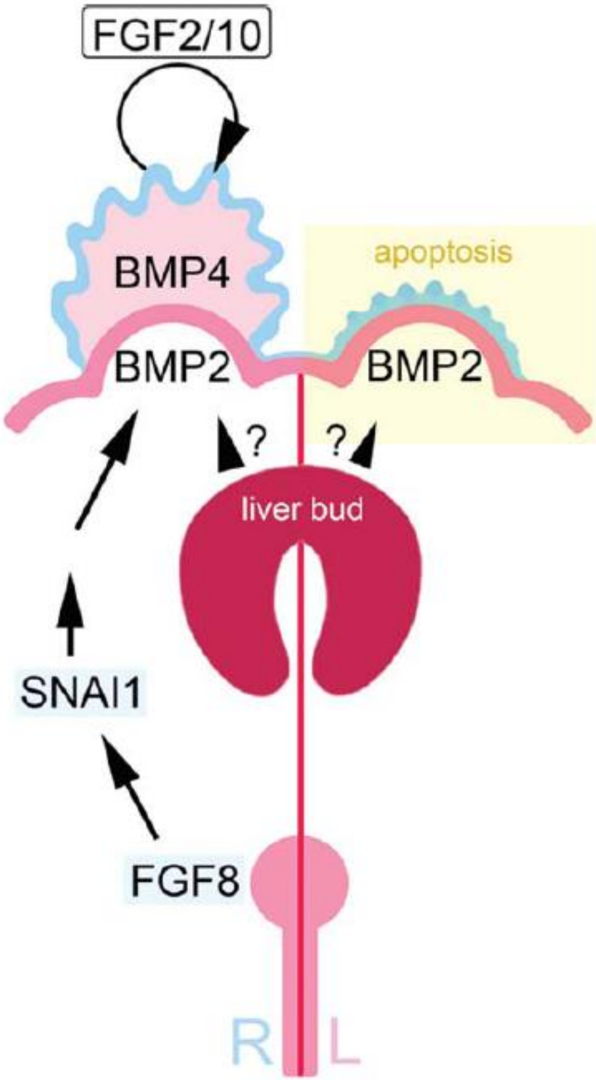
FGF promotes proliferation and survival

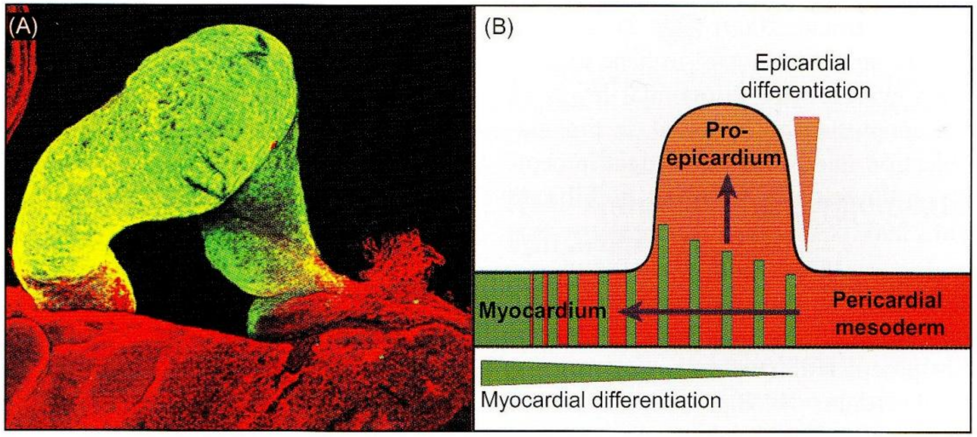


Reconstruction of proepicardial TBX18 expression domains after inhibition of FGF signals in vivo



BMP and FGF signals during PE development

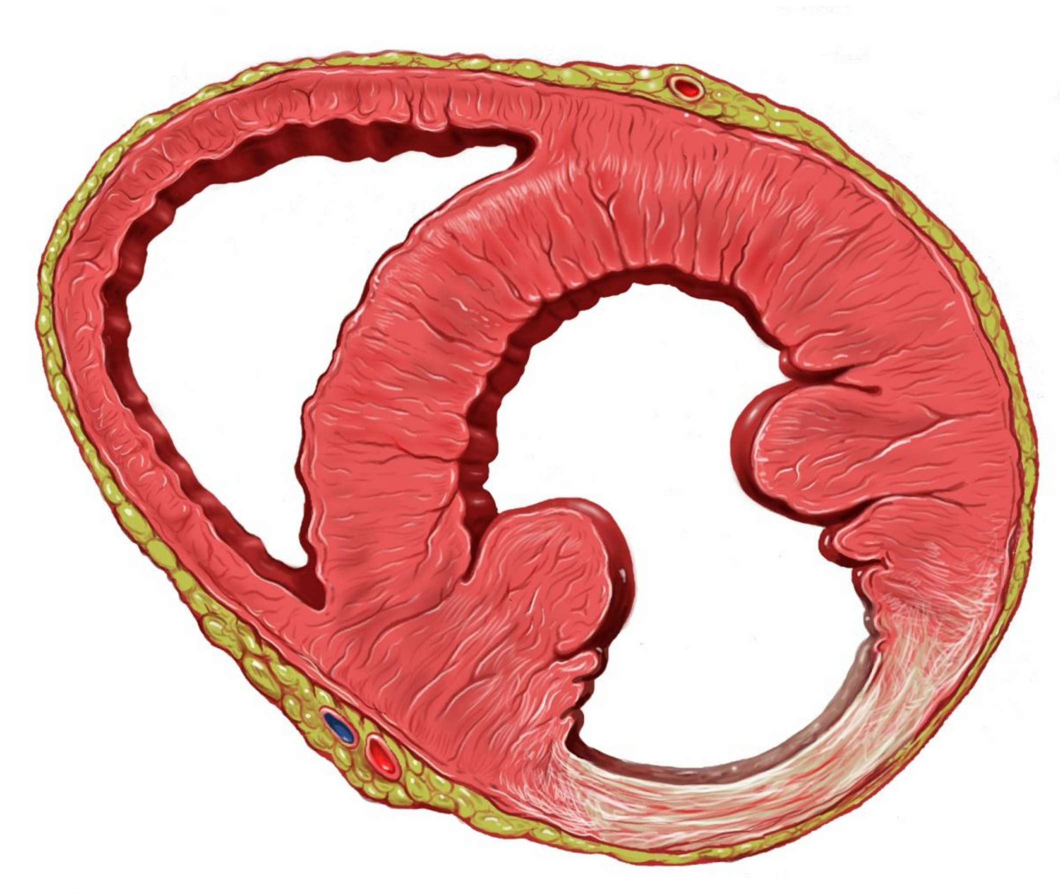




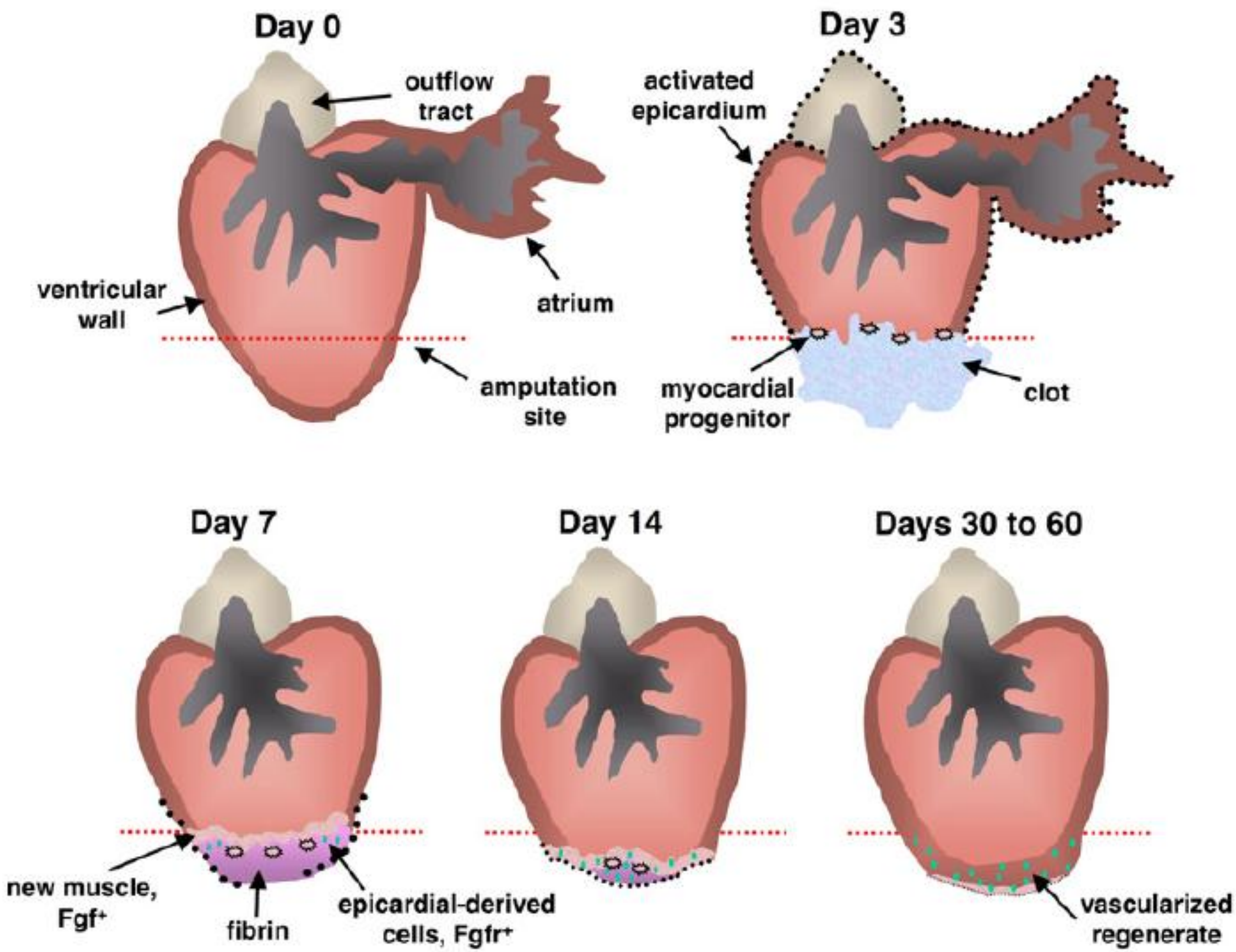
Summary 3

- BMP signalling promotes myocardial differentiation of a subset of PE cells
- High BMP dose > myocardium
- Low BMP dose > proepicardium
- FGF signalling is important for the proliferation and survival of PE cells

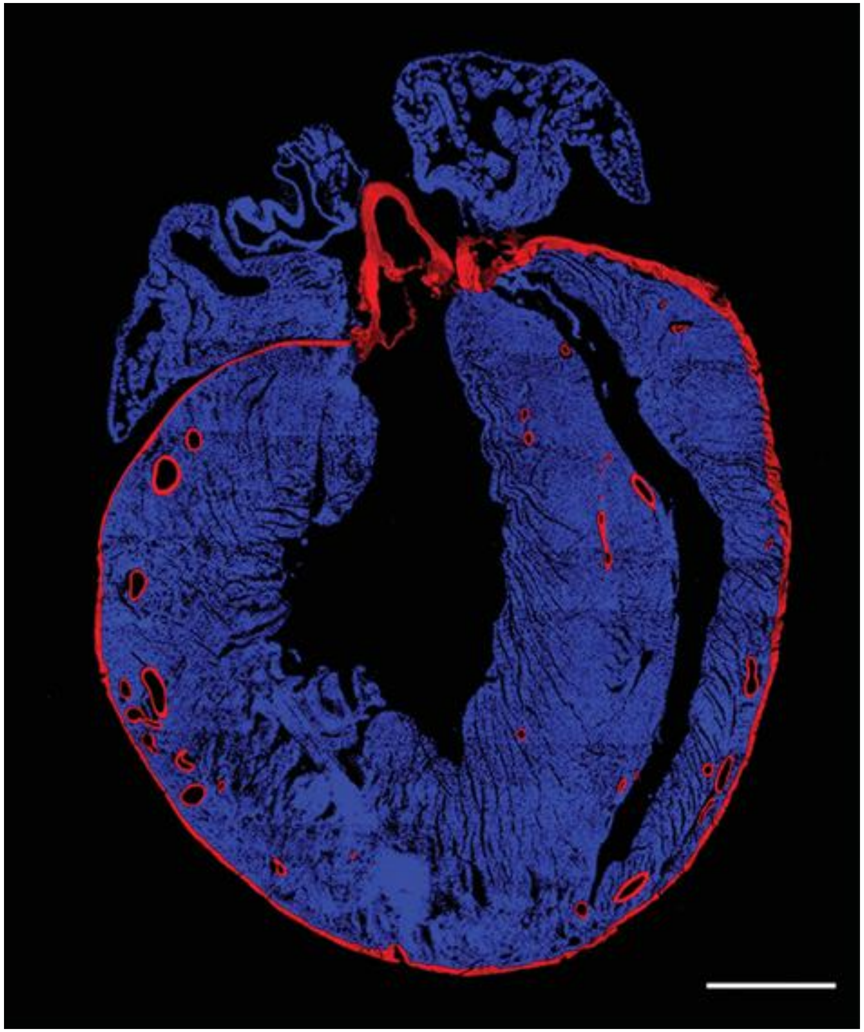
4. Neovascularization and regeneration



Process of myocardial and epicardial regeneration



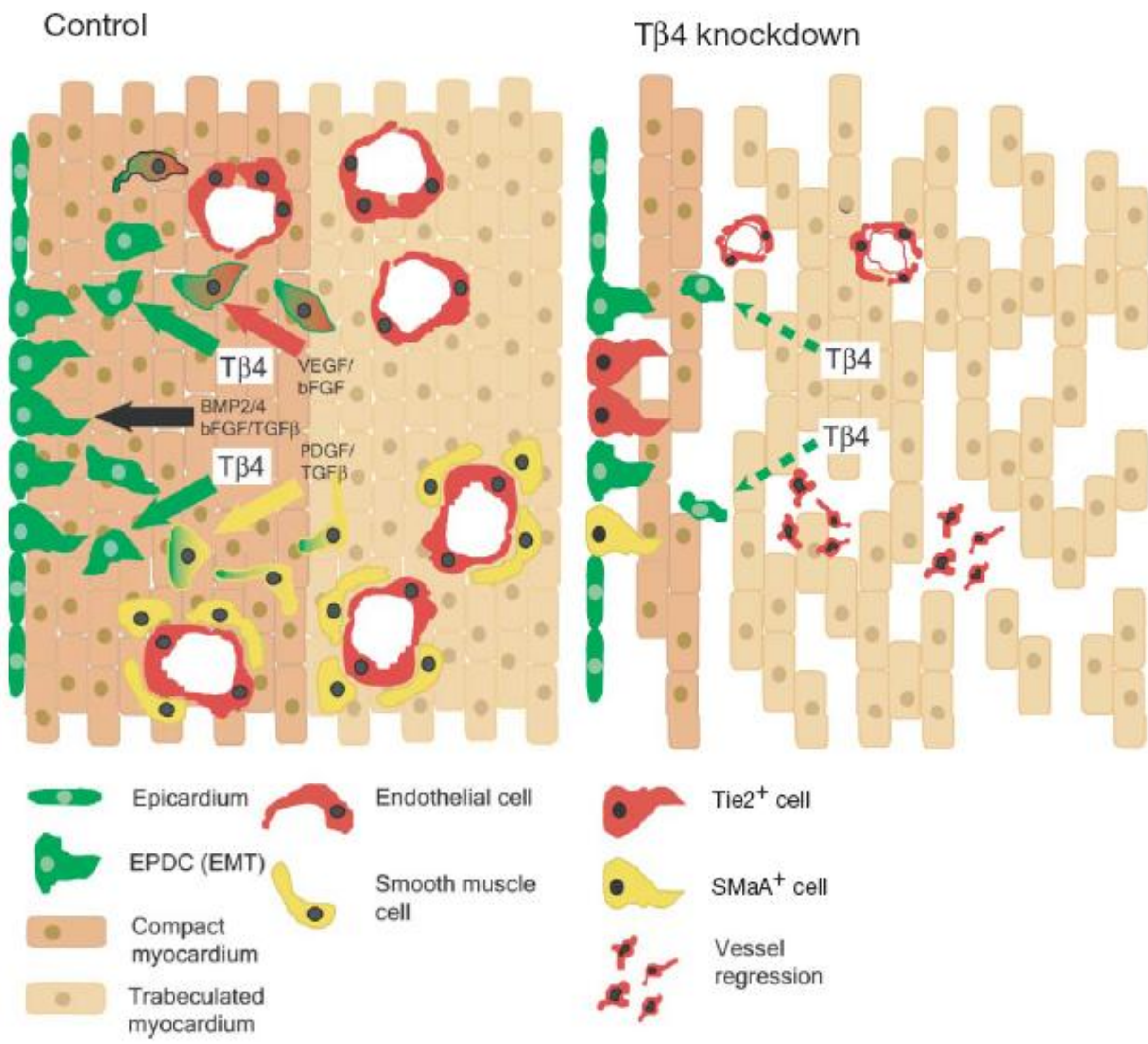
Thymosinß4 is an important regulator for neovascularization in the mouse



Thymosinß4

- Actin-monomer-binding protein which regulates cytoskeletal dynamics and directed cell movement
- Essential for migration of EPDCs (epicardial derived cells)
- Overexpression of Tß4 causes EPDCs to revert to their embryonic phenotype and give rise to endothelial and smooth muscle cells
- Thereby stimulating neovascularization of the heart

The function of Thymosinß4



Summary 4

- Cardiac failure is strongly connected to ischaemic damage caused by vascular insufficiency
- The zebrafish heart is exceptional for its natural capacity to regenerate muscle tissue facilitated by reactivation of coronary vascularization
- Therefore the investigation of early developmental regulation of epicardium formation provides insight into myocardial and epicardial de/redifferention processes in the adult heart
- Thymosinß4 has been shown to act as a powerful factor to regulate coronary vessels development as well as to promote neovascularization in the adult mouse heart

literature

- Lepilina, A., Coon, AN., Kikuchi, K., Holdway., JE, Roberts., RW, Burns., CG, Poss KD. (2006). A dynamic epicardial injury responnse supports progenitor cell activity during zebrafish heart regeneration. *Cell* 127, 607-19
- Männer, J. (1999). Does the subepicardial mesenchyme contribute myocardioblasts to the myocardium of the chick embryo heart? A quail-chick chimera study tracing the fate of the epicardial primordium. *Anat Rec* 255**,** 212-226.
- Männer, J., Perez-Pomares, J. M., Macias, D., and Munoz-Chapuli, R. (2001). The origin, formation and developmental significance of the epicardium: a review. *Cells Tissues Organs* 169, 89-103.
- Torlopp, A., Schlueter, J., Brand, T., (2010). Role of fibroblast factor signaling during proepicardium formation in the chick embryo. *Dev Dyn.* 239, 2393-403
- Schlueter, J., Brand, T. (2009). A rightsided pathway involving FGF8/SNAI1 controls asymmetric development of the proepicardium in the chick embryo. *PNAS* 106, 7485-90
- Schlueter J., Brand T. Left-right axis development: examples of similar and divergent strategies to generate asymmetric morphogenesis in chick and mouse embryos. *Cytogenet Genome Res* 117, 256-67. Review.
- Schulte I., Schlueter J., Abu-Issa R., Brand T., Männer J. (2007). Morphological and molecular left-right asymmetries in the development of the proepicardium: a comparative analysis on mouse and chick embryos. *Dev Dyn* 236, 684-95.
- Smart, N., Rossdeutsch, A., Riley ,PR. (2007). Thymosin beta4 and angiogenesis: modes of action and therapeutic potential. *Angiogenesi*s 10, 229-41. Review.