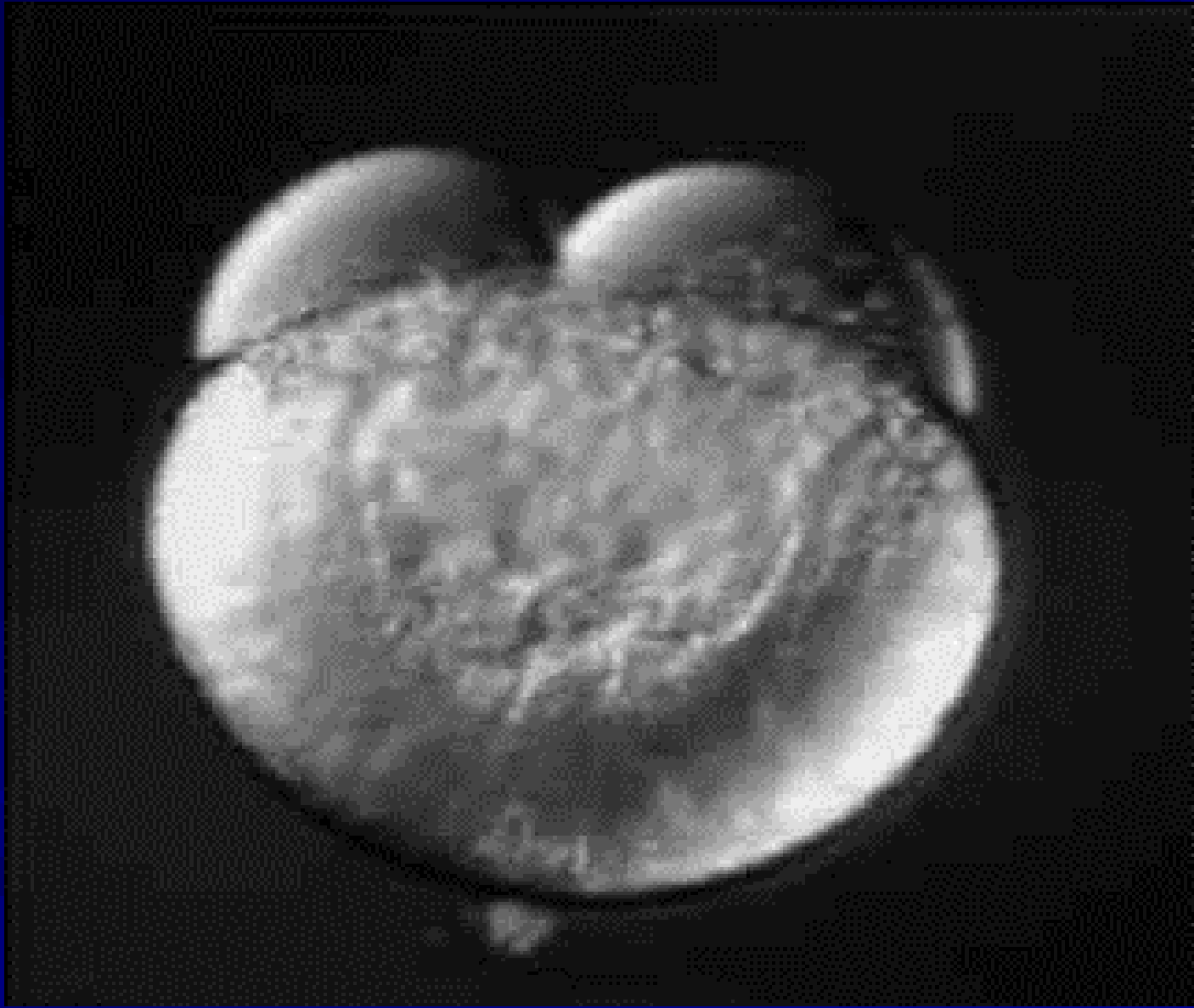


Metody studia buněčné signalizace

Vítězslav BRYJA

**Ústav experimentální biologie, PŘF MU
&
Oddělení cytokinetiky, Biofyzikální ústav AV ČR**

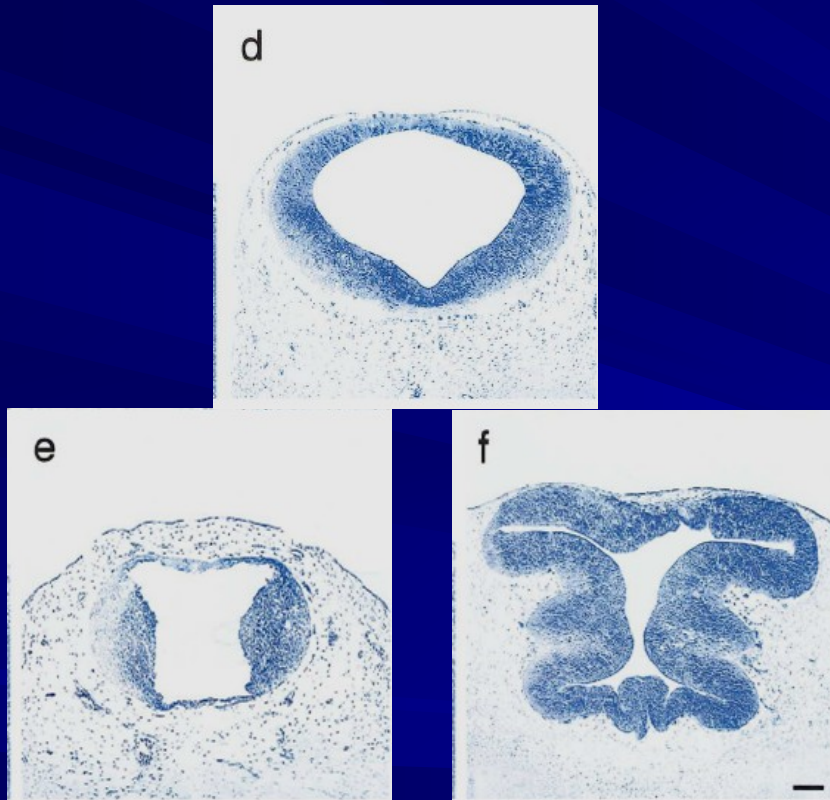


Wnt signalizace



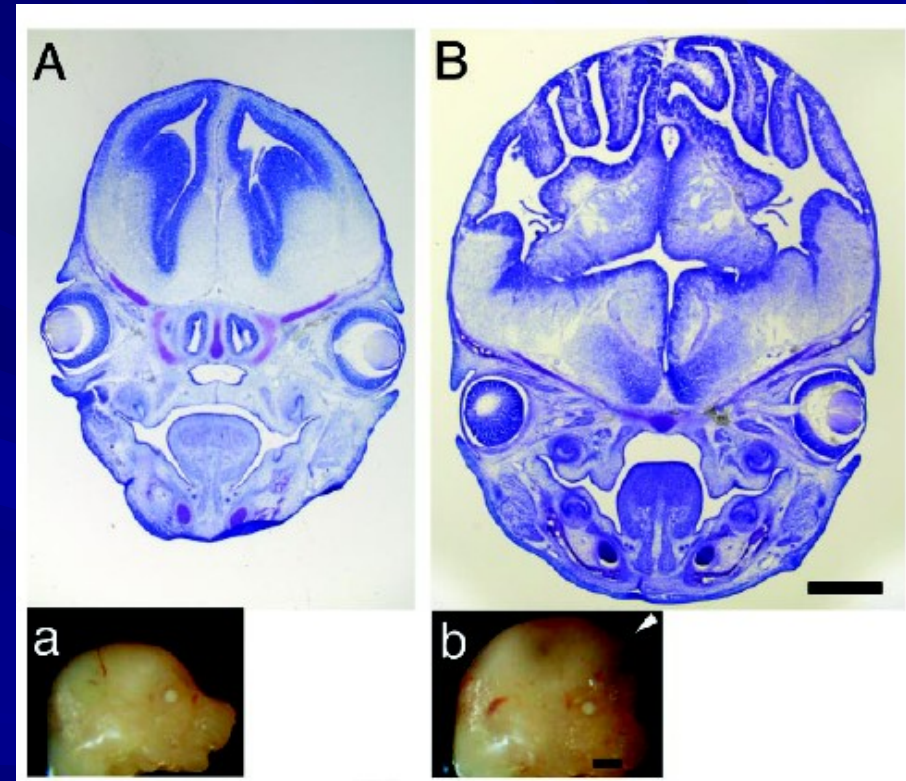
Důsledky aktivace Wnt signalizace v nervové soustavě:

midbrain (Brn4-promotor)



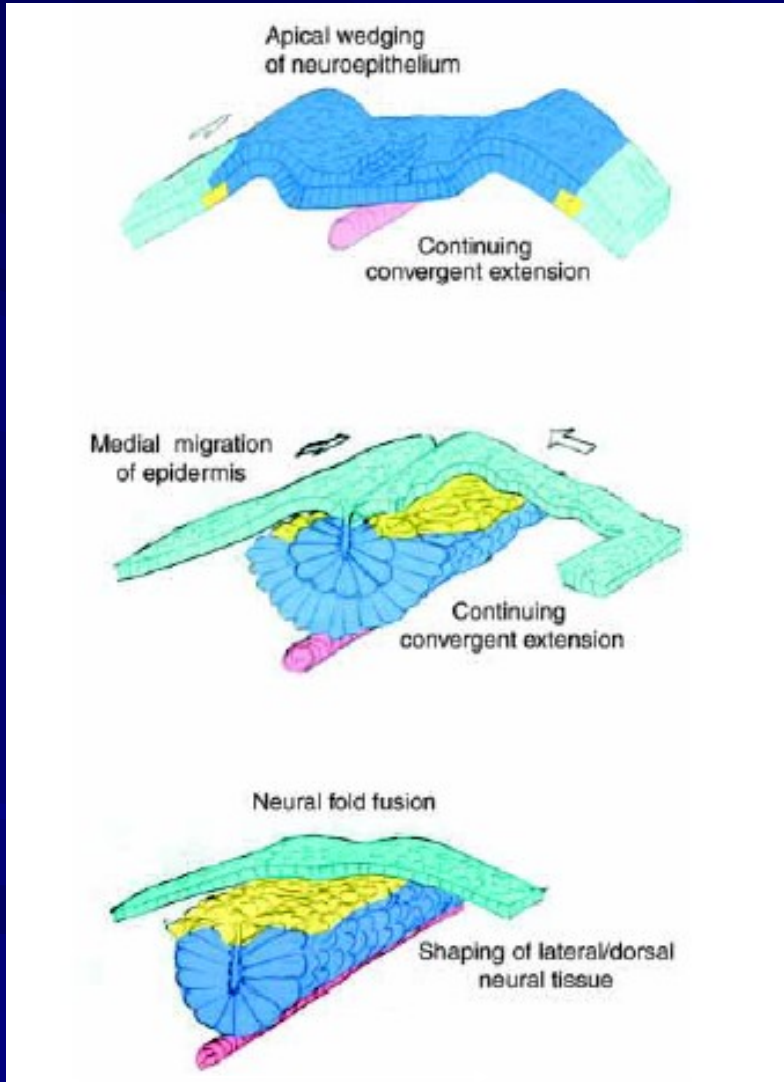
Zechner et al., 2003: Dev. Biol.;258:406-418.

cortex (nestin enhancer)



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

Nakanonická/PCP (Planar cell polarity) dráha způsobuje defekty v uzavírání nervové trubice



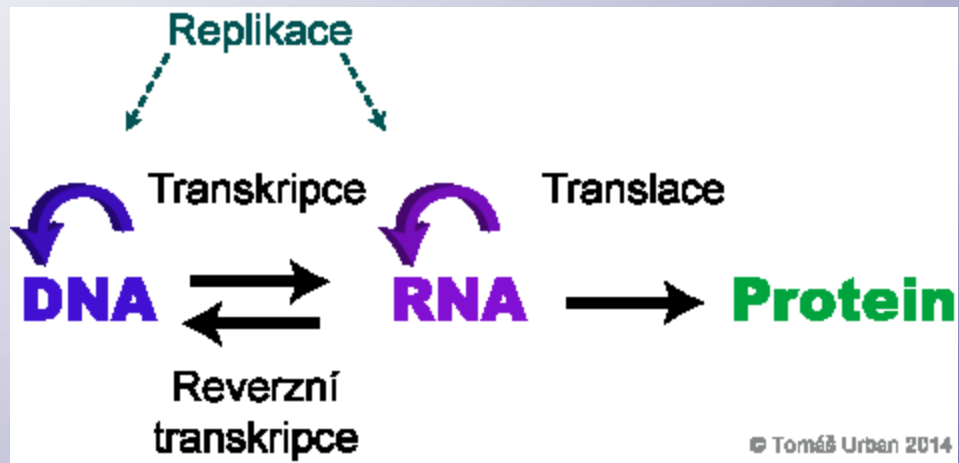
Exencephaly



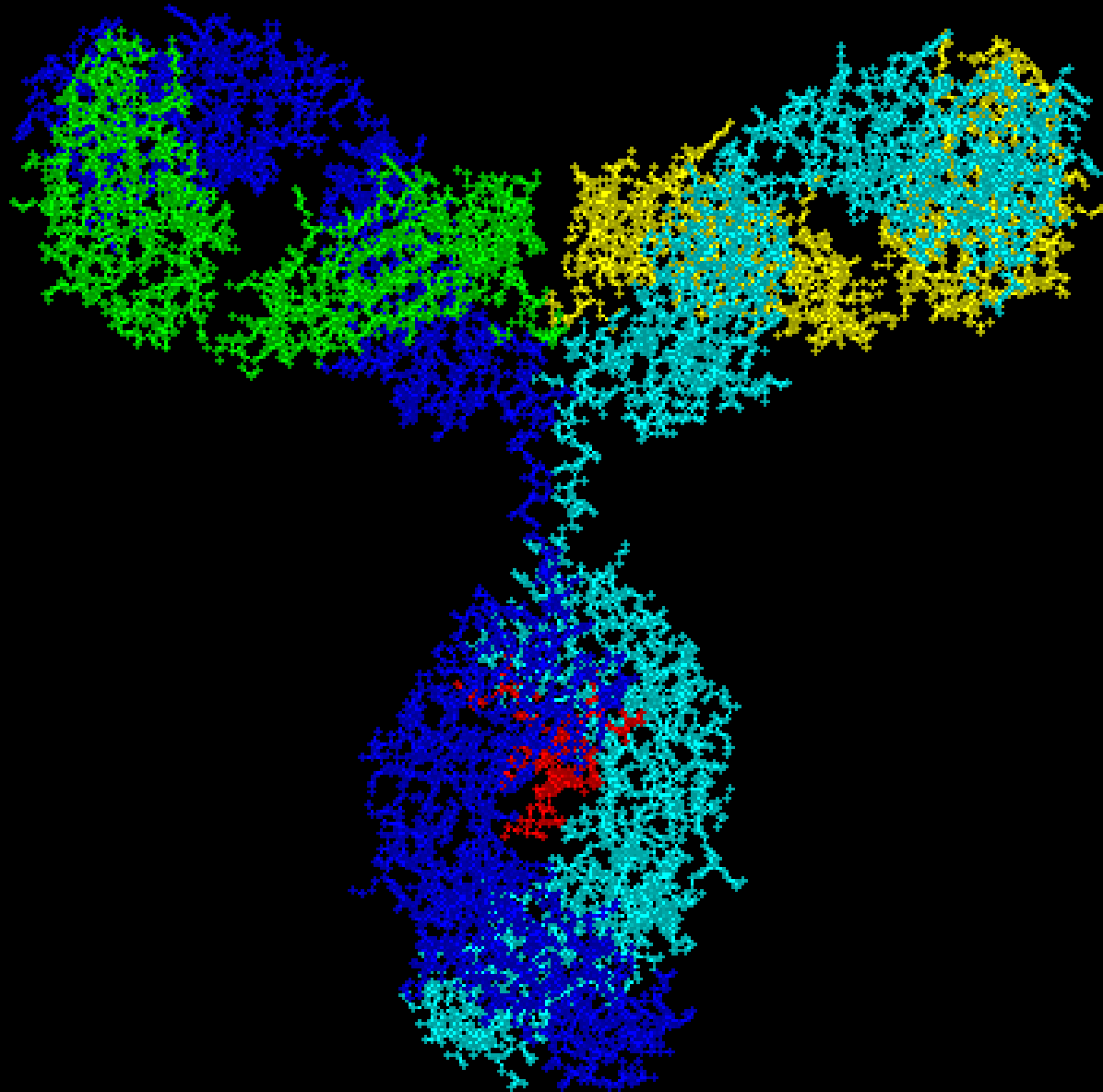
Open neural tube



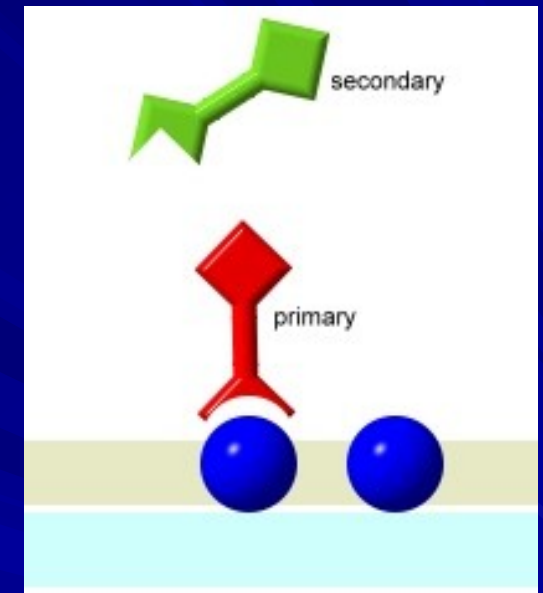
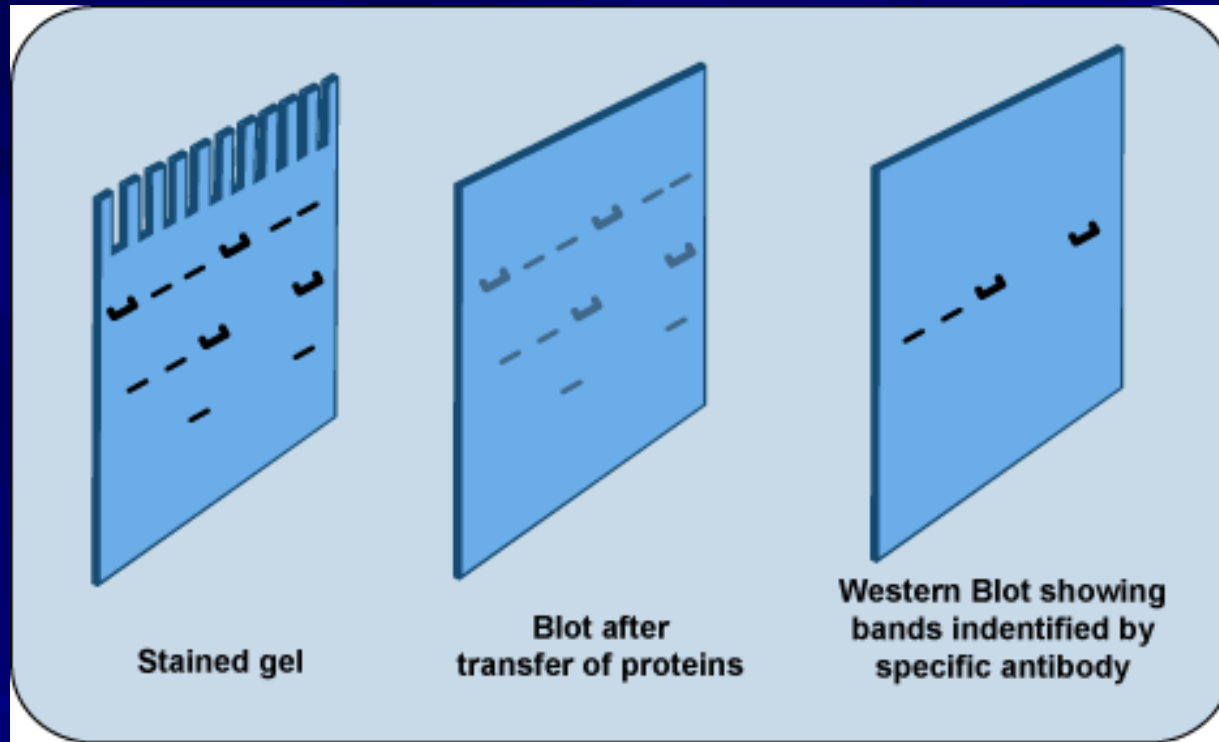
Centrální dogma molekulární biologie

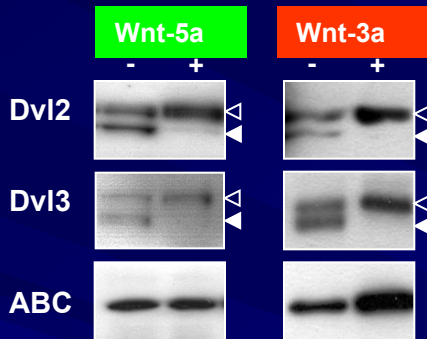


Protilátka
(imunoglobulin)



Metoda 1: Western blotting





ABC – active β -catenin = β -catenin dephosphorylated on GSK3 β target sites

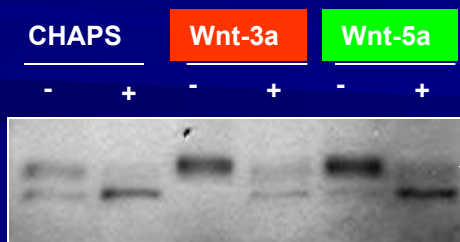
Dvl – Dishevelled – activated by phosphorylation detected as phosphorylation dependent mobility shift

◁ PS-Dvl

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

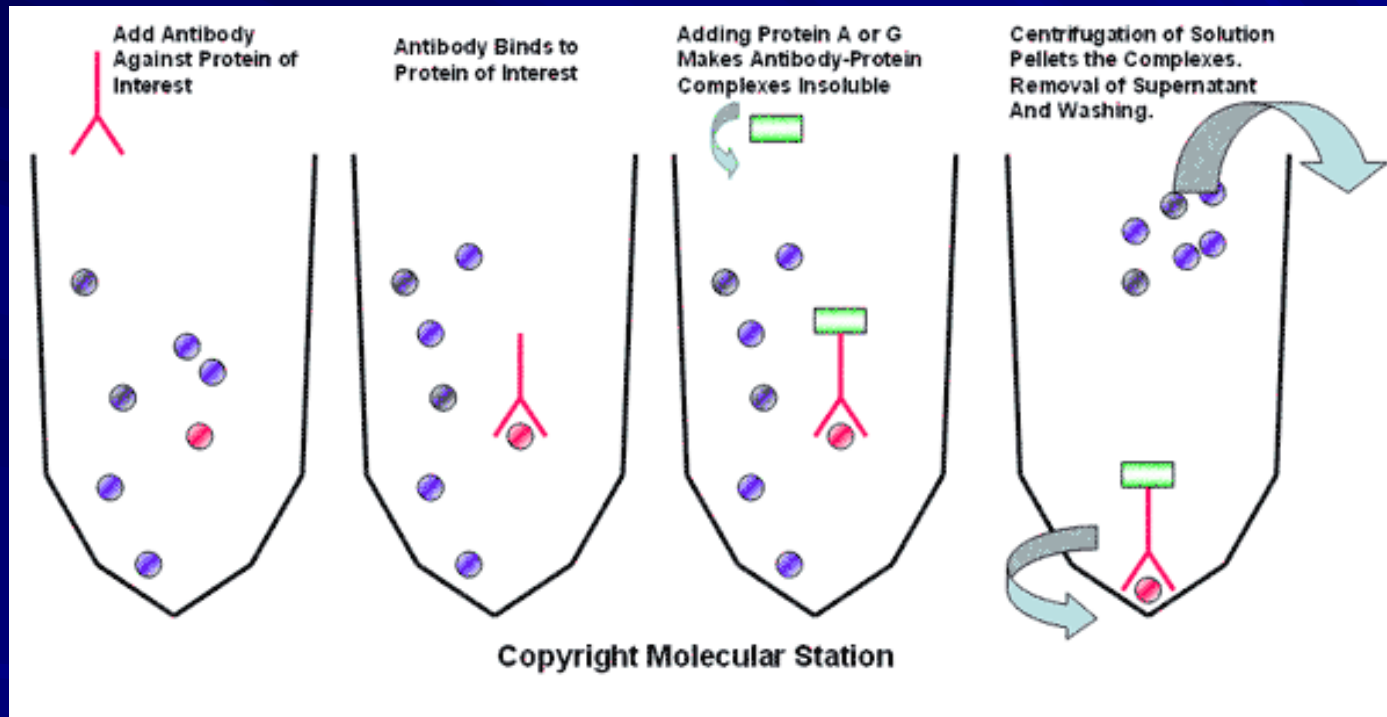
D4476 (100 μ M)

Dvl2



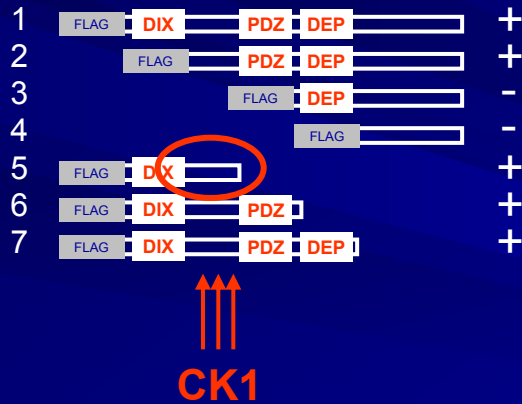
Both **Wnt-3a** and **Wnt-5a** activate Dvl2 and Dvl3 via casein kinase 1 (CK1)

Metoda 2: Immunoprecipitace



β -arrestin váže Dishevelled

Flag-Dvl3 constructs

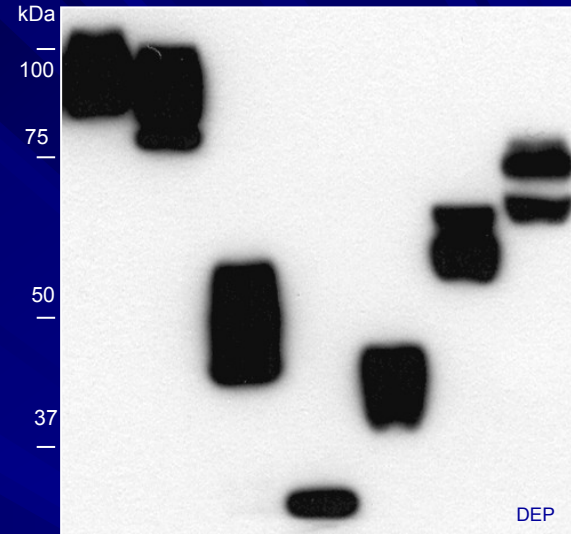


Flag-Dvl3 mutants

1 2 3 4 5 6 7

TCL:

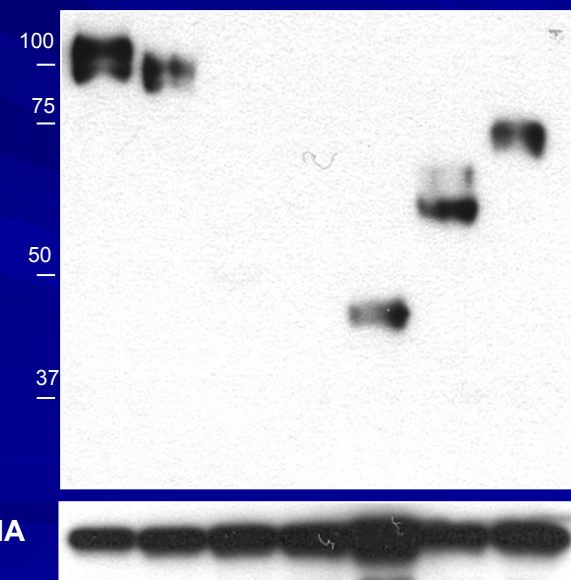
WB:
Flag



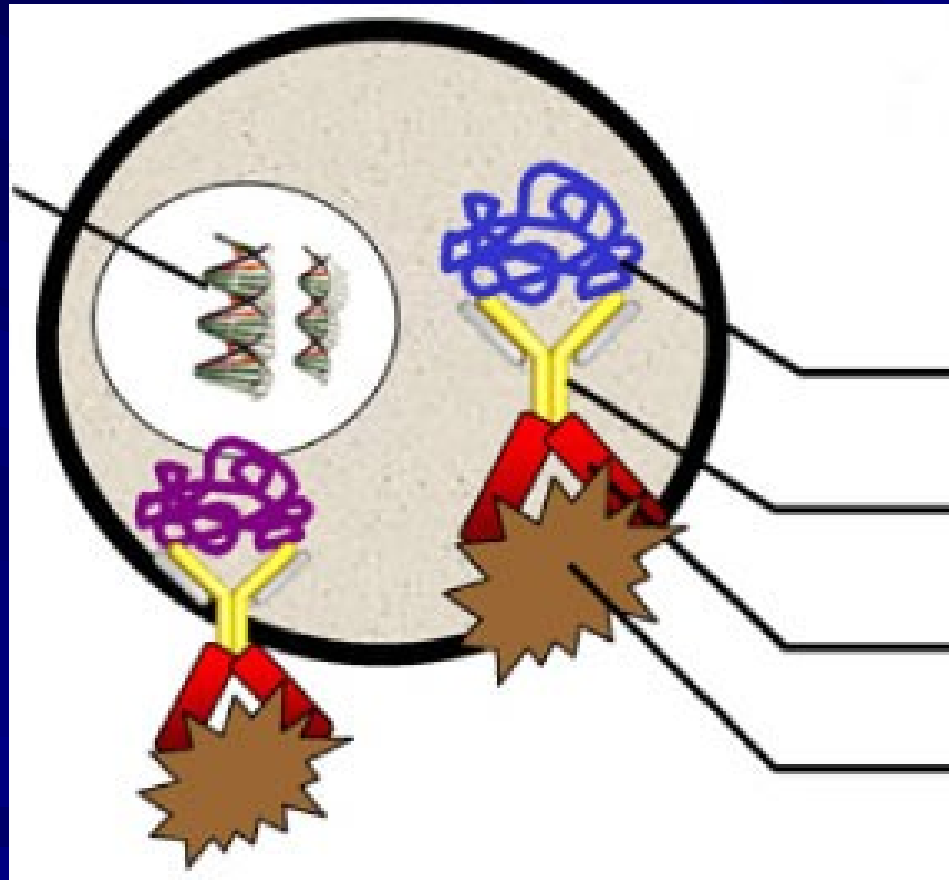
IP: HA- β -arrestin

WB:
Flag

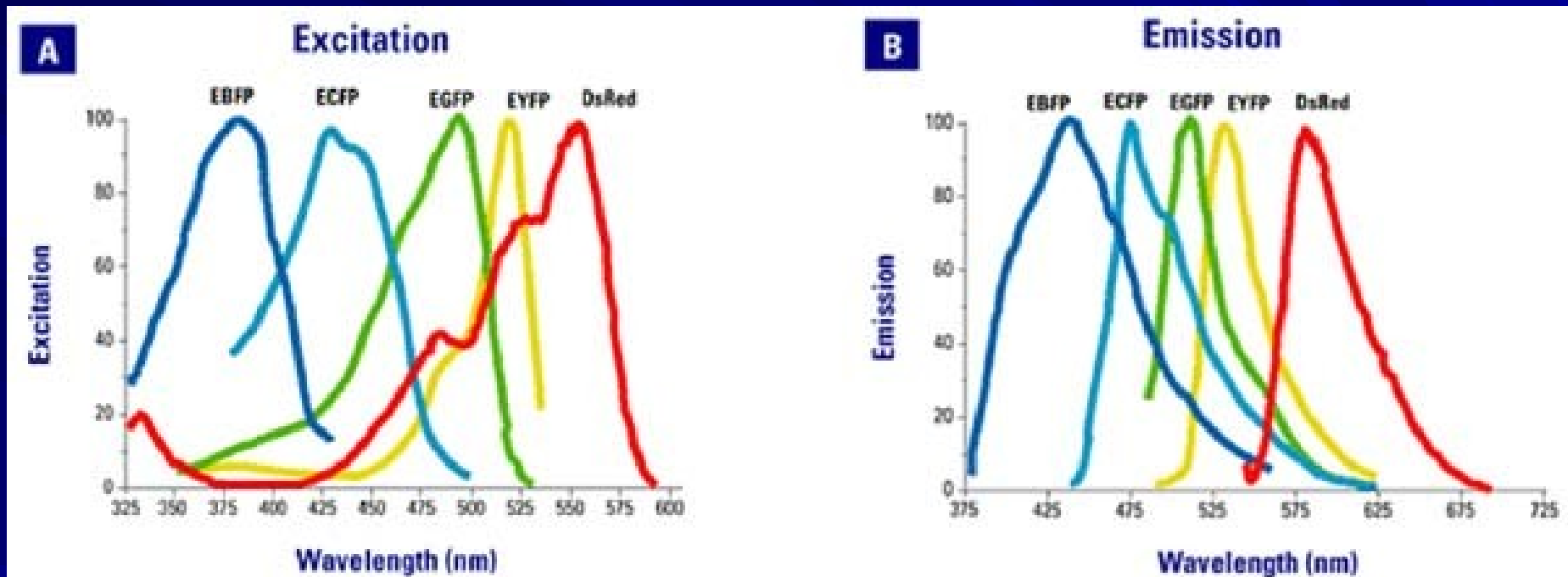
WB: HA



Metoda 3: Immunocytochemie



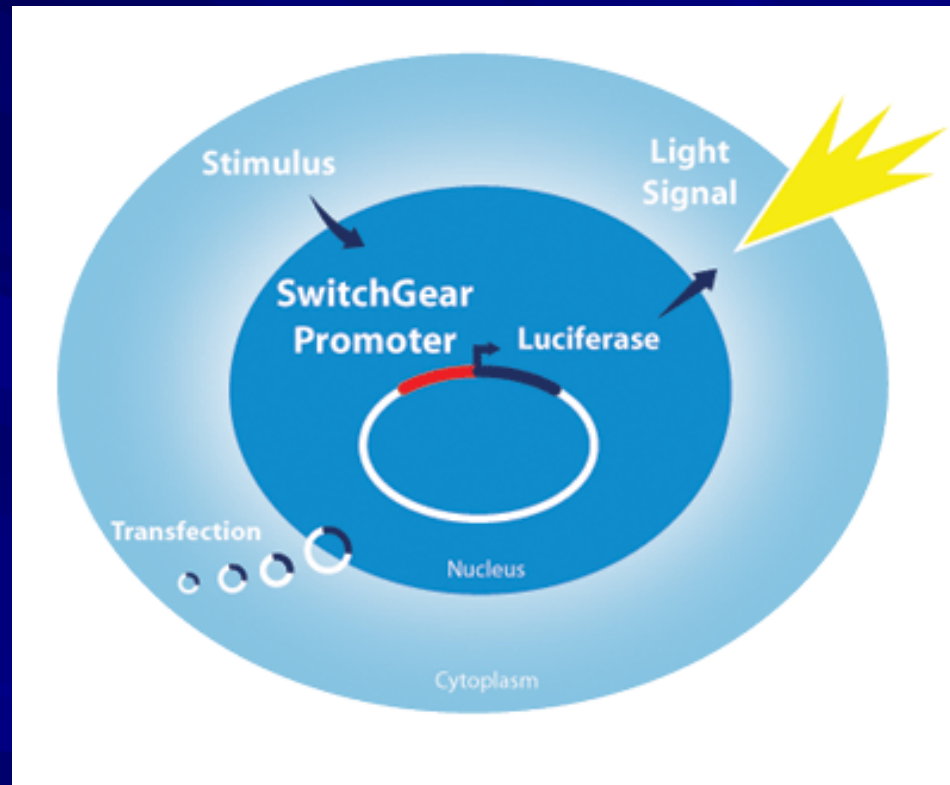
Fluorescenční proteiny



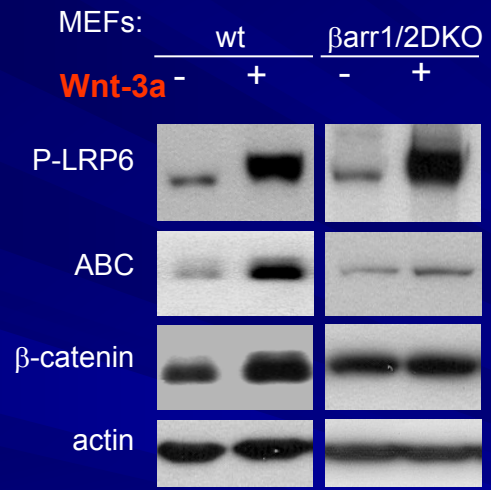
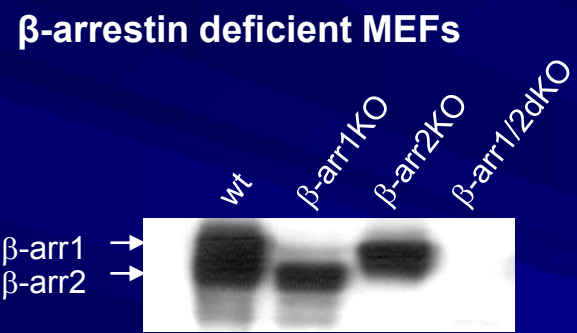
β -arrestin co-localizes with Dvl in the cytoplasm



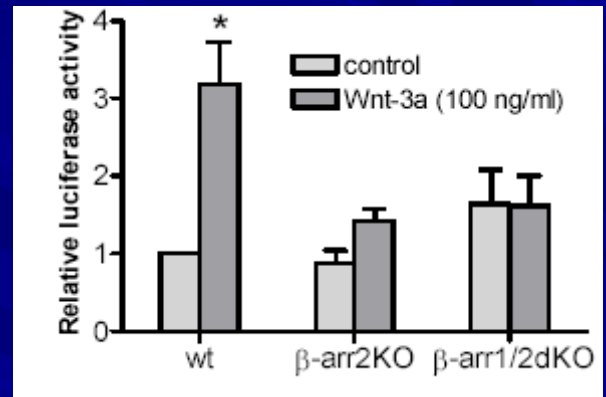
Metoda 4: Reportérové eseje – analýza aktivace promotoru



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

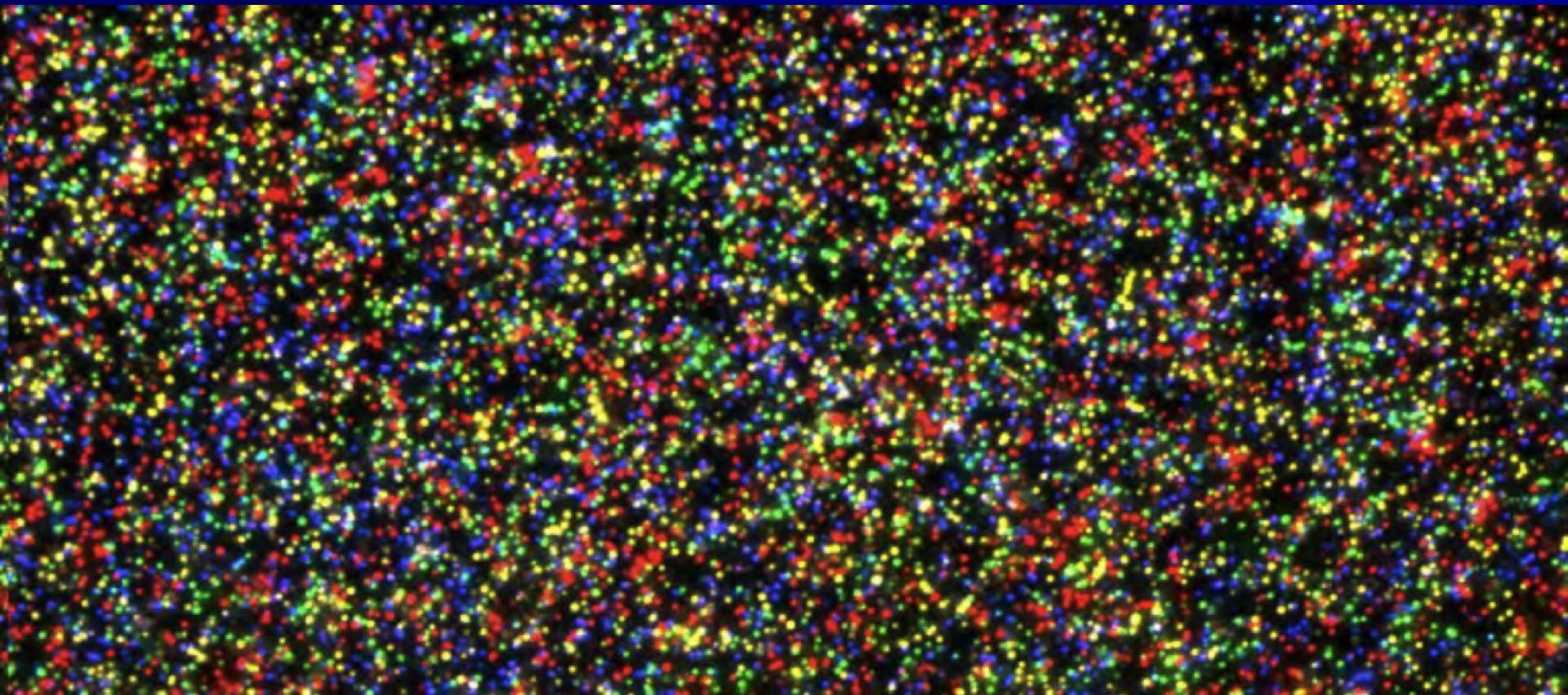


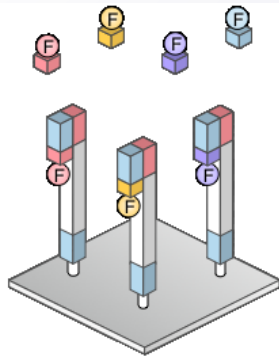
TopFlash reporter - β -catenin transcriptional activity



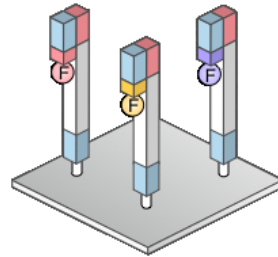
Is this relevant for Wnt signal transduction in vivo?

Metoda 5: RNA sekvenování (RNA Seq)

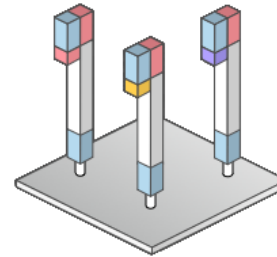




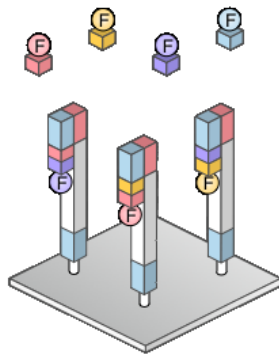
Primers are extended by one base



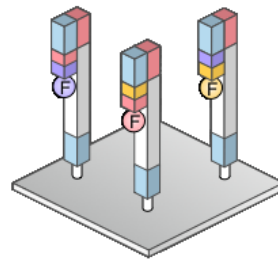
Excess nucleotides are washed away



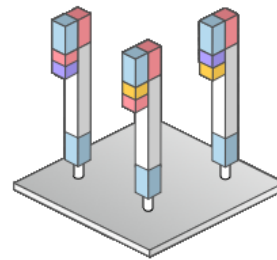
Fluorophore branches are removed



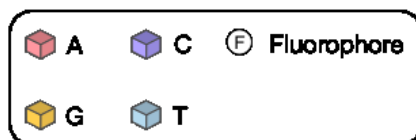
More nucleotides are added



Excess nucleotides are washed away



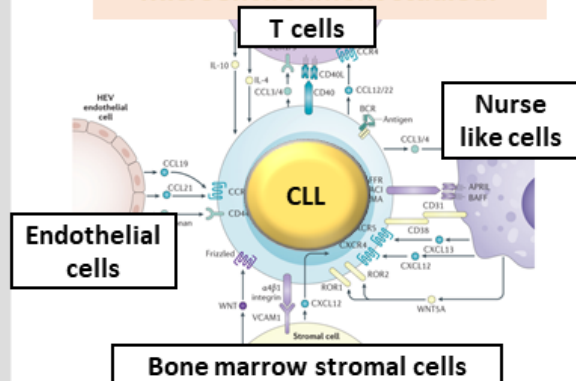
Fluorophore branches are removed



RNA sekvenování – vícedruhové kultivace mezi druhy

CK1 inhibition: In vitro direct co-culture system with primary patient CLL cells

Microenvironment studied:



Global RNA Seq analysis (SA3.1)

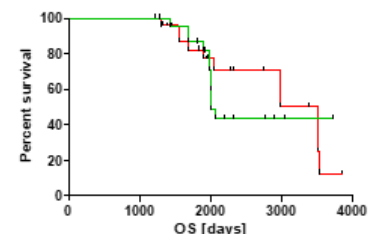
- Species-specific sequencing of human, mouse and mixed samples
- Data processing - pipeline designed to separate human and mouse reads

Focused functional analysis (SA3.2)

- Migration/invasion
- Adhesion
- Cytotoxicity
- Immunological synapse

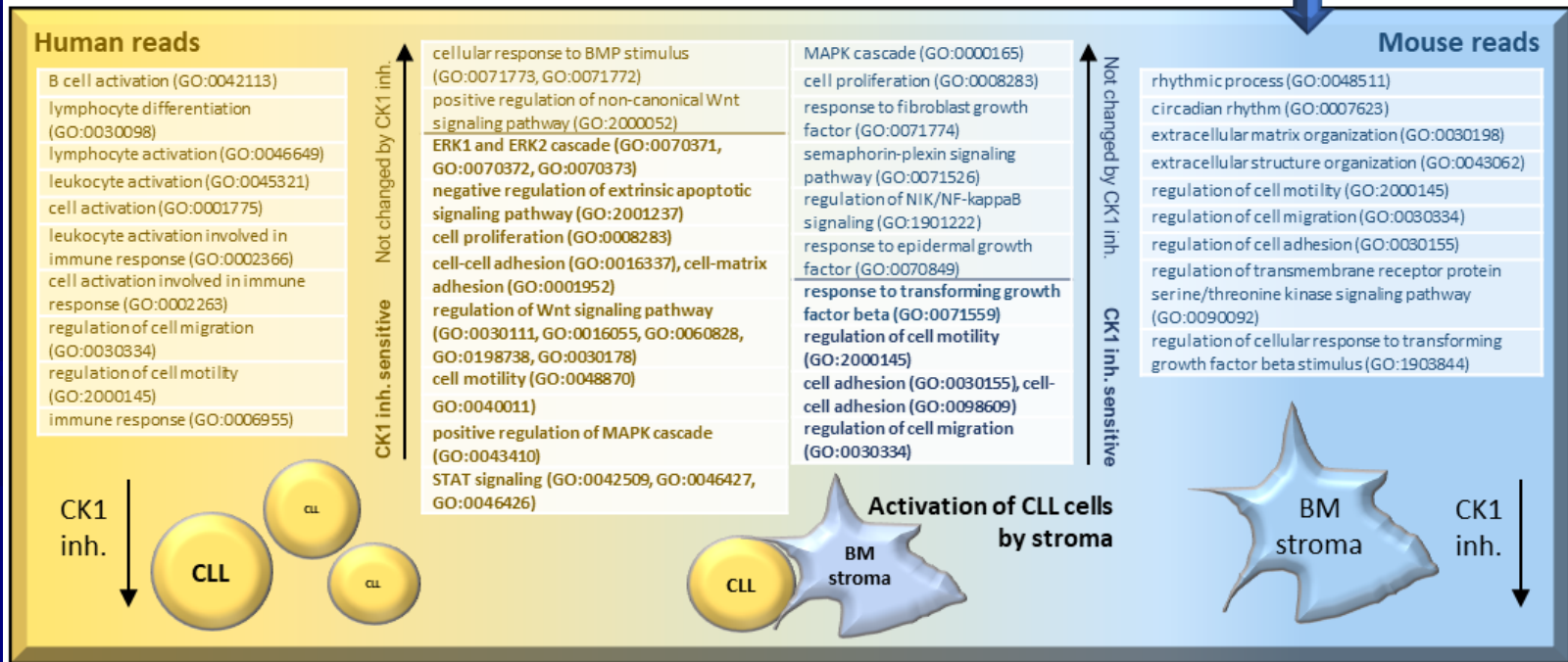
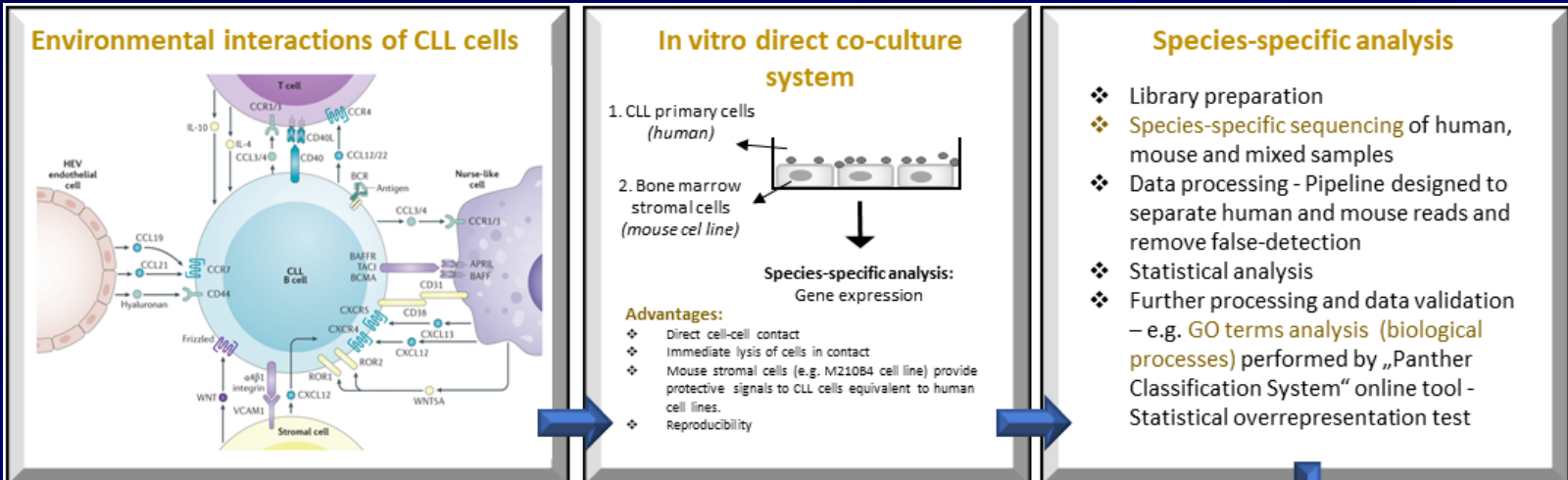
Validation on the larger patient cohort (SA3.3)

“hand picking” of genes/proteins with the clinically important function in CLL



Identification of processes controlled by CK1 inhibition in CLL cells as well as in the cells forming the microenvironment

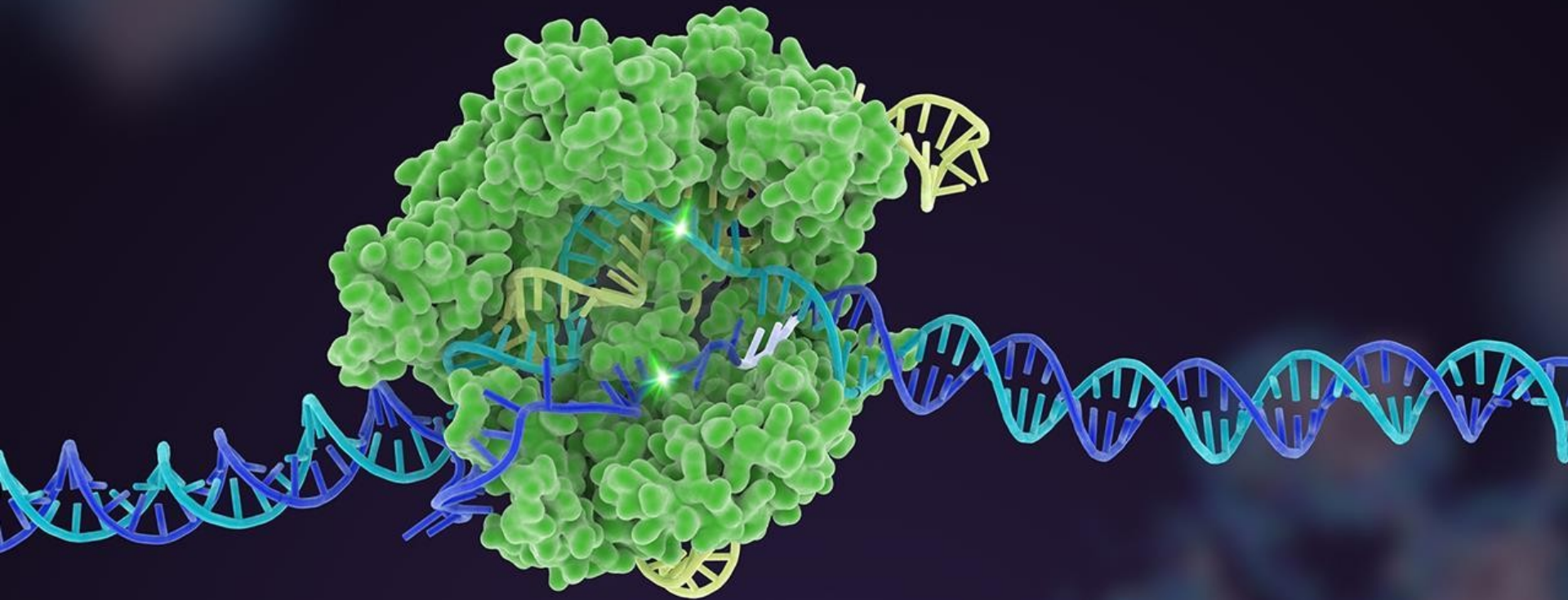
RNA sekvenování mezi druhy



Metody č. 6: Genetické modifikace buněčných linií a myši

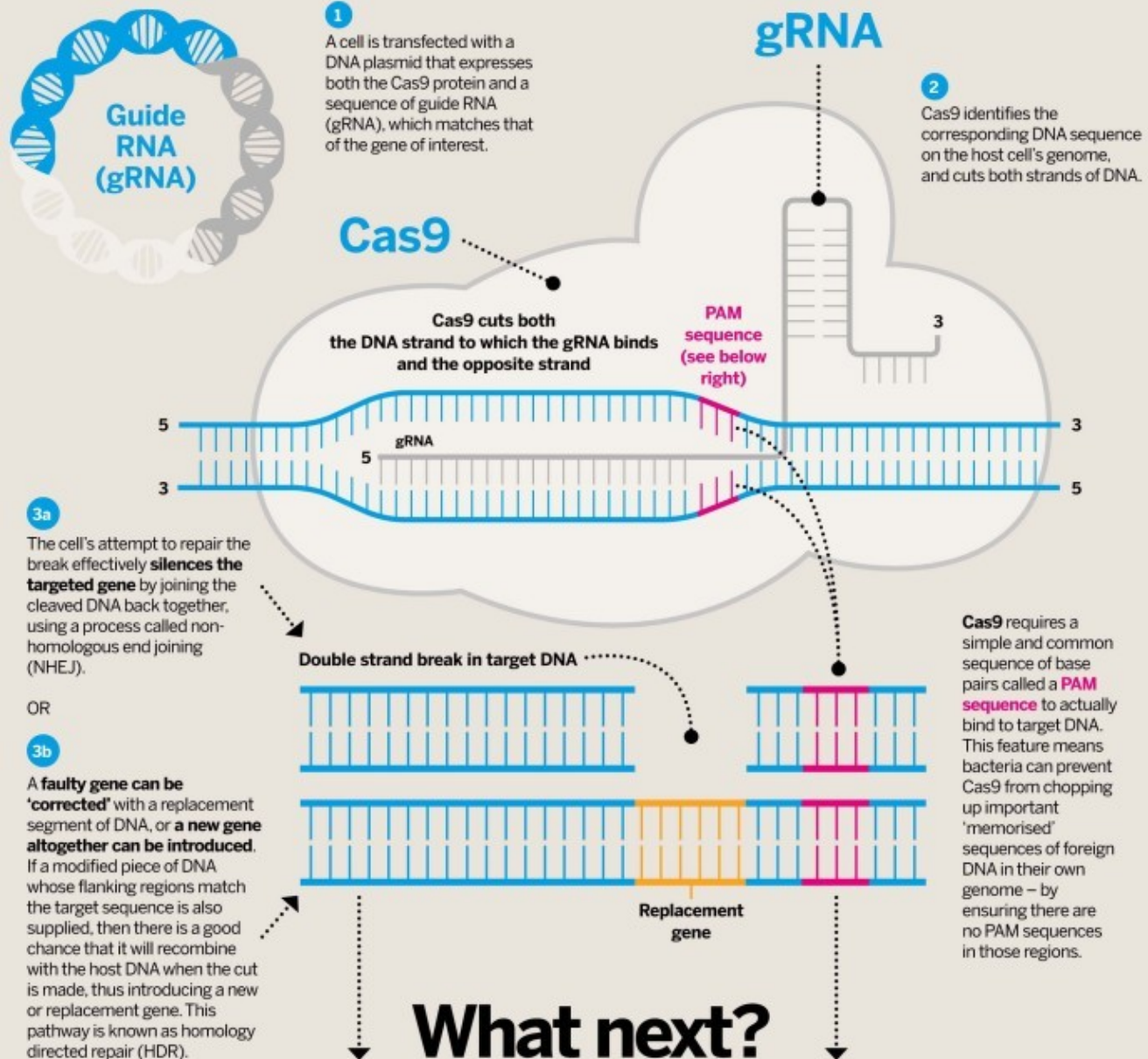
2014: Crispr/Cas9-mediated gene editing

METHOD OF THE YEAR



CRISPR-Cas9

How the genome editor works



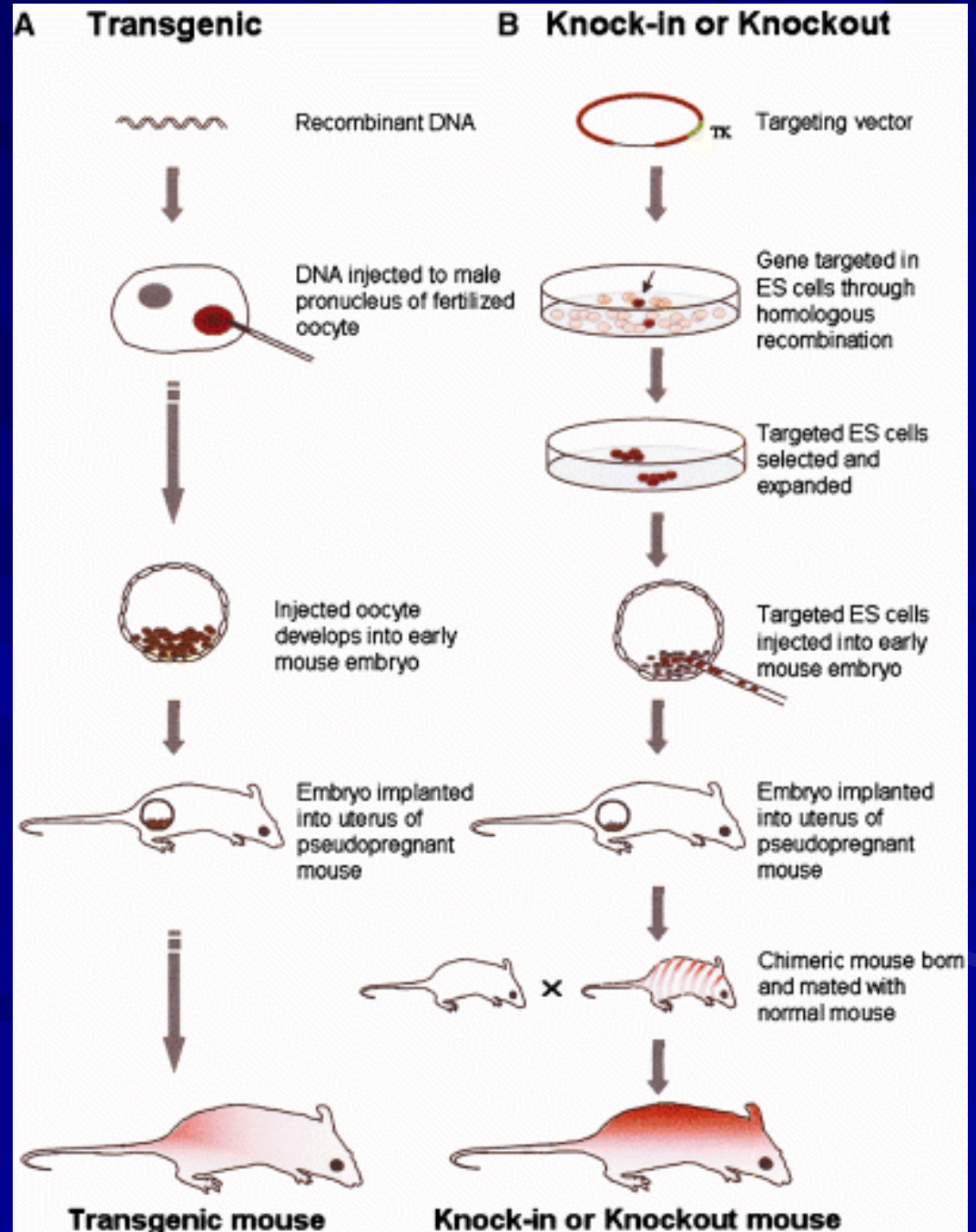
Transgenní myš

Nobelova cena 2007

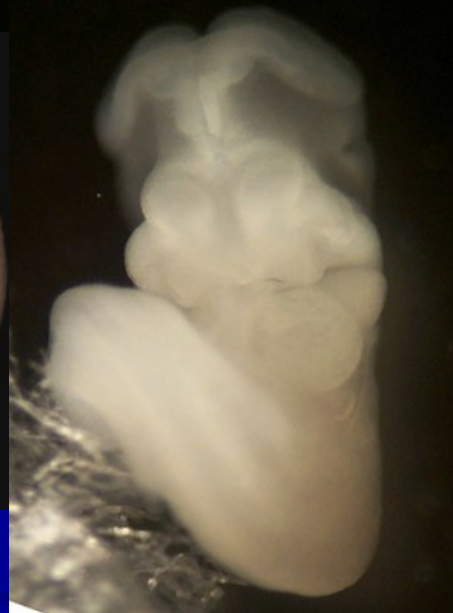
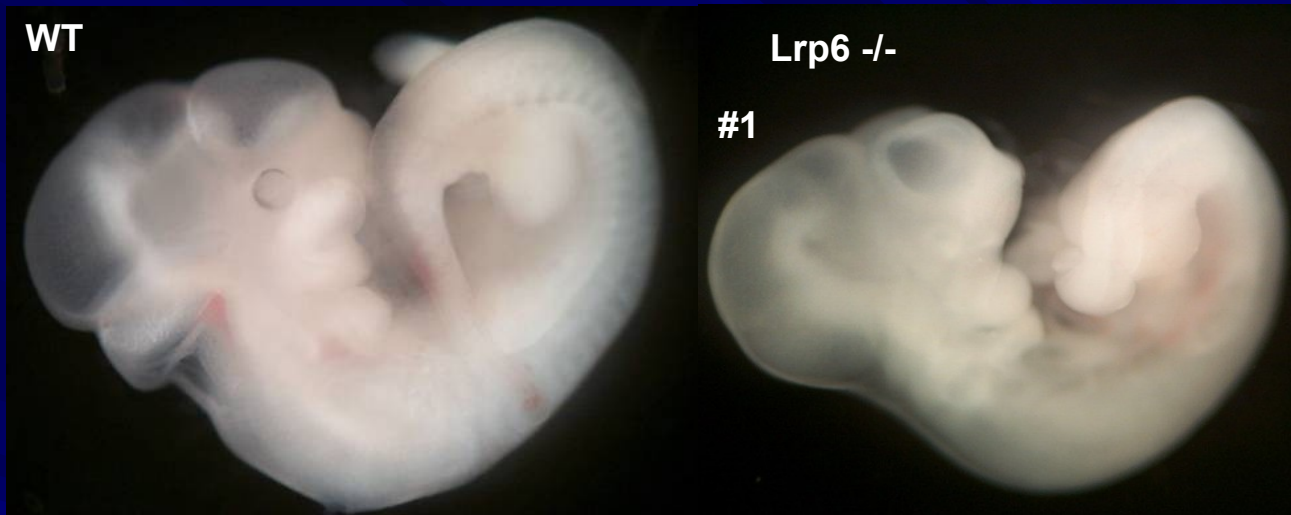
Mario R. Capecchi,
Martin J. Evans and
Oliver Smithies

za

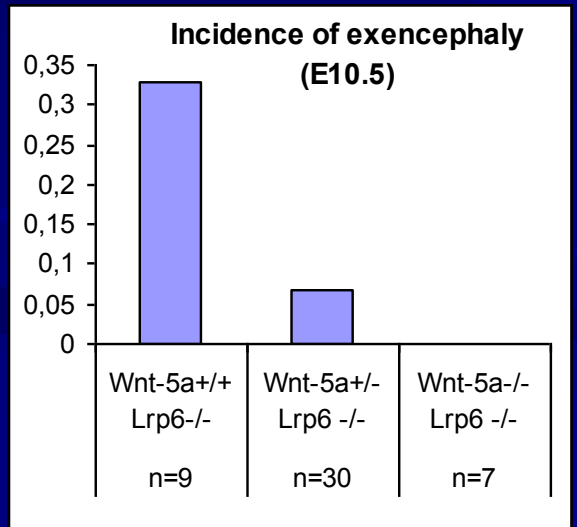
„principles for
introducing specific
gene modifications in
mice by the use of
embryonic stem cells“



Lrp6 KO embryos display exencephaly....

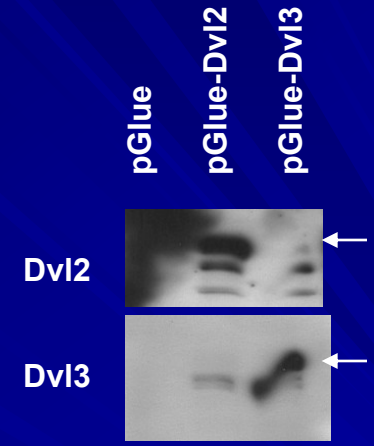
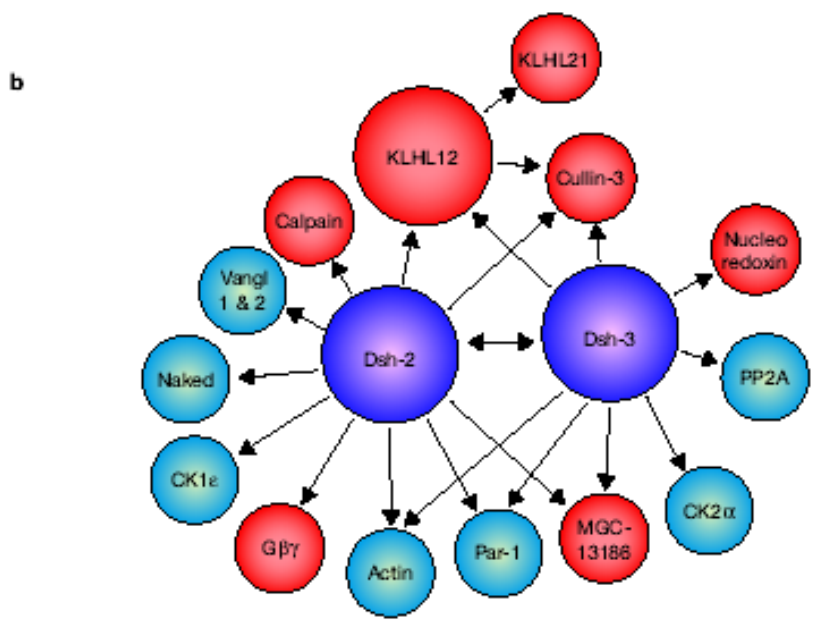
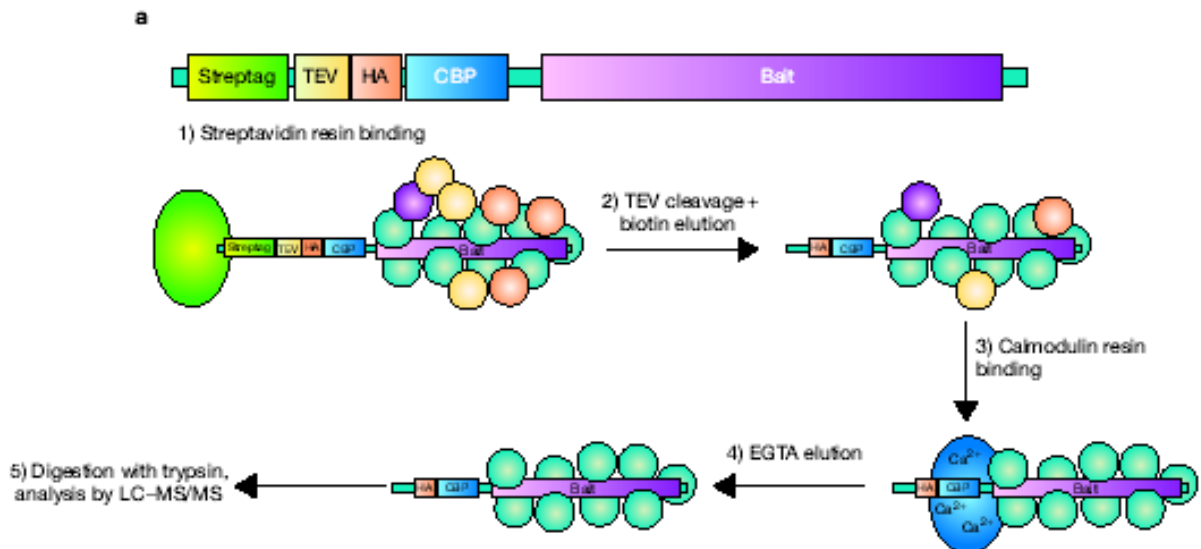


....which is rescued by loss of Wnt5a

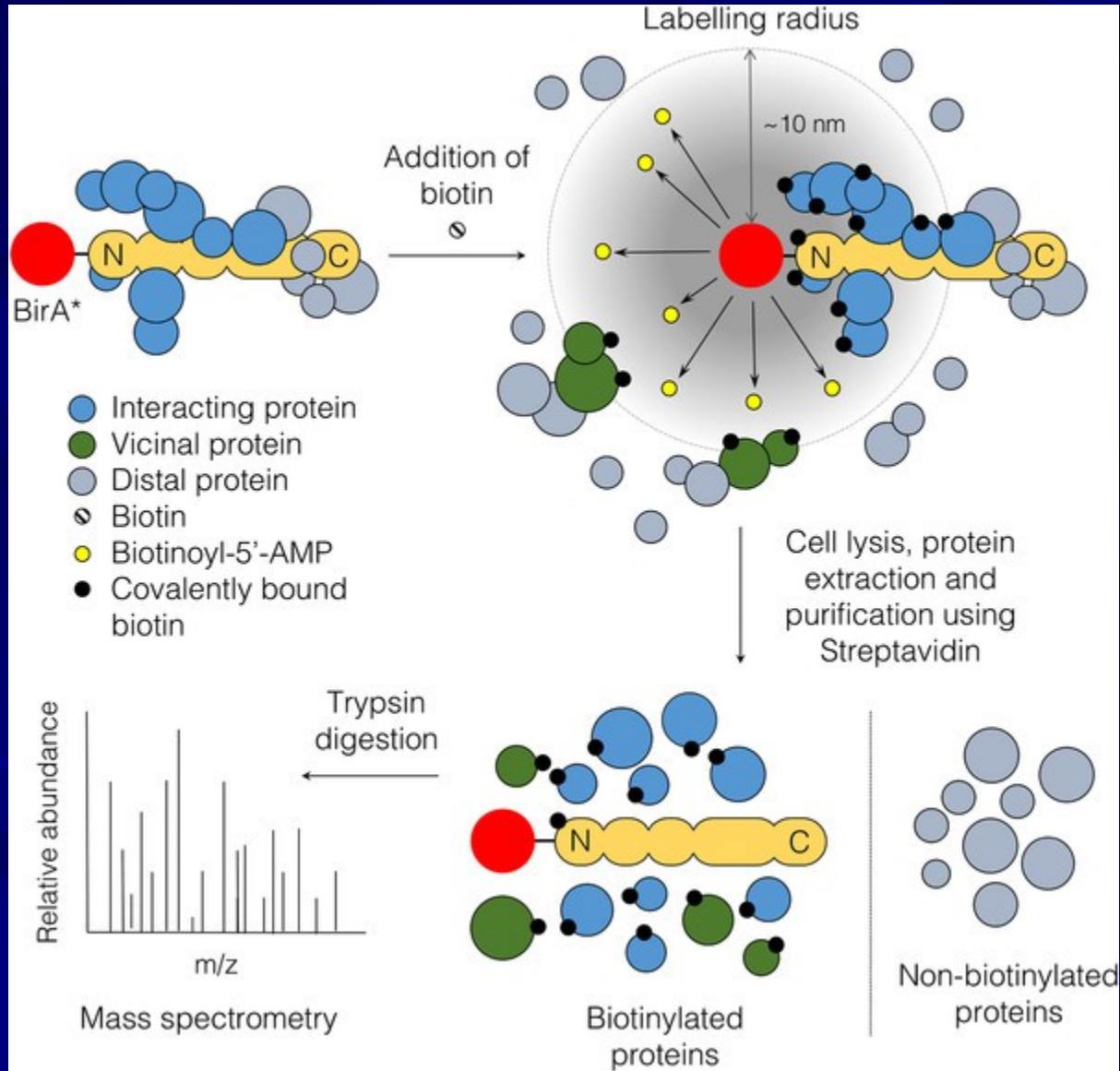


Metody č. 7: Afinity purifikace a hmotnostní spektroskopie

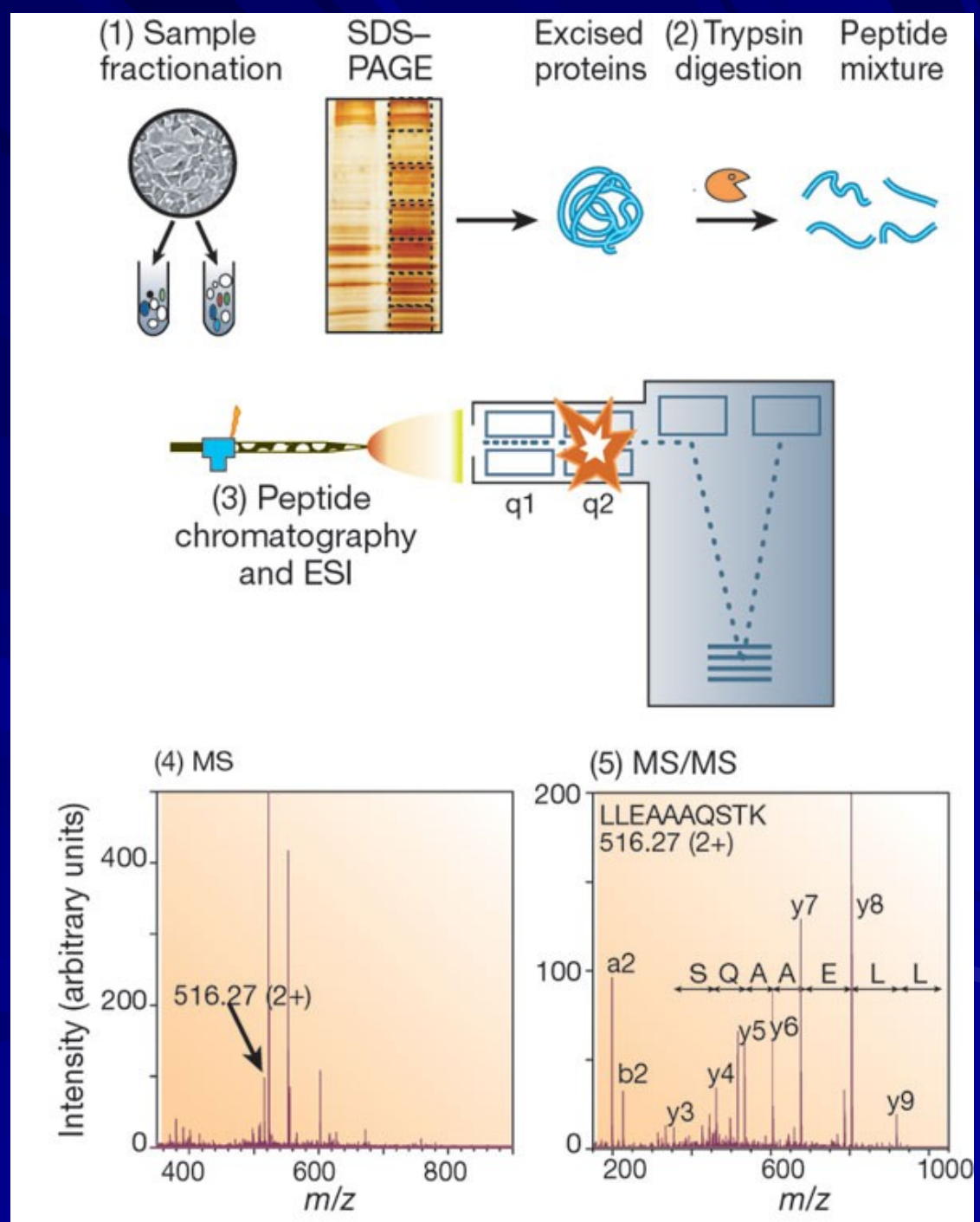
Afinitní purifikace



BioID – proximity labeling



Hmotnostní spektroskopie (Mass Spec)



Děkuji za pozornost!

Celogenomové
techniky

Molekulární
mechanismus

Celoproteomové
techniky

