Metody studia Wnt signalizace

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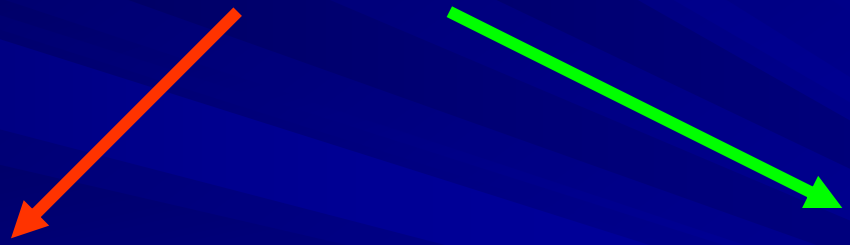
&
Oddělení cytokinetiky, Biofyzikální ústav AV ČR


MOVIE
Zebrafish.mov

Wnts (Wingless/Int)

- family of ligands
- 19 members in human and mouse
- glycosylated and palmitoylated extracellular proteins
- short range of action, bind to extracellular matrix
- only in multicellular animals



canonical

(eg. Wnt-1 or Wnt-3a)

non-canonical

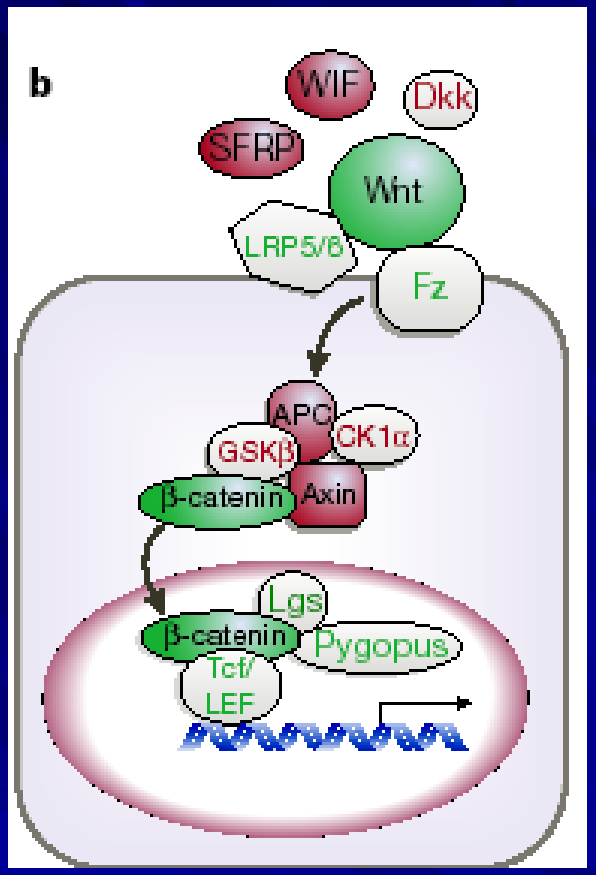
(eg. Wnt-5a)

Canonical Wnt signalling

- eg. Wnt-1 or Wnt-3a

- induce axis duplication in Xenopus
- induce transformation of mammary cell line C57mg
- signals via nuclear translocation of β-catenin

Canonical Wnt signalling and tumours



according to Beachy et al., Nature 2004

Wnt pathway		
Colon	Adenocarcinoma	Tumorigenesis by inactivation of APC, Axin; tumorigenesis by stabilization of β-catenin; epigenetic inactivation of SFRPs
Liver	Hepatoblastoma	Tumorigenesis (in mouse) by inactivation of APC and by stabilization of β-catenin
Blood	Multiple myeloma	Cell-growth inhibition by dominant negative TCF4; growth stimulation by Wnt ligand
Hair follicle	Pilomatricoma	Tumorigenesis (in mouse) by overexpression of β-catenin
Bone	Osteosarcoma	Dkk3 and LRP5 expression inhibits tumour cell growth *in vitro*
Lung	Non-small-cell carcinoma	Apoptosis and cell-growth inhibition by short intefering RNA and a blocking antibody against Wnt2
Pleura	Mesothelioma	Apoptosis and cell-growth inhibition by transfection of SFRP

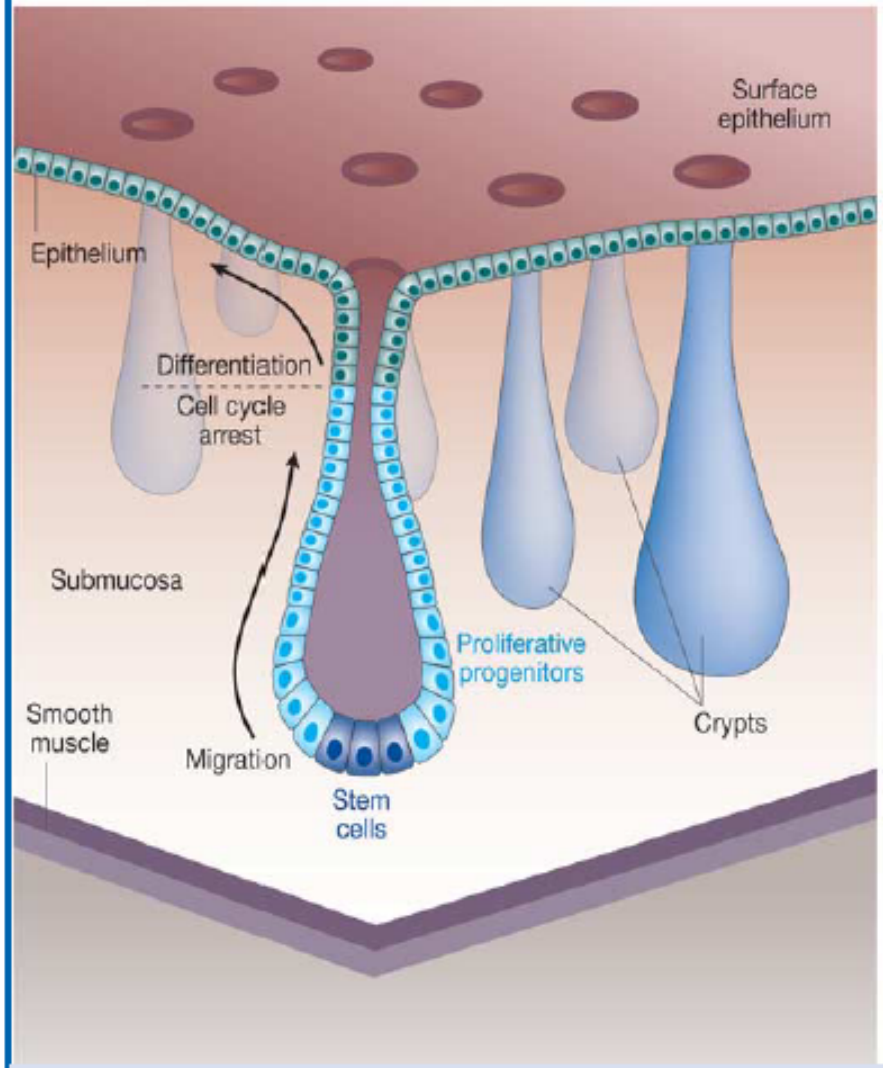


Figure 3 Tissue anatomy of the colonic epithelium. Putative stem cells (dark blue) reside at the crypt bottom. Proliferating progenitor cells occupy two-thirds of the crypt. Differentiated cells (green) populate the remainder of the crypt and the flat surface epithelium. (Adapted from ref. 89.)

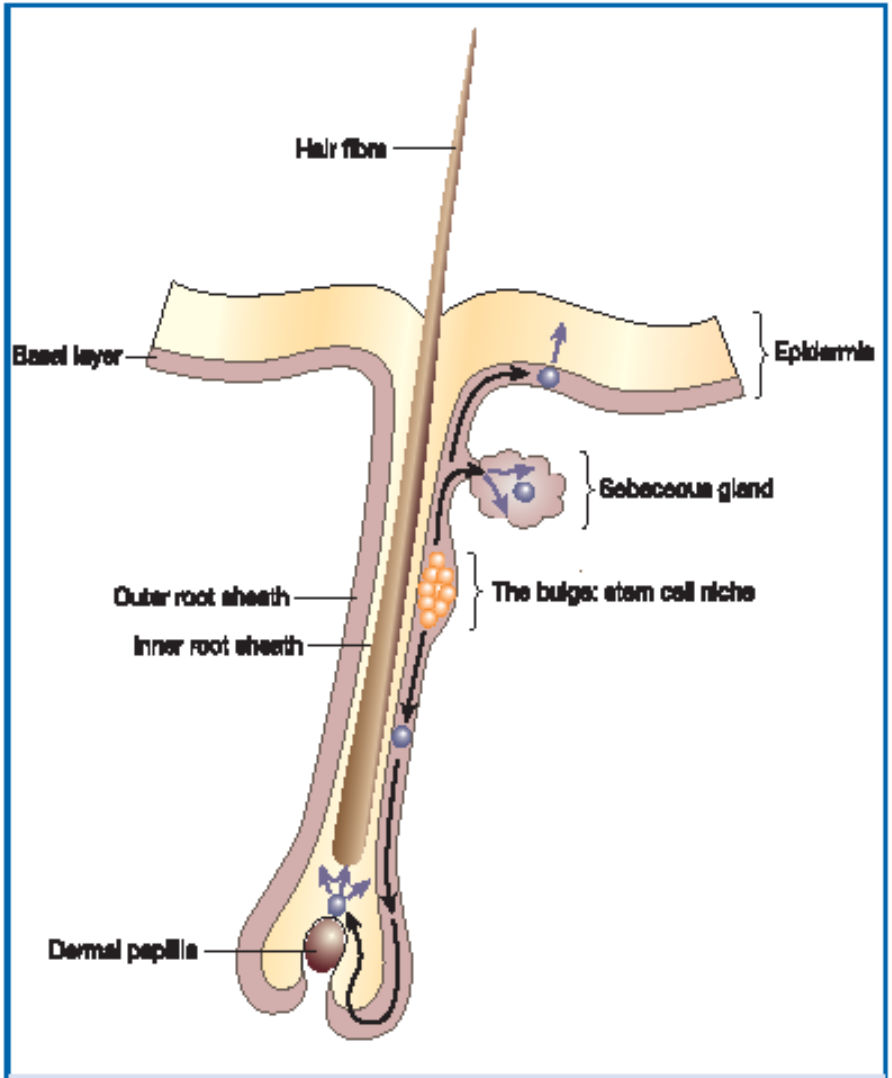
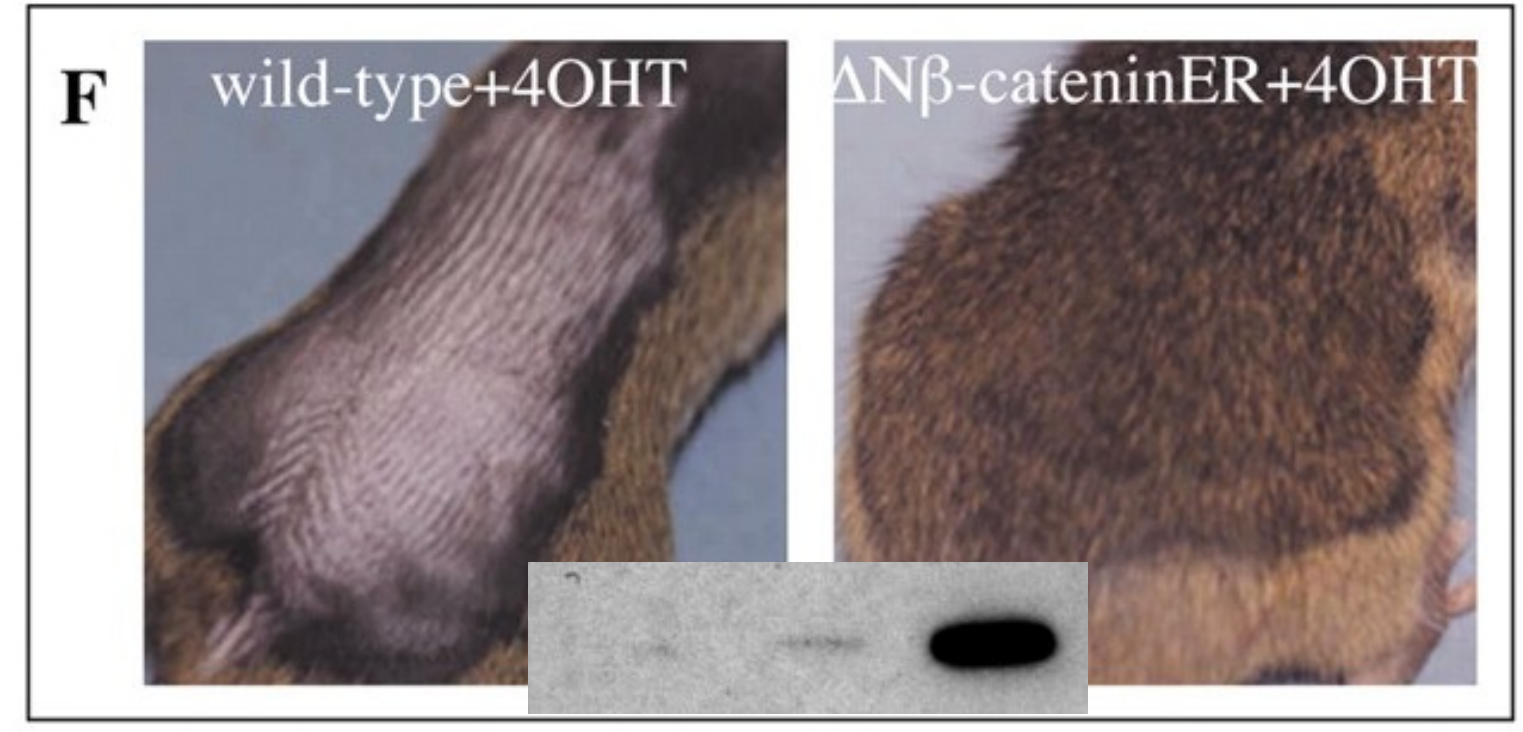


Figure 4 The hair follicle. Stem cells reside in the bulge niche. Cells can migrate upwards from here to populate the sebaceous gland and the interfollicular epidermis. Cells that migrate downwards enter the matrix where they rapidly proliferate and then differentiate to form the hair. (Adapted from ref. 90.)

Reya & Clevers 2005, Nature

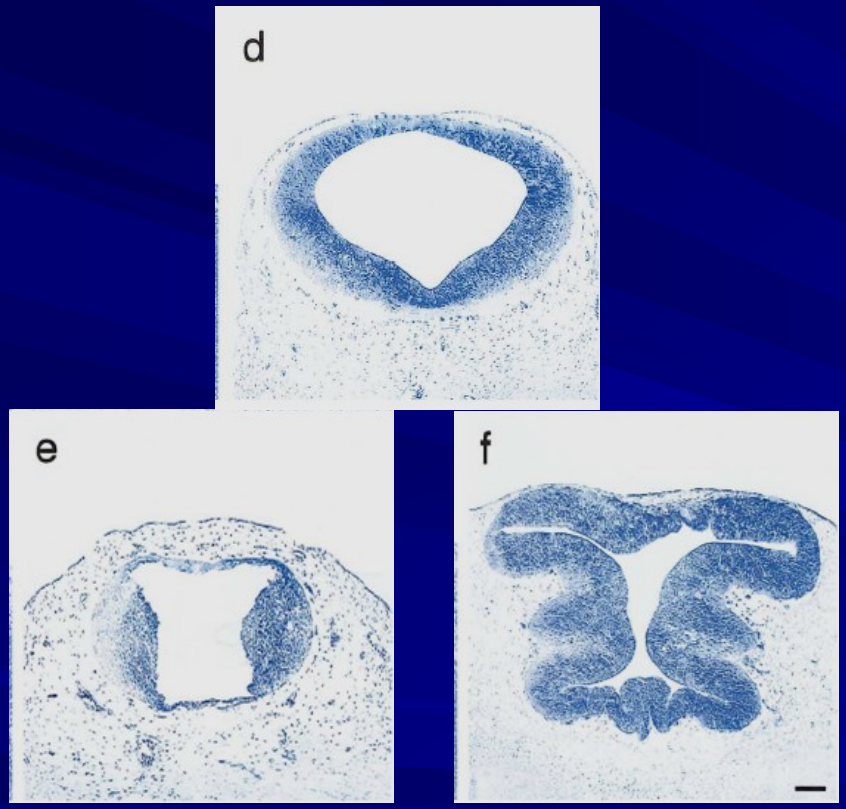
Consequences of β-catenin activation in the epidermis (keratin 14 promotor):



Lo Celso, C. L. et al. Development 2004;131:1787-1799

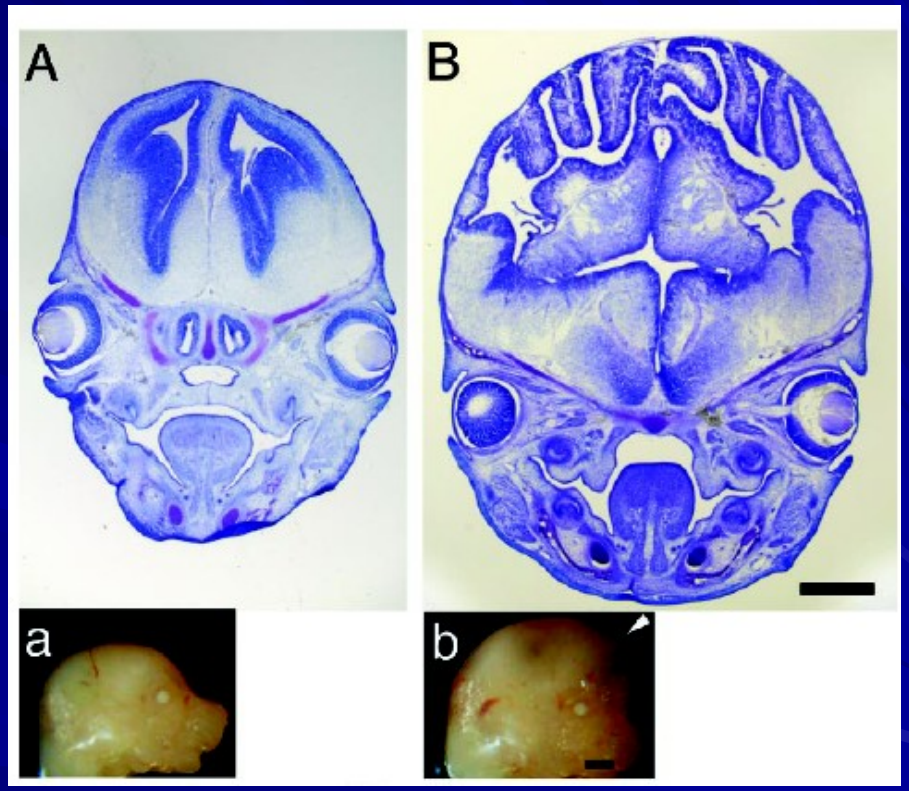
Consequences of β-catenin activation in the brain:

midbrain (Brn4-promotor)



Zechner et al., 2003: Dev. Biol.;258:406-418.

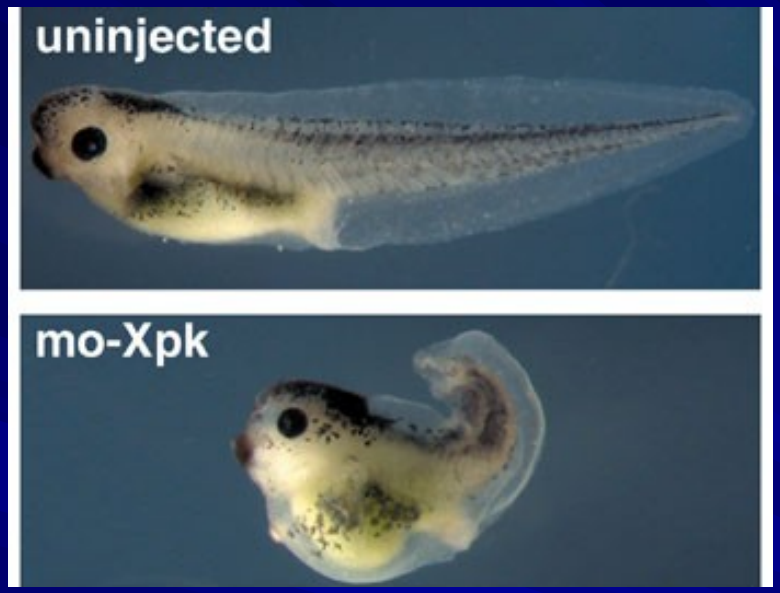
cortex (nestin enhancer)



Chenn & Walsh, 2002: Science;297:365-369.

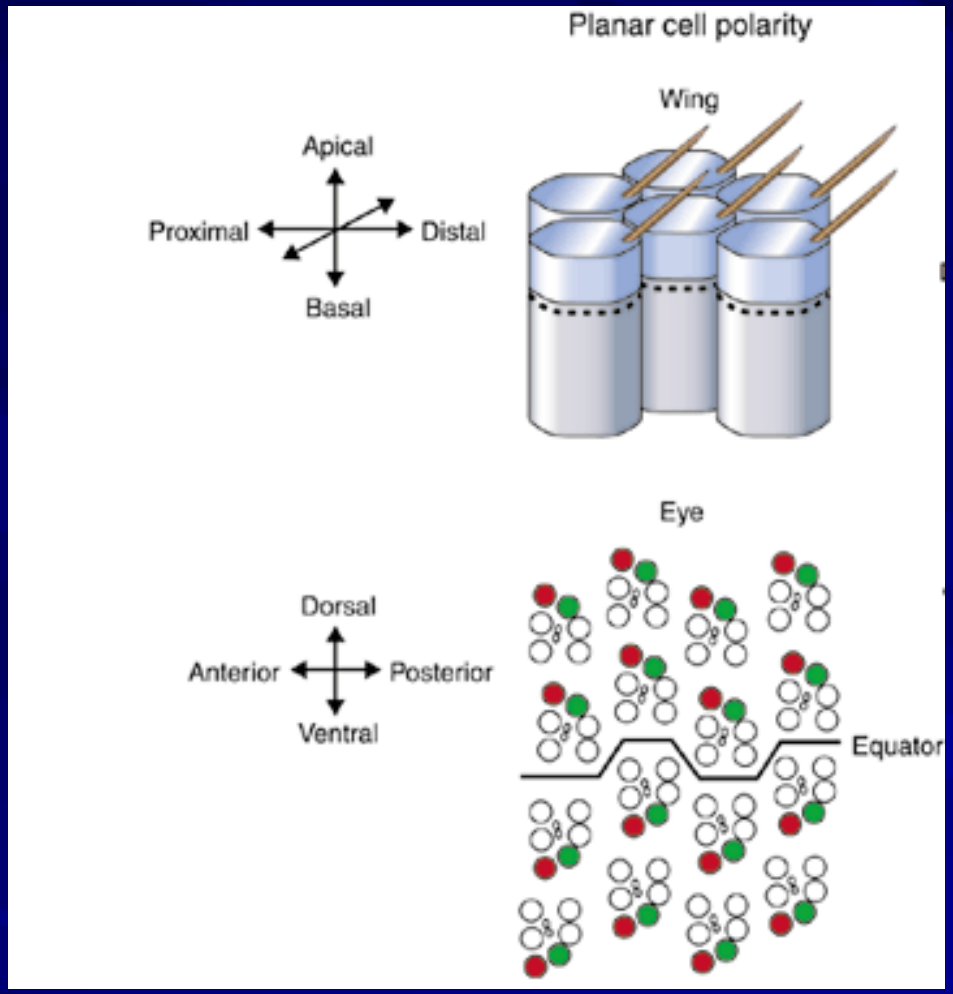
Non-canonical Wnt signalling

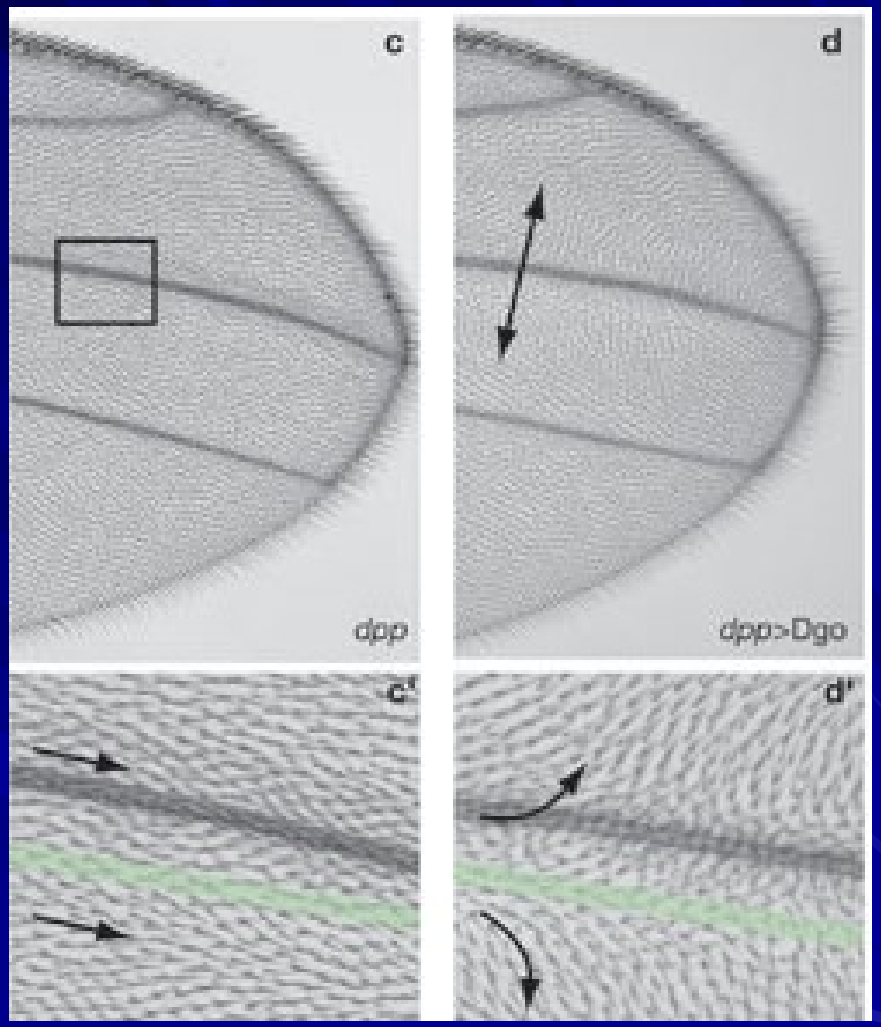
- e.g. Wnt5a



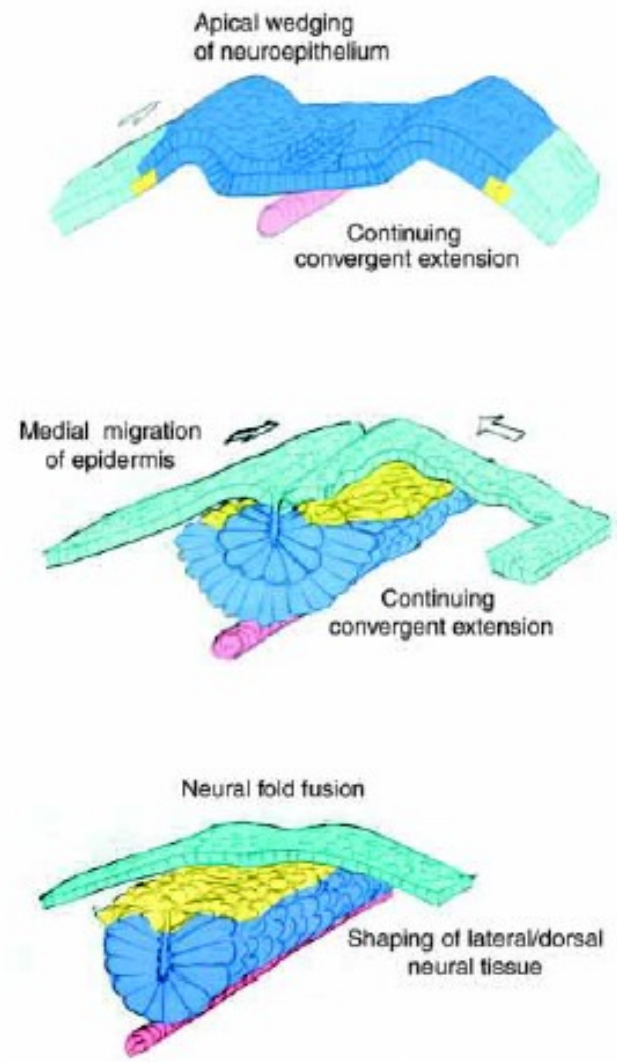
- do not induce axis duplication in Xenopus
- do not induce transformation of mammary cell line C57mg
- do not signal via nuclear translocation of β-catenin

Drosophila – PCP (planar cell polarity)

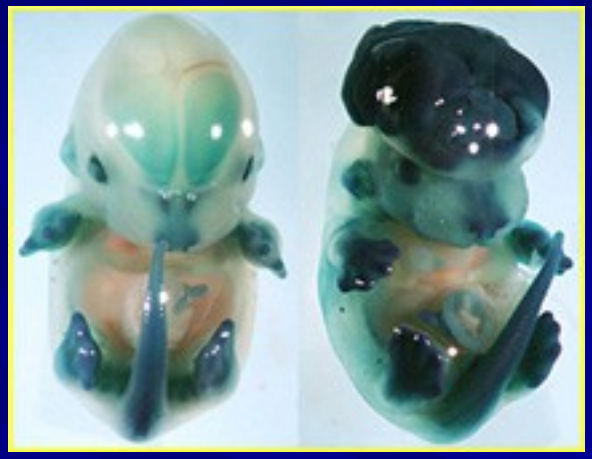




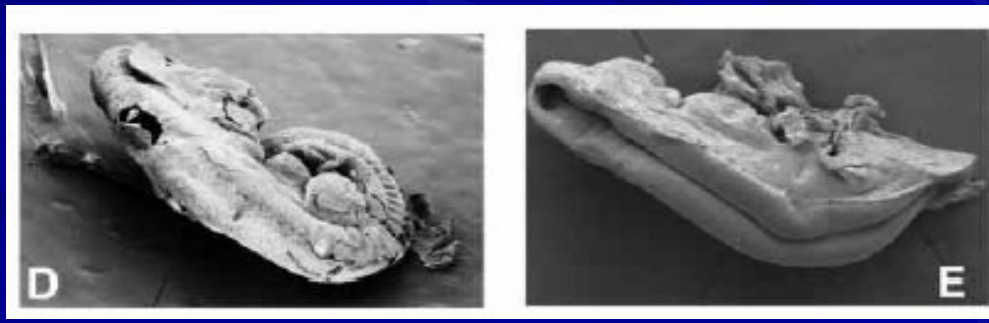
Non-canonical/PCP (Planar cell polarity) pathway defects cause neural tube closure phenotypes in mouse (and human)



Exencephaly

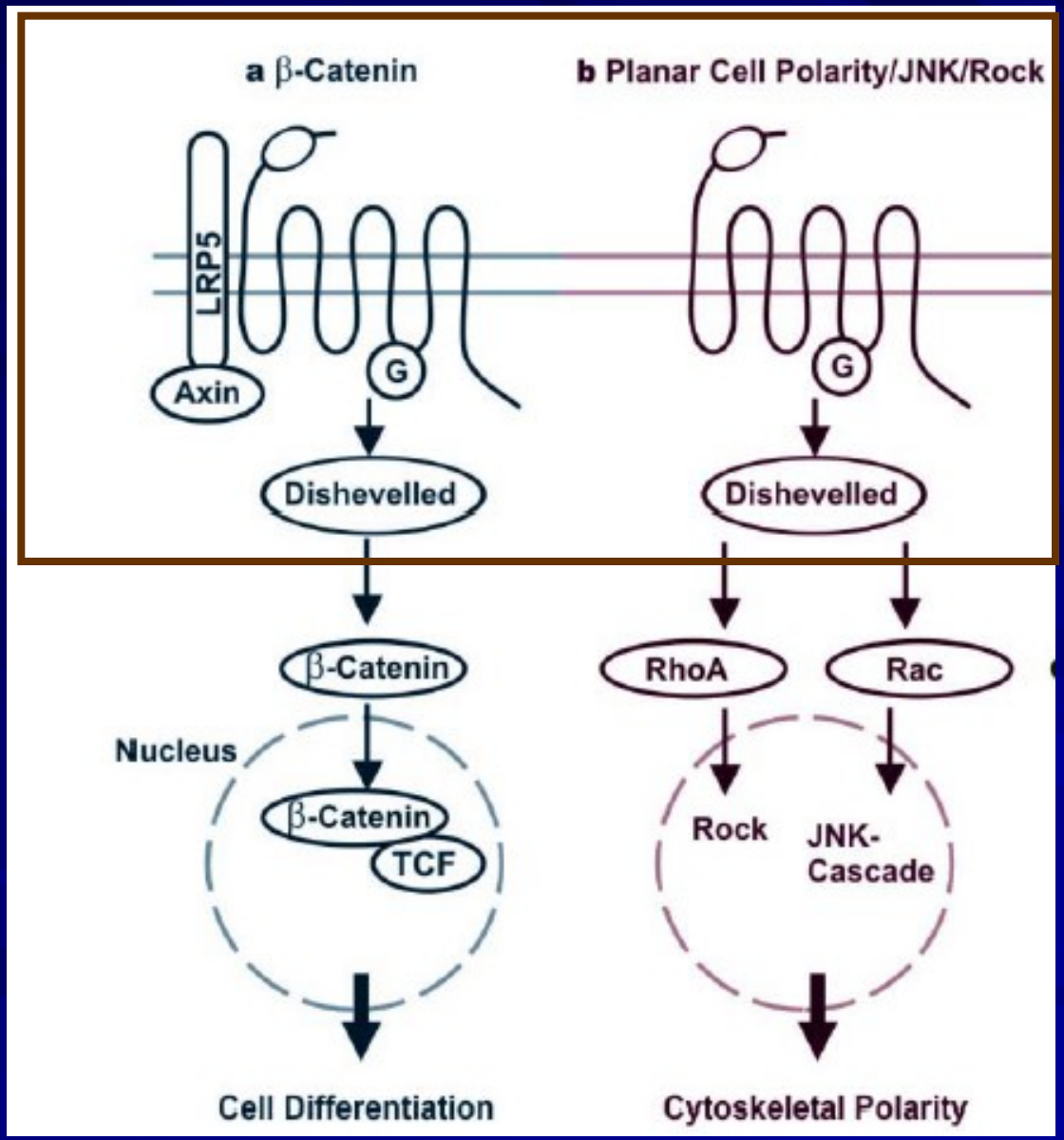


Open neural tube



Hamblet et al., 2002, Development

Fundamental question of Wnt signalling: How the specificity is achieved?



Vincan, 2004, Frontiers in Bioscience

DISHEVELLED

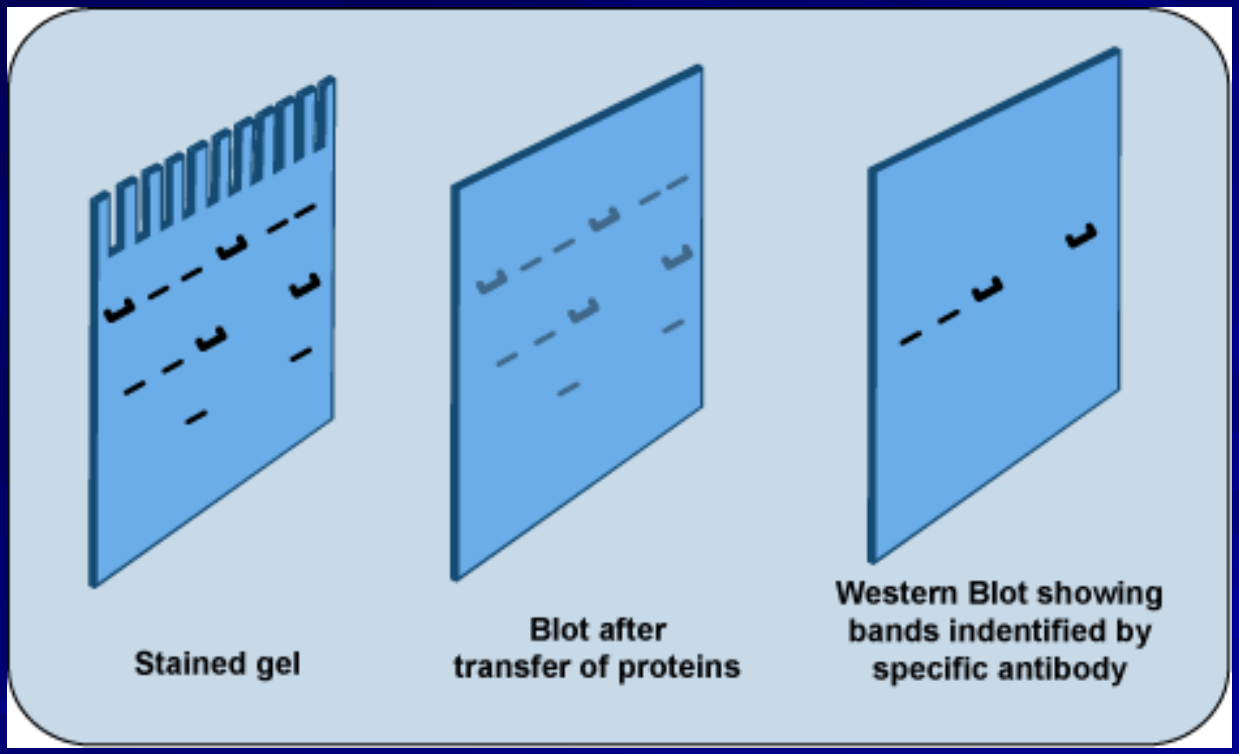
(Dvl – mouse, XDsh – Xenopus, Dsh – Drosophila)

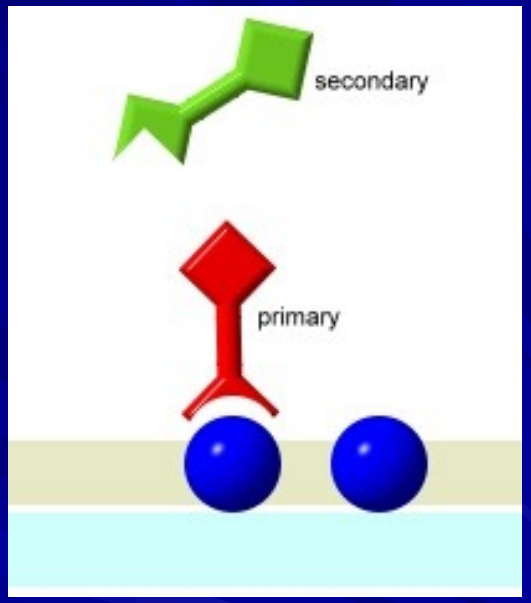
-phosphoprotein – phosphorylated by numerous kinases, significance often unknow

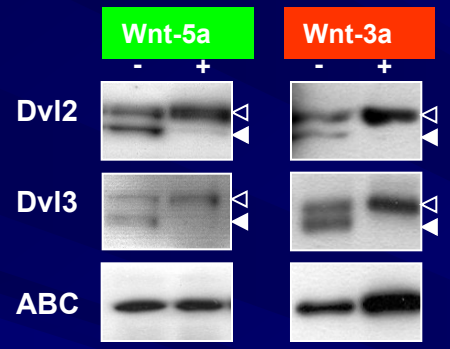
- acts between Frizzled and downstream signalling components

- required for signal transduction of all Wnt signalling pathways

Metoda 1: Western blotting





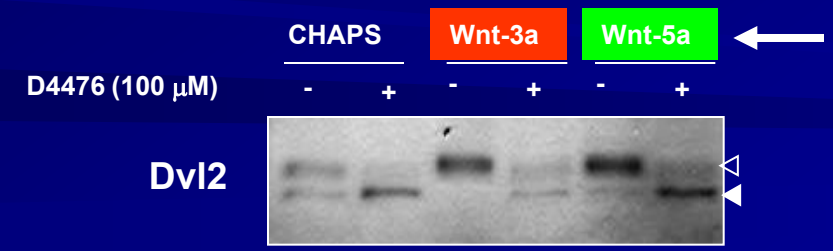


ABC – active β-catenin = β-catenin dephosphorylated on GSK3β target sites

Dvl – Dishevelled – activated by phosphorylation detected as phosphorylation dependent mobility shift

◁ PS-Dvl

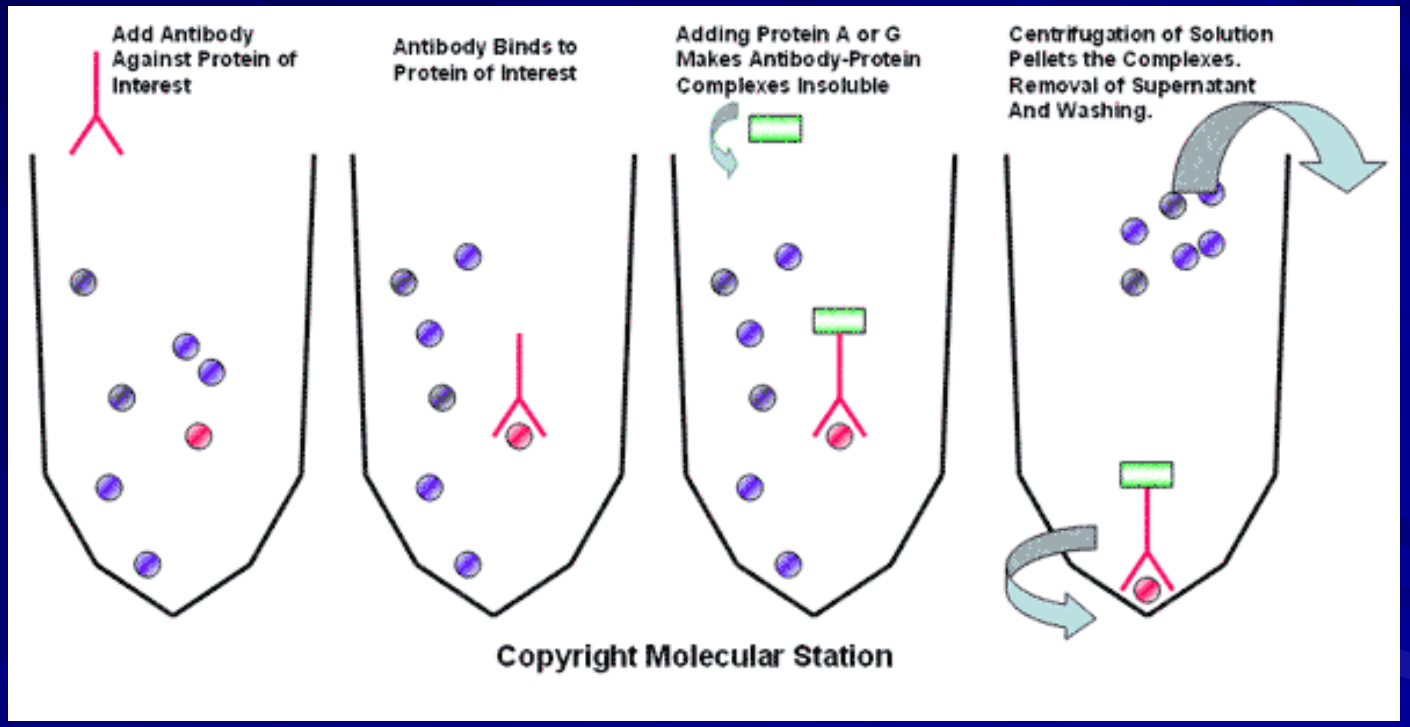
Compound	Target	Concn	Activity
PTX	Galpha i/o	100 ng/ml	No
PDBu	PKC activator	1 μM	No
Wortmannin	PI3K	50 nM	No
LY294002	PI3K	50 μM	No
PD98059	MEK1/2	10 μM	No
UO126	MEK1/2	10 μM	No
SB203580	p38	10 μM	No
JNKII inhib	JNK	6 μM	No
Genistein	PKC	50 μM	No
chelerythrine	PKC	10 μM	No
Ro-31 8220	PKC	1 μM	No
BIM I	PKC	500 nM	No
KN93	CamKII	10 μM	No
I3M	GSK-3	2 μM	No
Kenpaullone	GSK-3	6 μM	No
H89	PKA	10 μM	No
8-Br-cAMP	cAMP pathway activator	10 μM	No
8CPT-2Me-cAMP	EPAC activator	30 μM	No
SQ22536	Adenylyl cyclase	100 μM	No
MDL12330	Adenylyl cyclase	10 μM	No
PP2	Src-like	10 μM	No
AG1276	EGFR	10 μM	No
ET-18-OCH3	PLC	10 μM	No
D4476	**Casein kinase 1**	**100 μM**	**Yes**
staurosporin	Ser/Thr kinases, PKC	2 μM	No



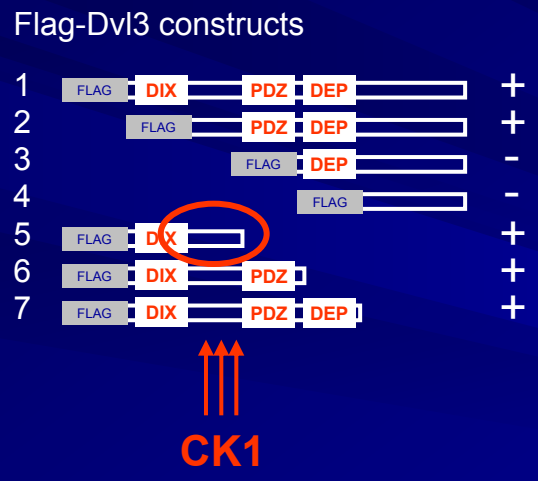
Both Wnt-3a and Wnt-5a activate Dvl2 and Dvl3 via casein kinase 1 (CK1)

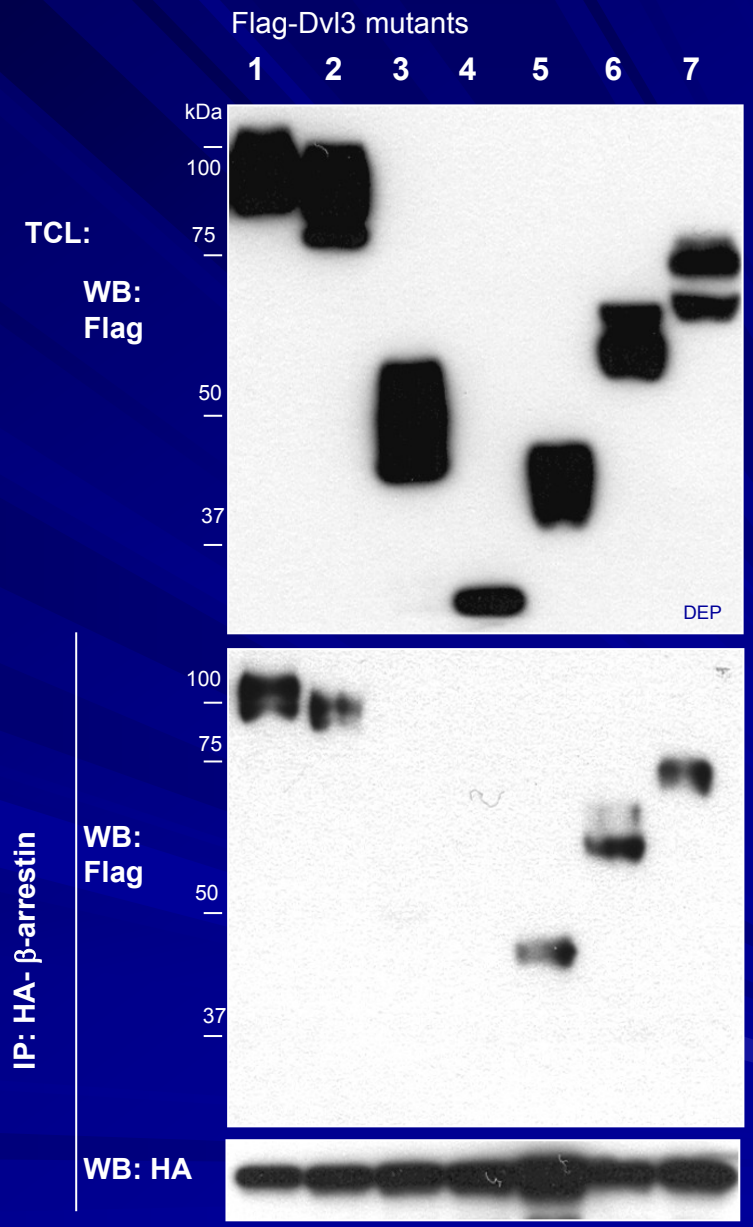
Bryja et al., J. Cell Sci., 2007

Metoda 2: Immunoprecipitace

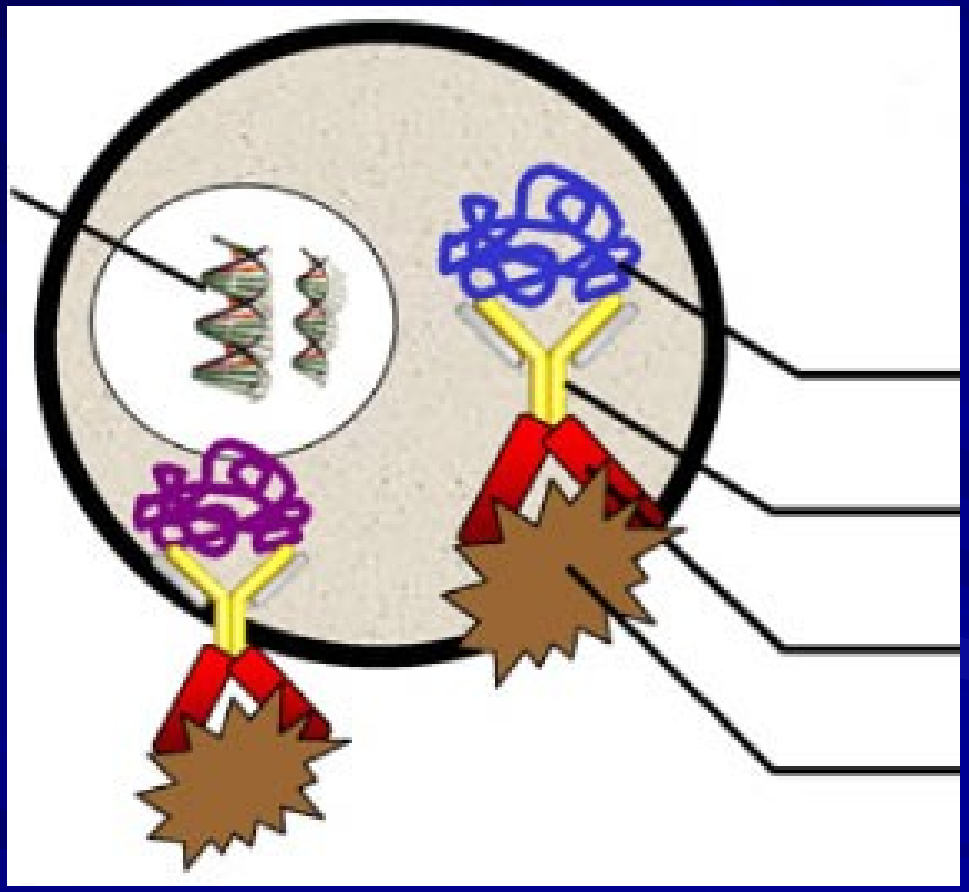


β-arrestin binds Dishevelled

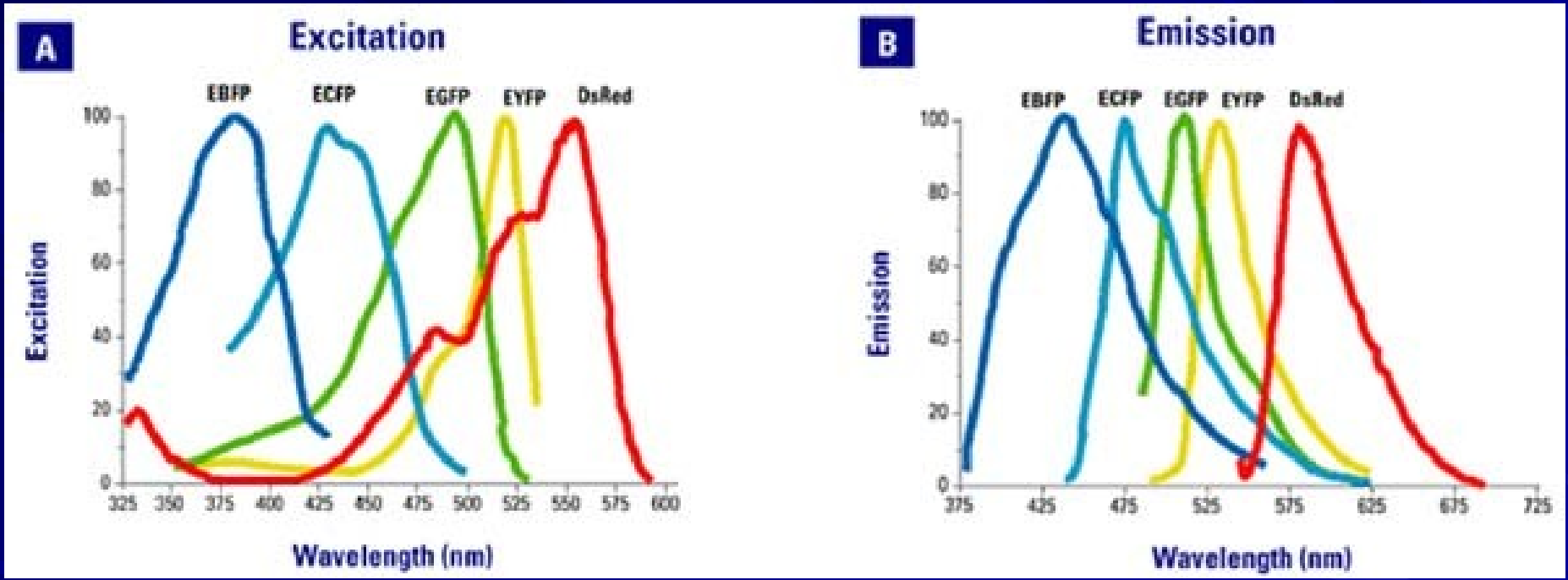




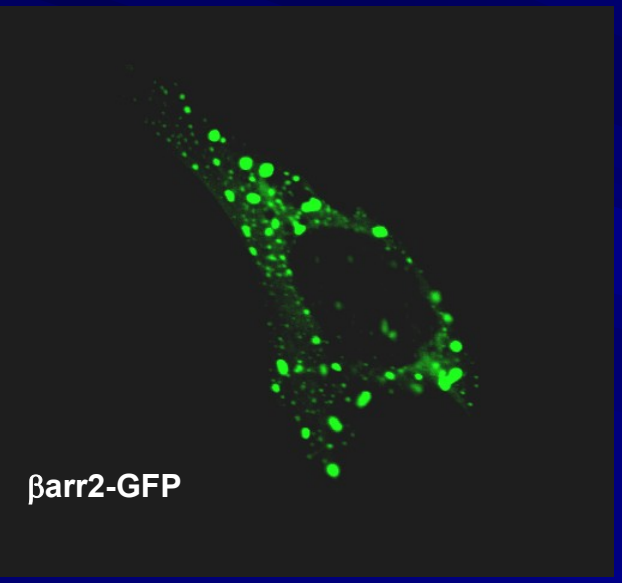
Metoda 3: Immunocytochemistry

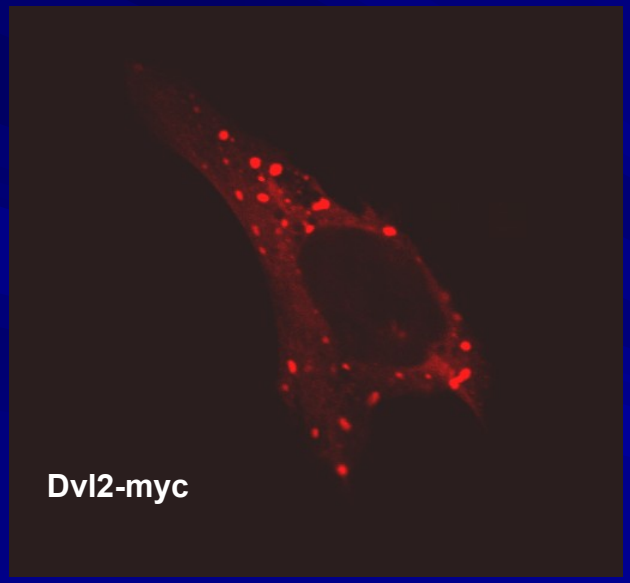


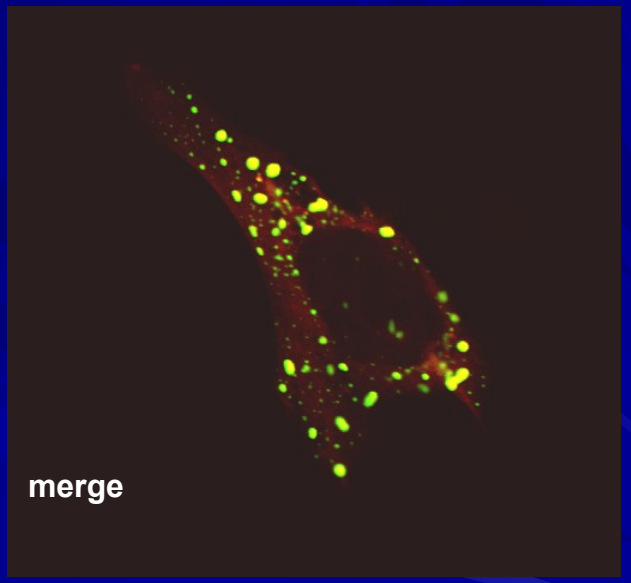
Fluorescenční proteiny



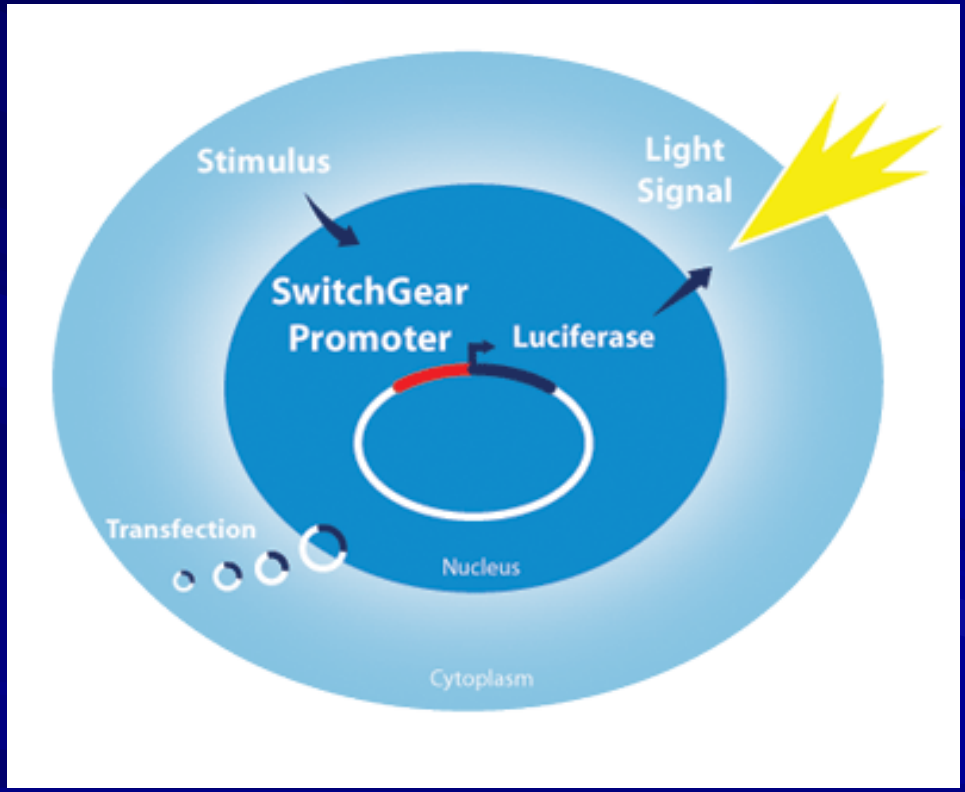
β-arrestin co-localizes with Dvl in the cytoplasm





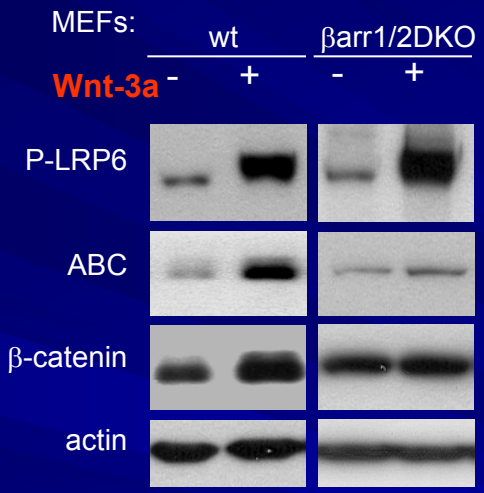


Metoda 4: Reporter assays

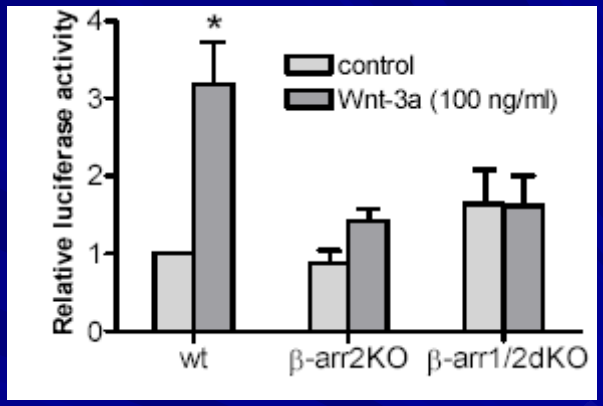


1. β-arrestin is required for β-catenin activation in vitro

β-arrestin deficient MEFs

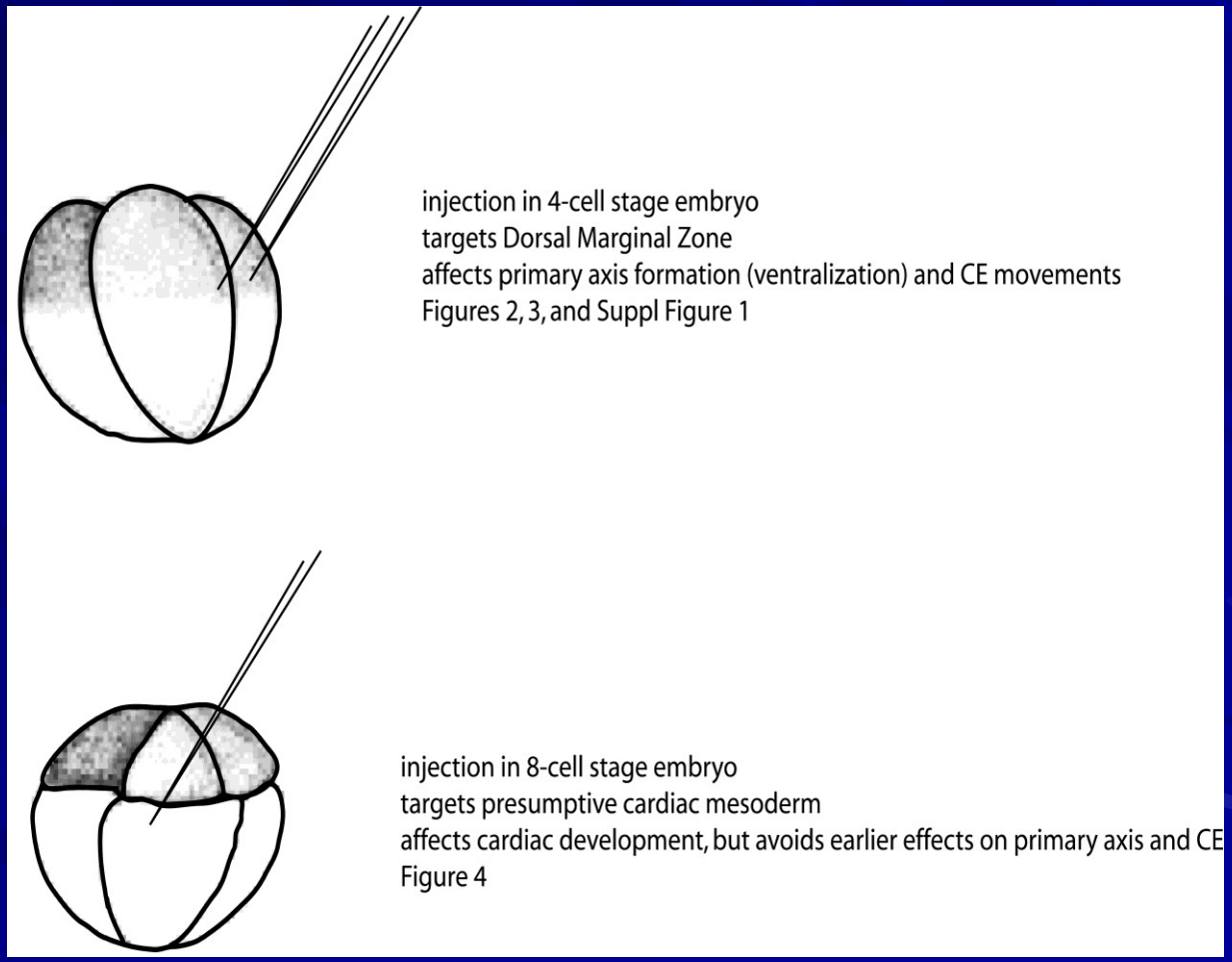


TopFlash reporter - β-catenin transcriptional activity



Is this relevant for Wnt signal transduction in vivo?

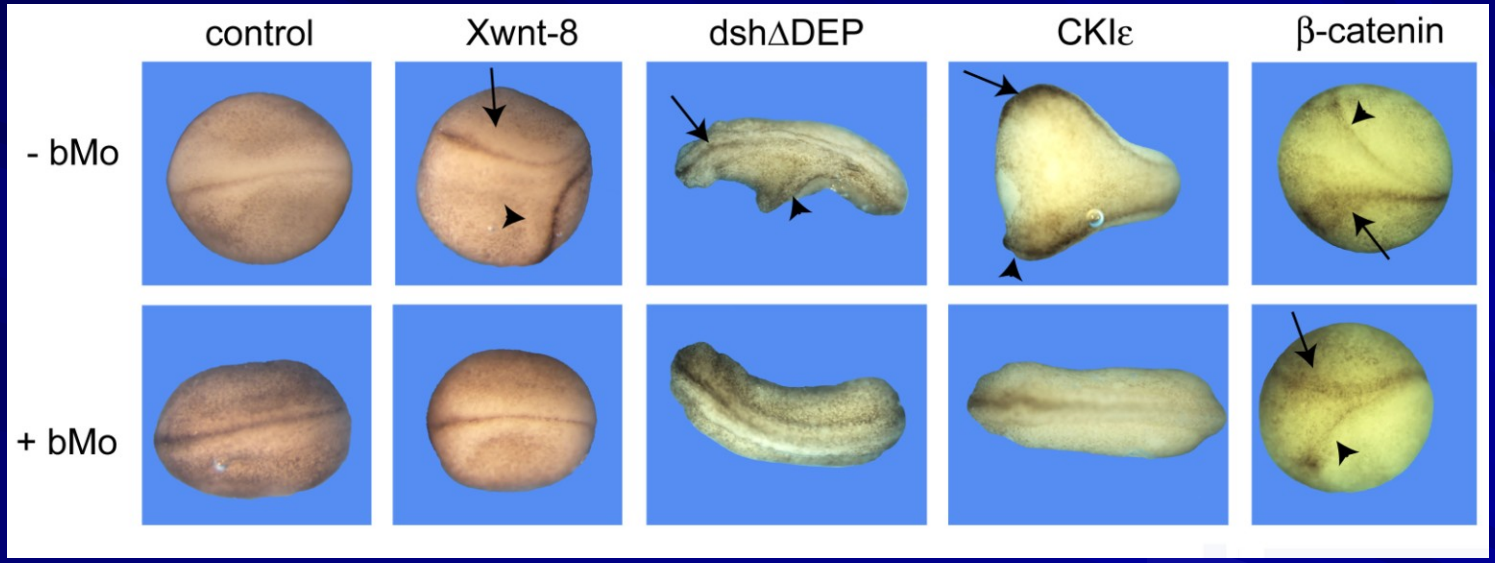
Metoda č. 5: In vivo analysis in Xenopus



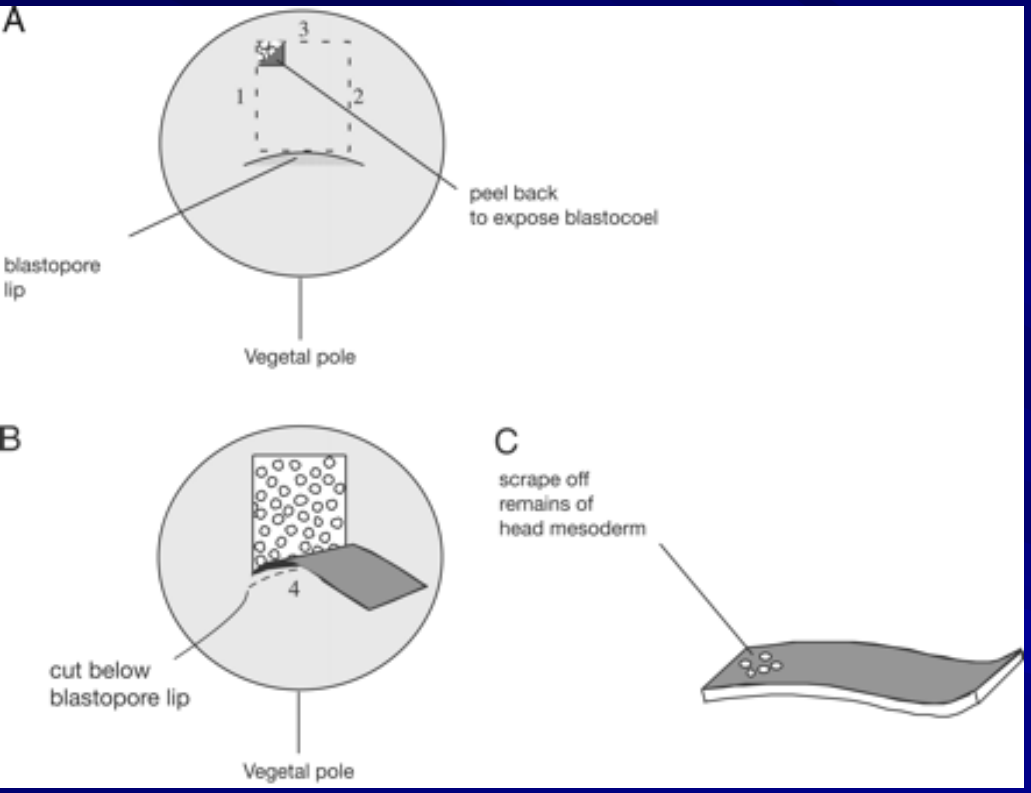
Metoda č. 5: In vivo analysis in Xenopus

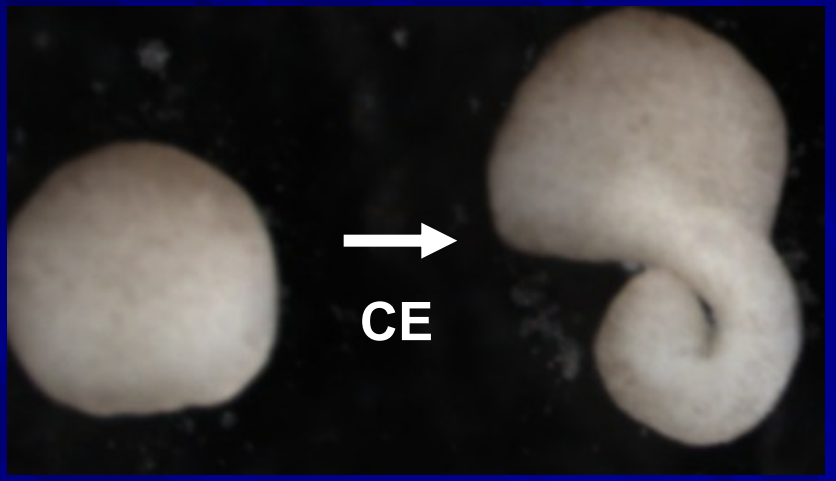
Is β-arrestin important for the Wnt/β-catenin signalling in vivo?

β-arrestin knockdown in Xenopus (axis duplication assay):

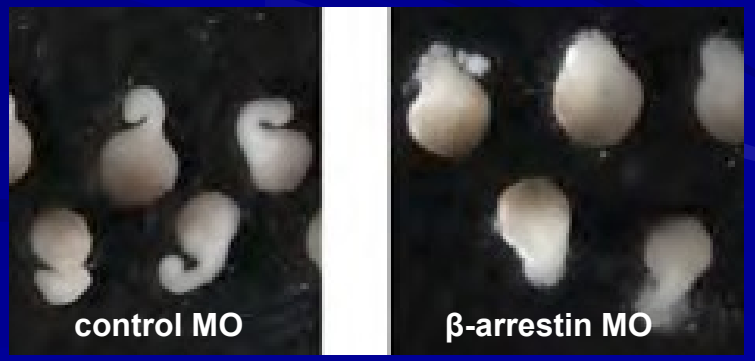


Keller explants (Xenopus)





β-arrestin regulates convergent extension movements in vivo



Metody č. 6: Genetické modifikace myši

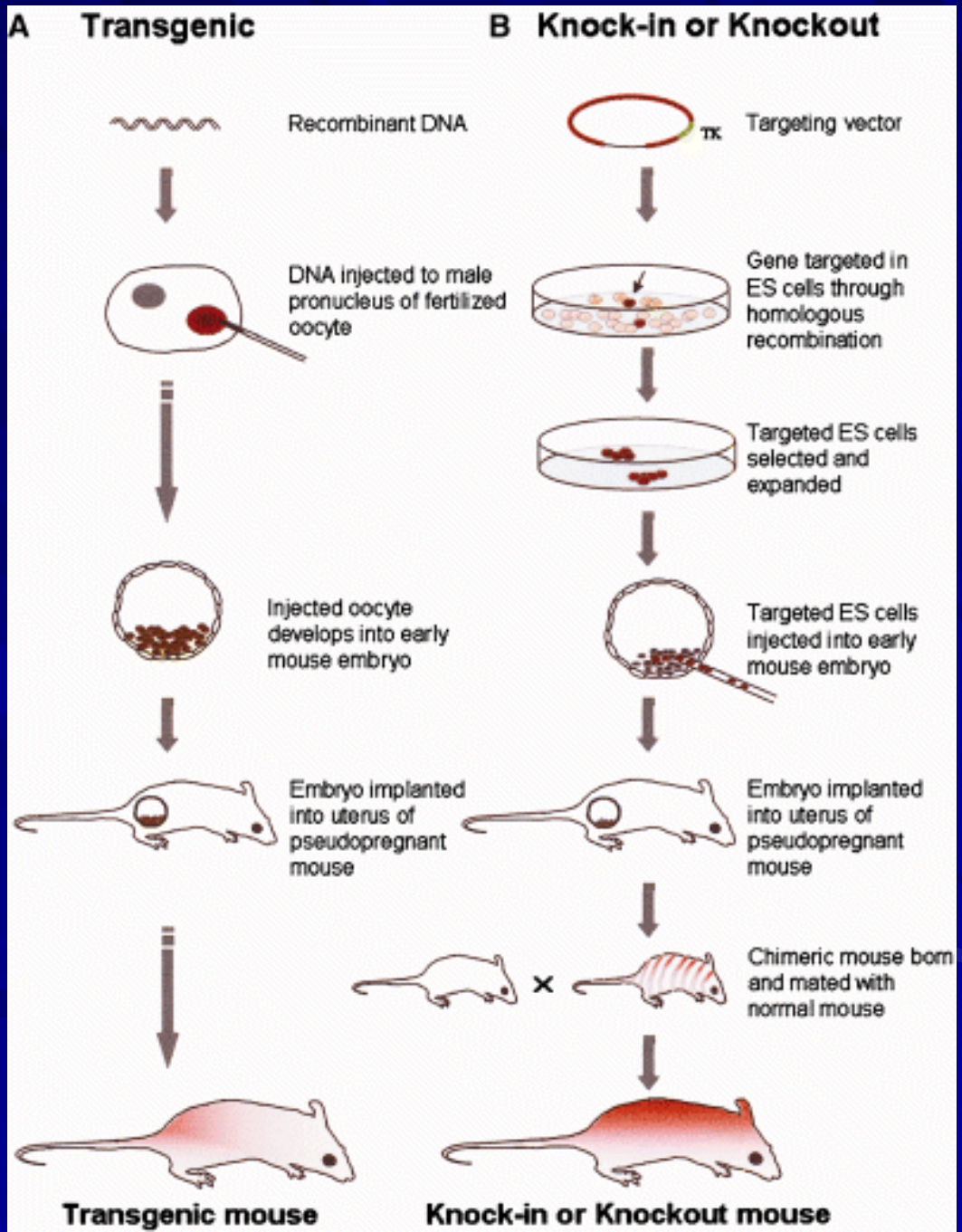
Transgenní myš

Nobelova cena 2007

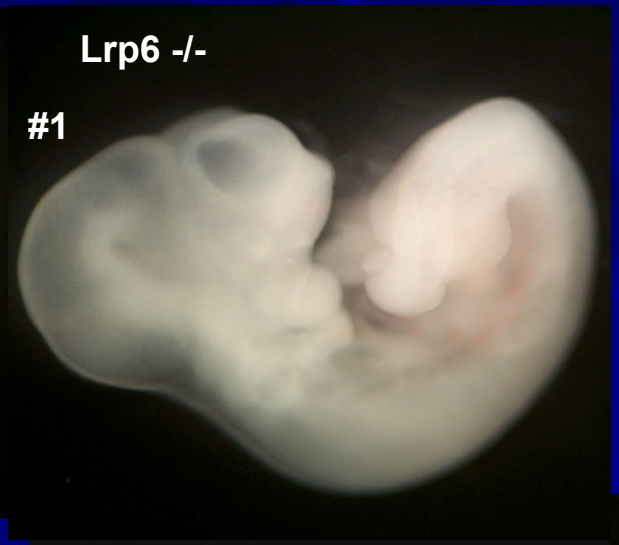
Mario R. Capecchi, **Martin J. Evans** and **Oliver Smithies**

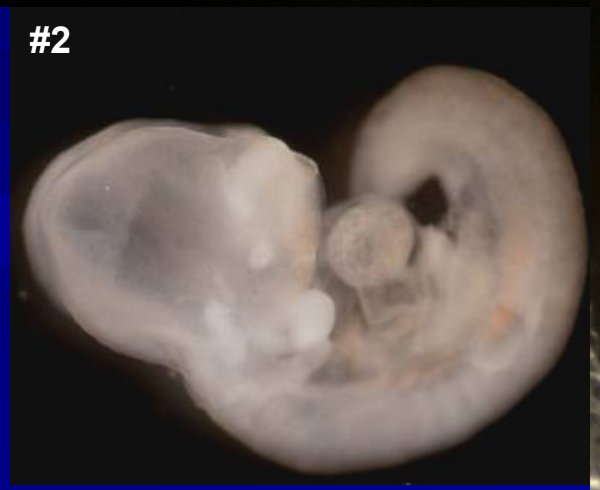
za

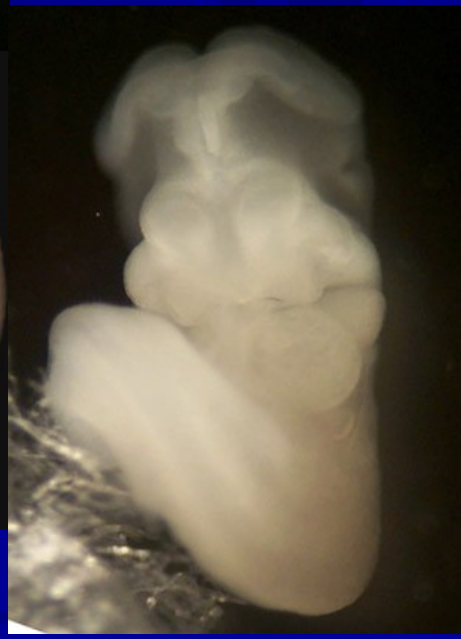
„principles for introducing specific gene modifications in mice by the use of embryonic stem cells“



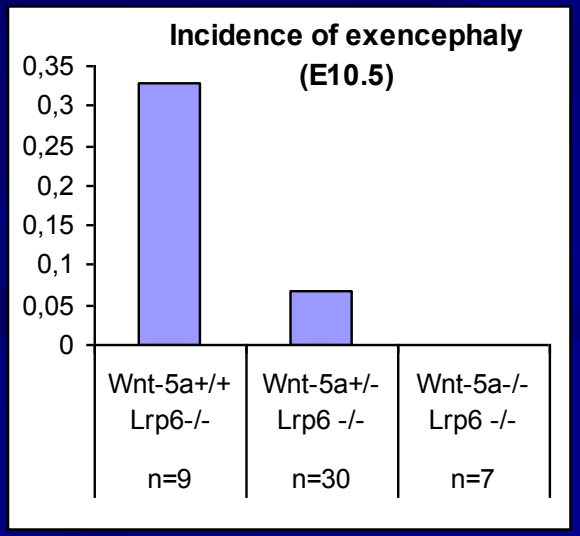
Lrp6 KO embryos display exencephaly....



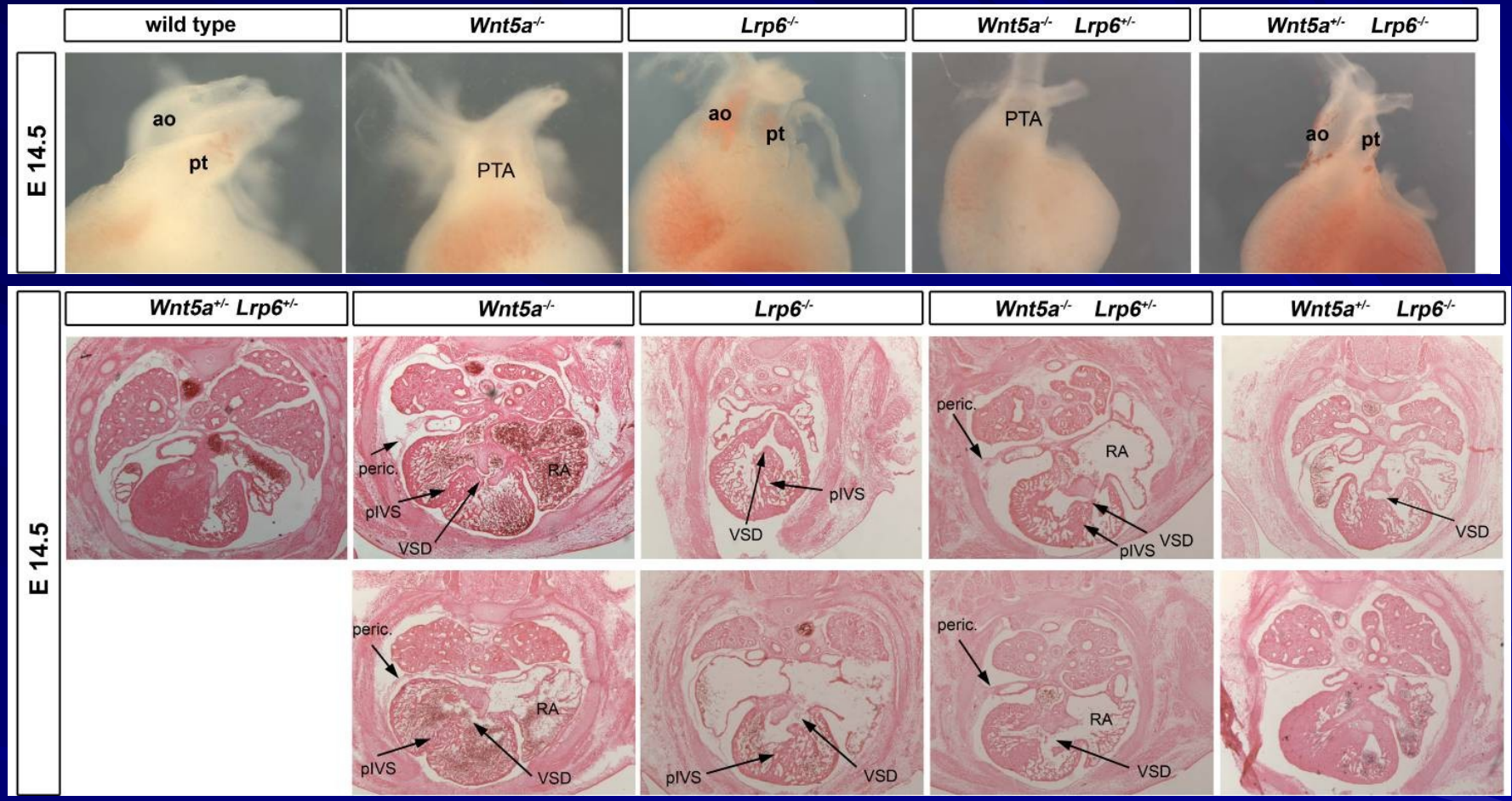




....which is rescued by loss of Wnt5a



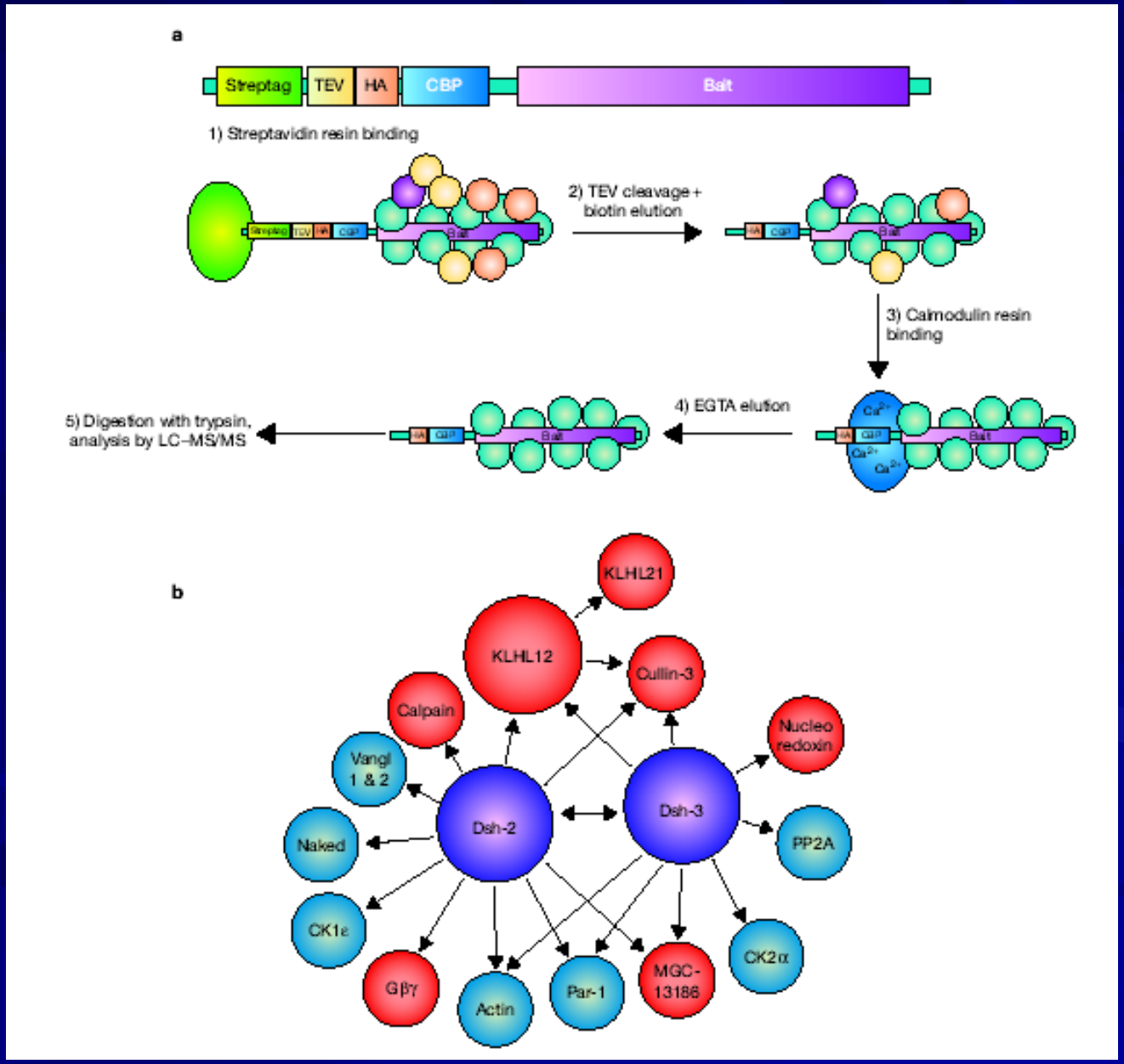
Lrp6 and Wnt5a KO show similar heart abnormalities associated with non-canonical Wnt signalling defects; heart abnormalities in Lrp6 KO are rescued by loss of Wnt5a

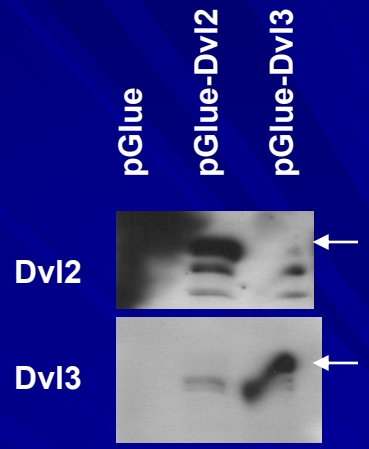


Genotype	*Wnt5a -/- Lrp6+/+*	*Wnt5a -/- Lrp6+/-*	*Wnt5a+/+ Lrp6 -/-*	*Wnt-5a +/- Lrp6-/-*	others
outflow tract defects/total number of embryos	7/7	8/8	6/6	2/4	0/6

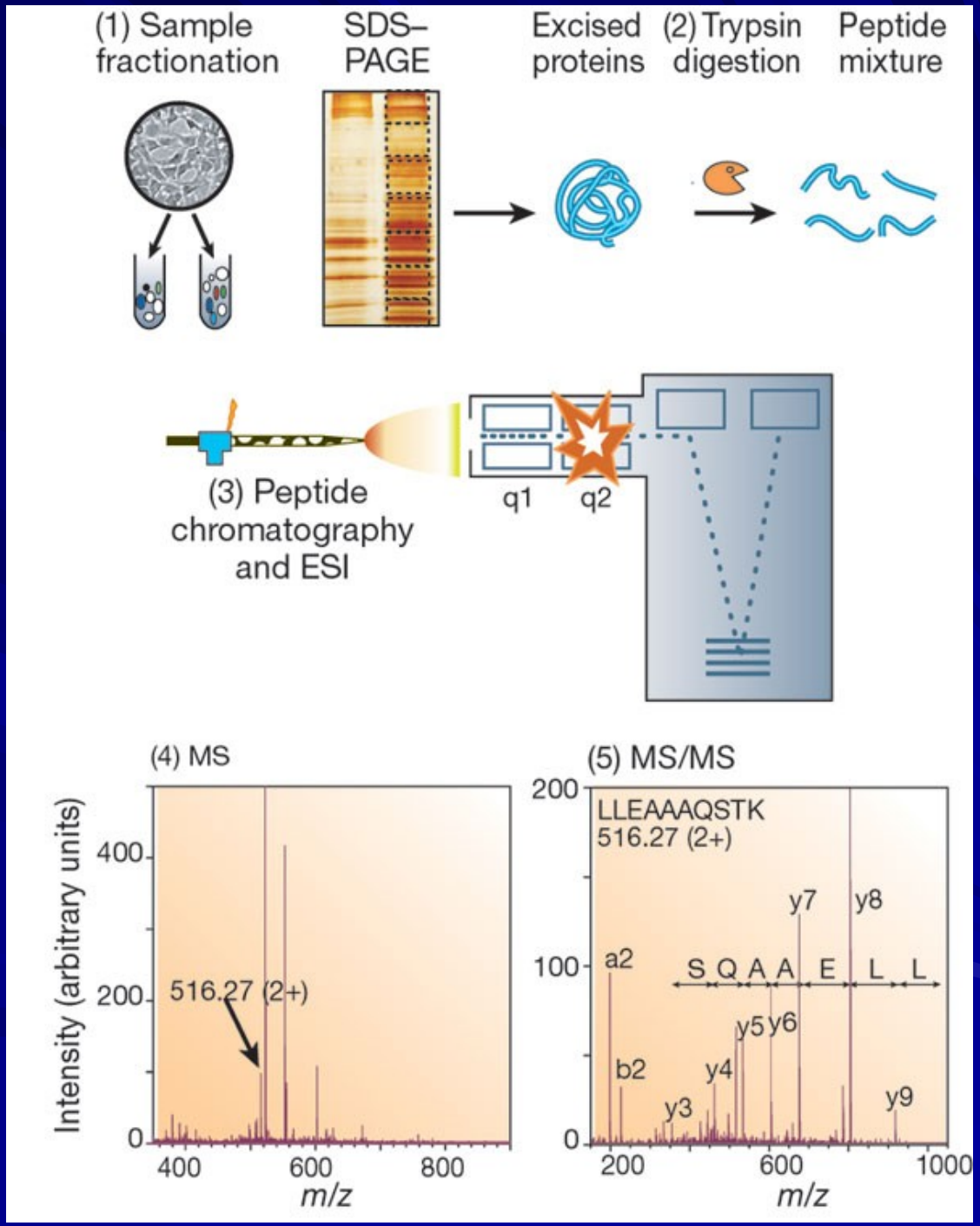
Metody č. 7: Afinitní purifikace a hmotnostní spektroskopie

Afinitní purifikace





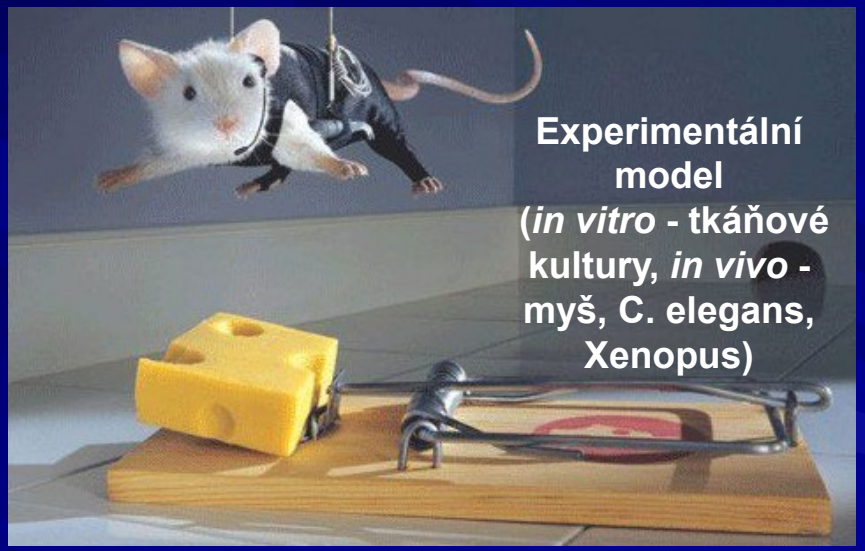
Hmotnostní spektroskopie (Mass Spec)



Děkuji za pozornost!

Celogenomové techniky

Molekulární mechanismus



Celoproteomové techniky