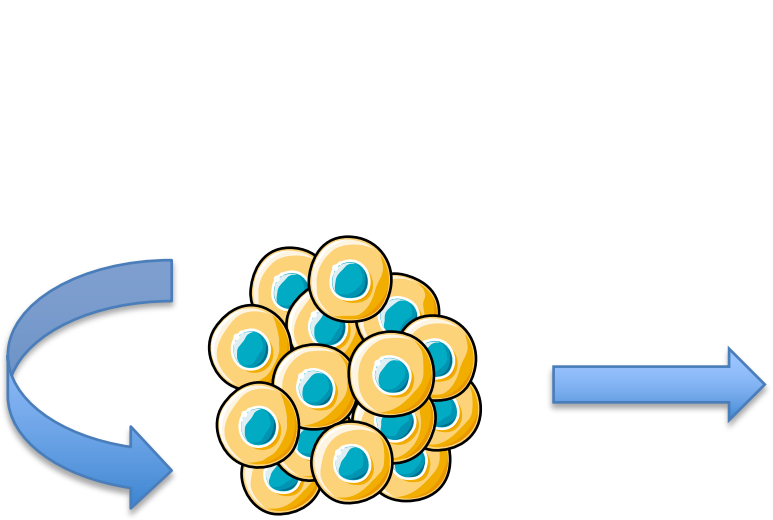
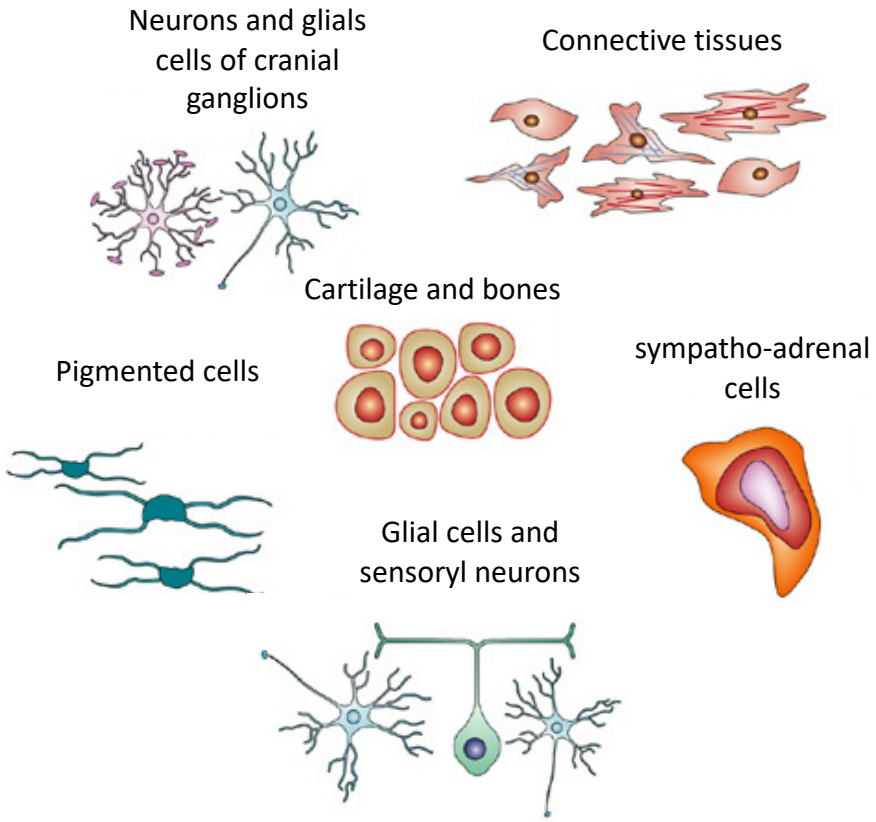
Mini research project 3 :
Are Hedgehog and/or Wnt signaling pathways involved in neural crest cell specification and/or migration?

Neural crest cell derivatives

Neural crests = Transient stem cell population in the vertebrate embryo

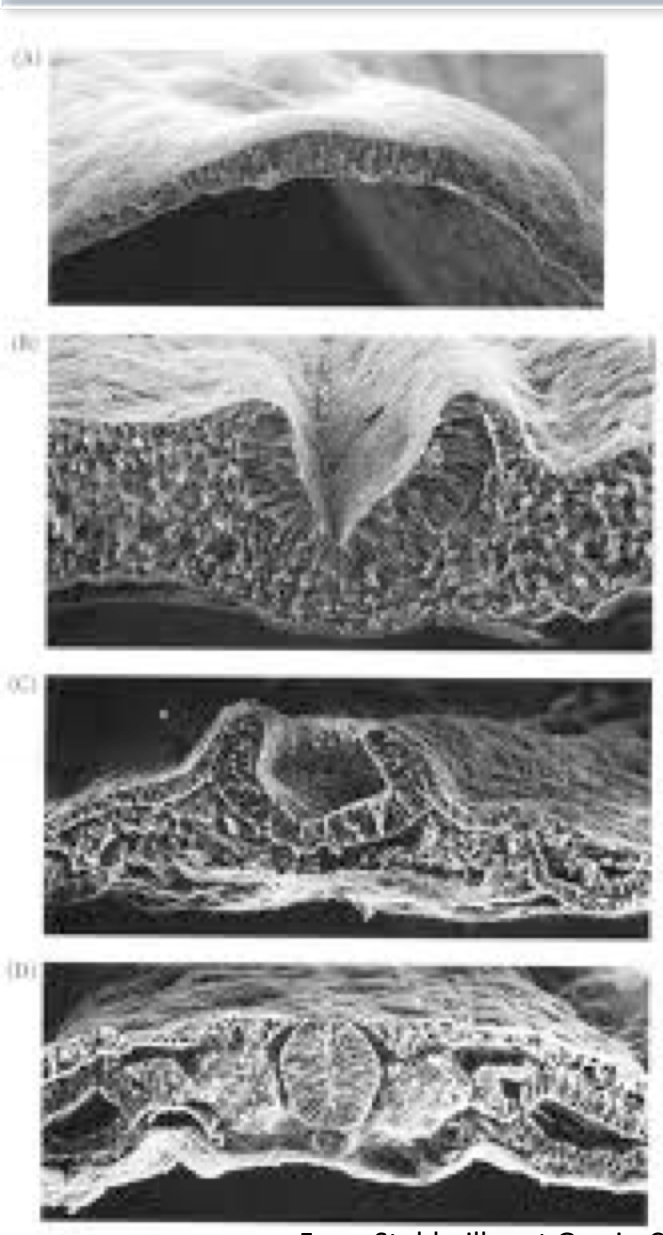


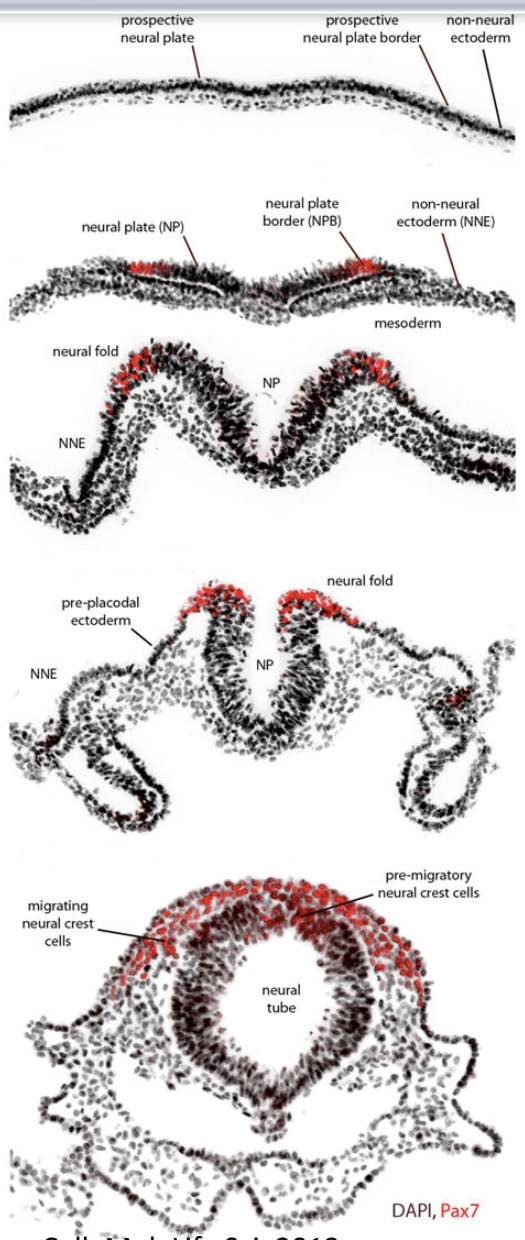


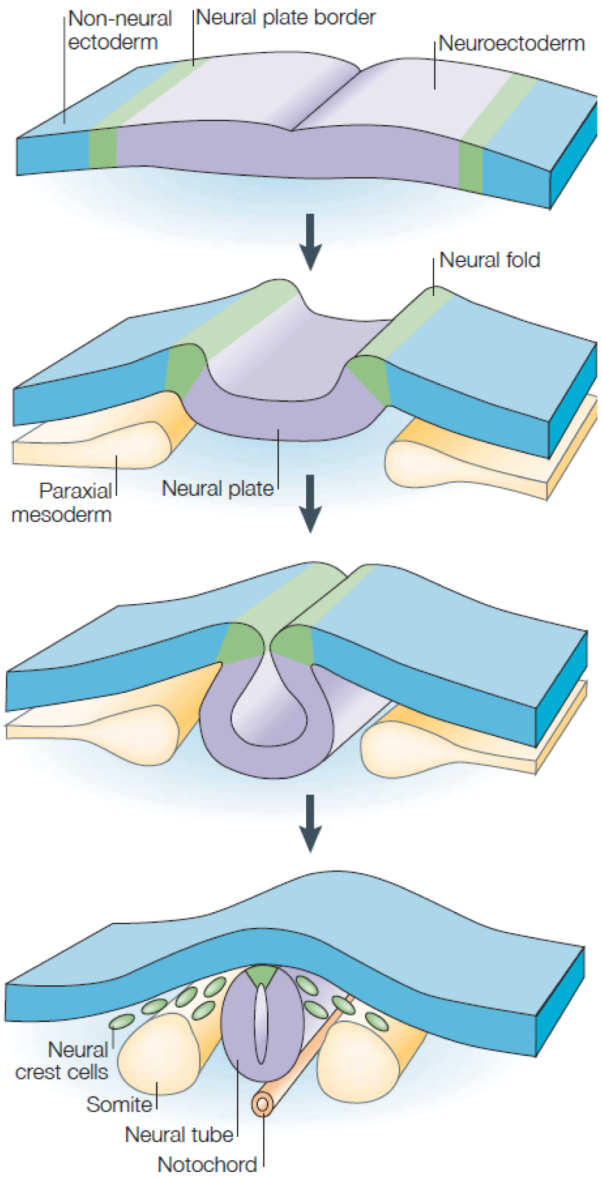
Self renewing

Multipotency

Sequential steps of Neural Crest development



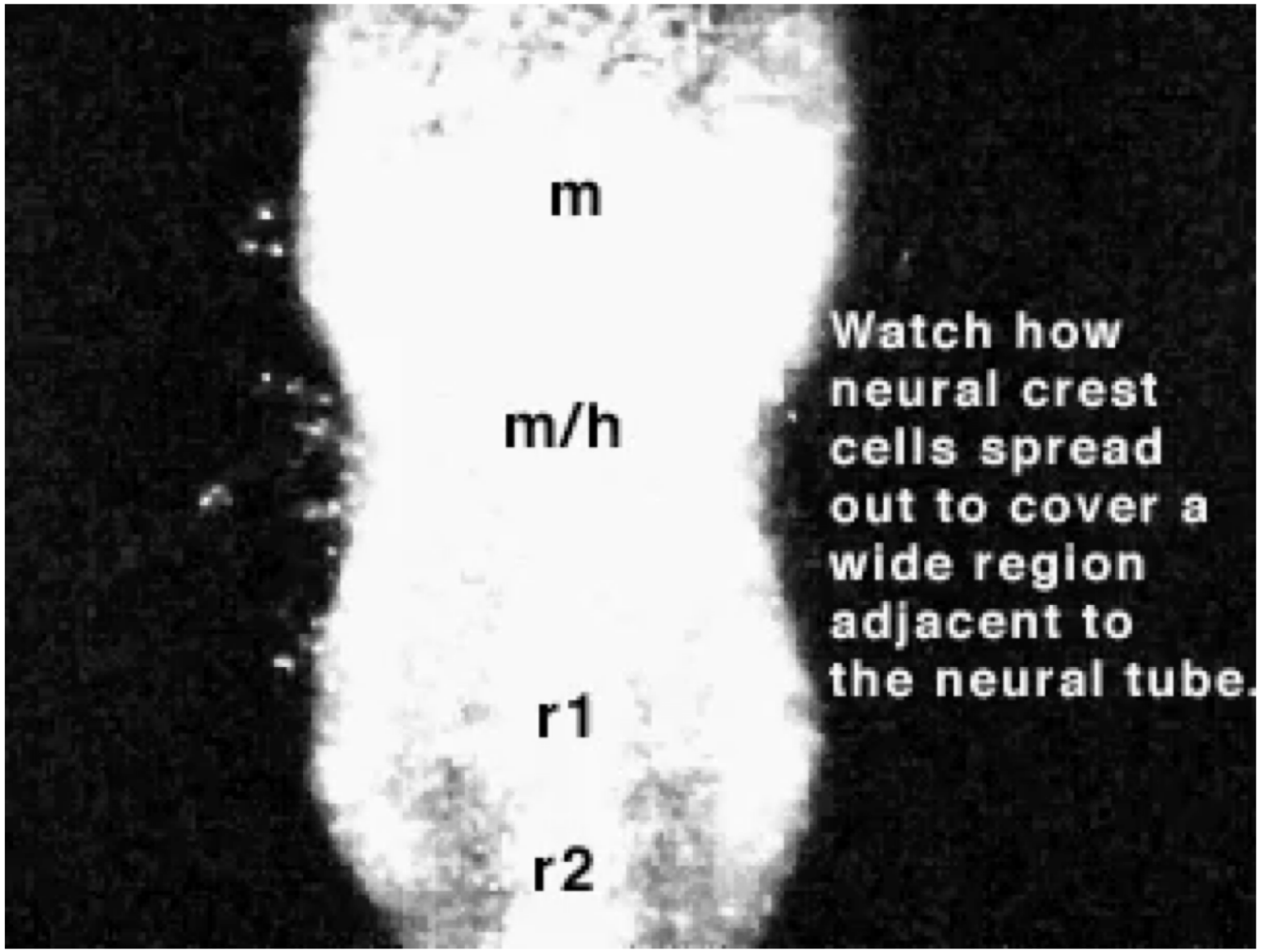




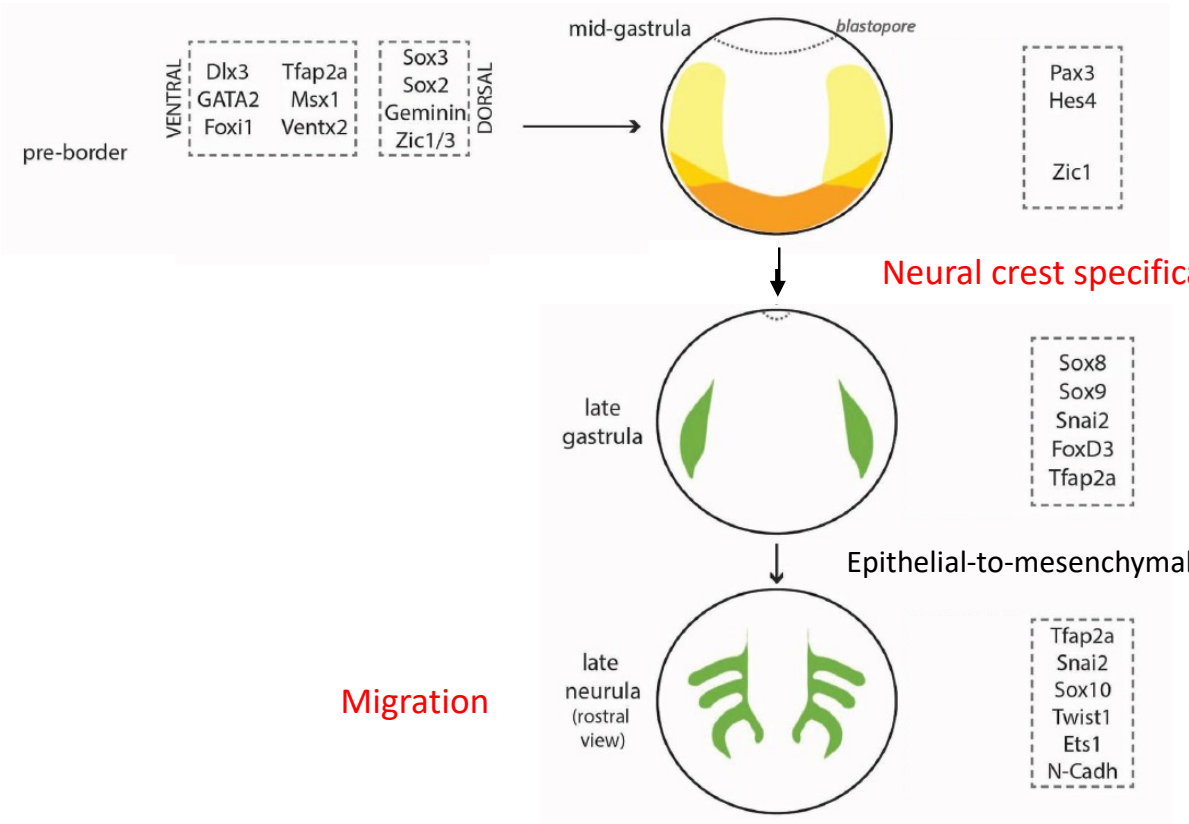
From Stuhlmiller et Garcia-Castro, Cell. Mol. Life Sci, 2012

From Gammil and Bronner-Fraser, Nature reviews, 2003

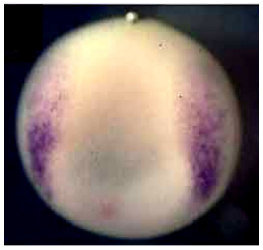
Migration step



Sequential steps of Neural Crest development

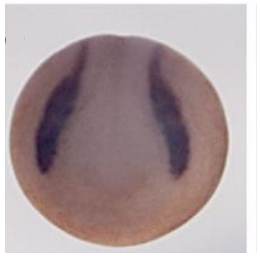


pax



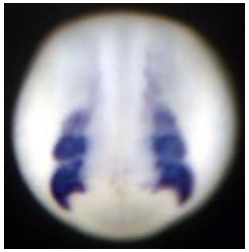
Neural border

snai2



Neural crests

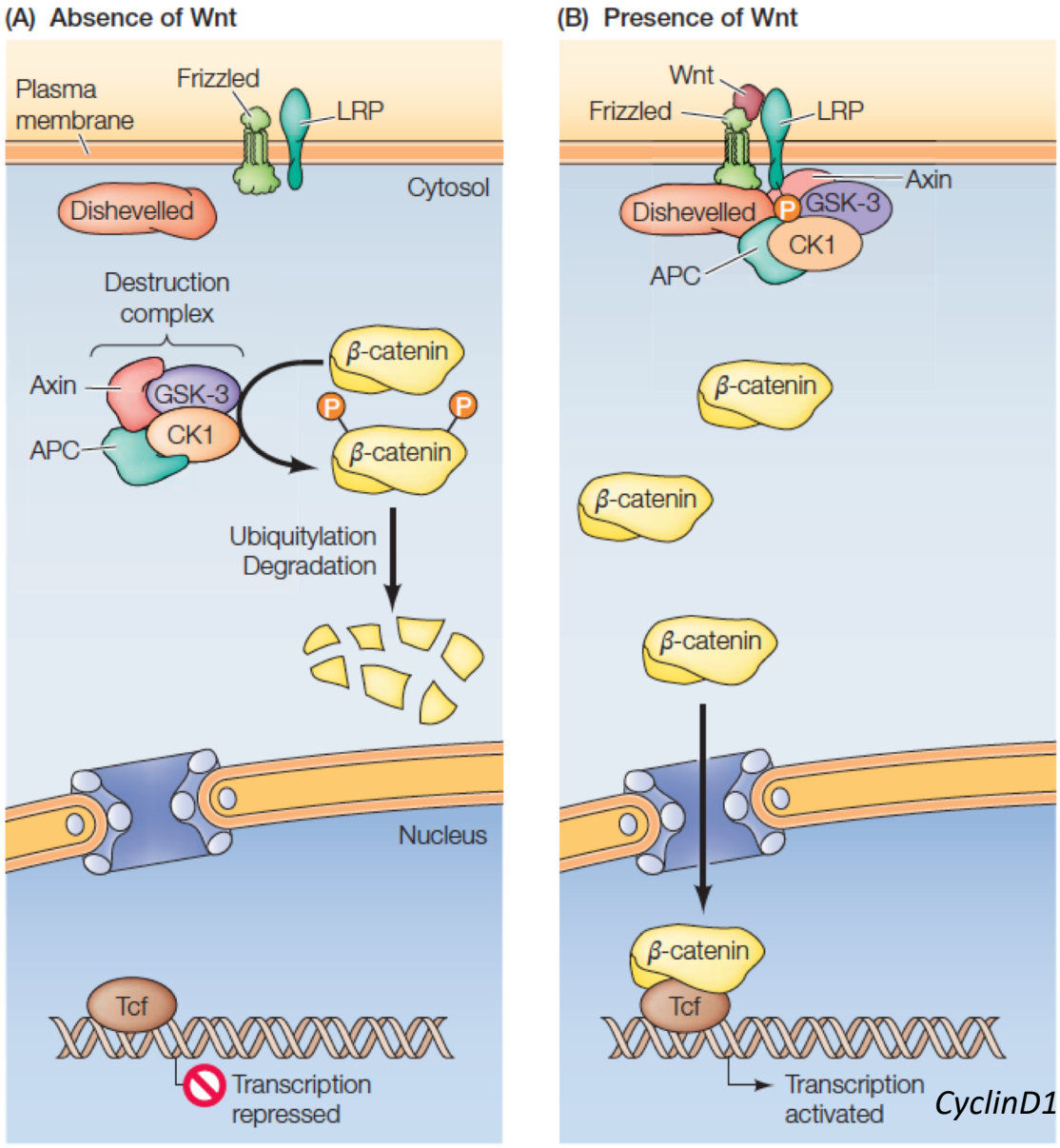
twist



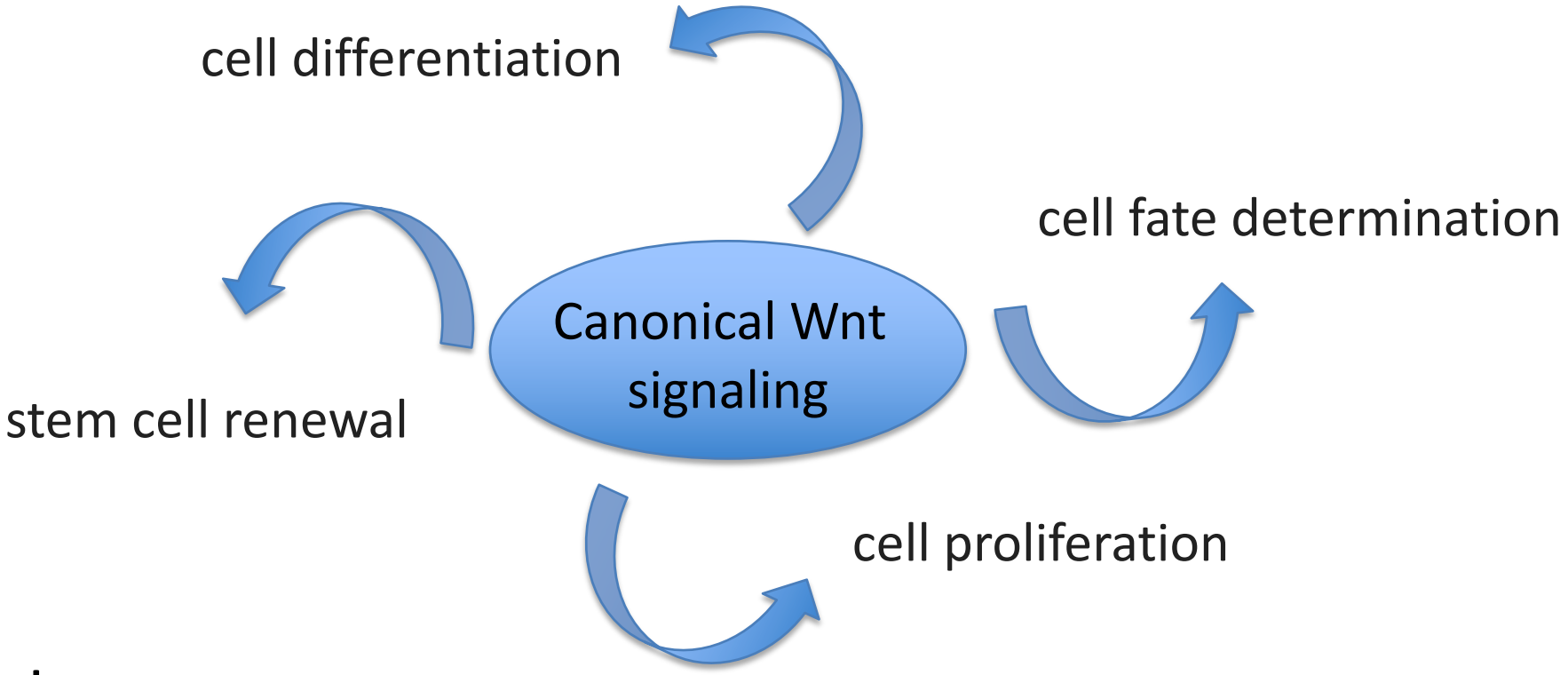
Migrating neural crest cells

Adapted from Seal and Monsoro-Burq, 2020

Wnt signaling



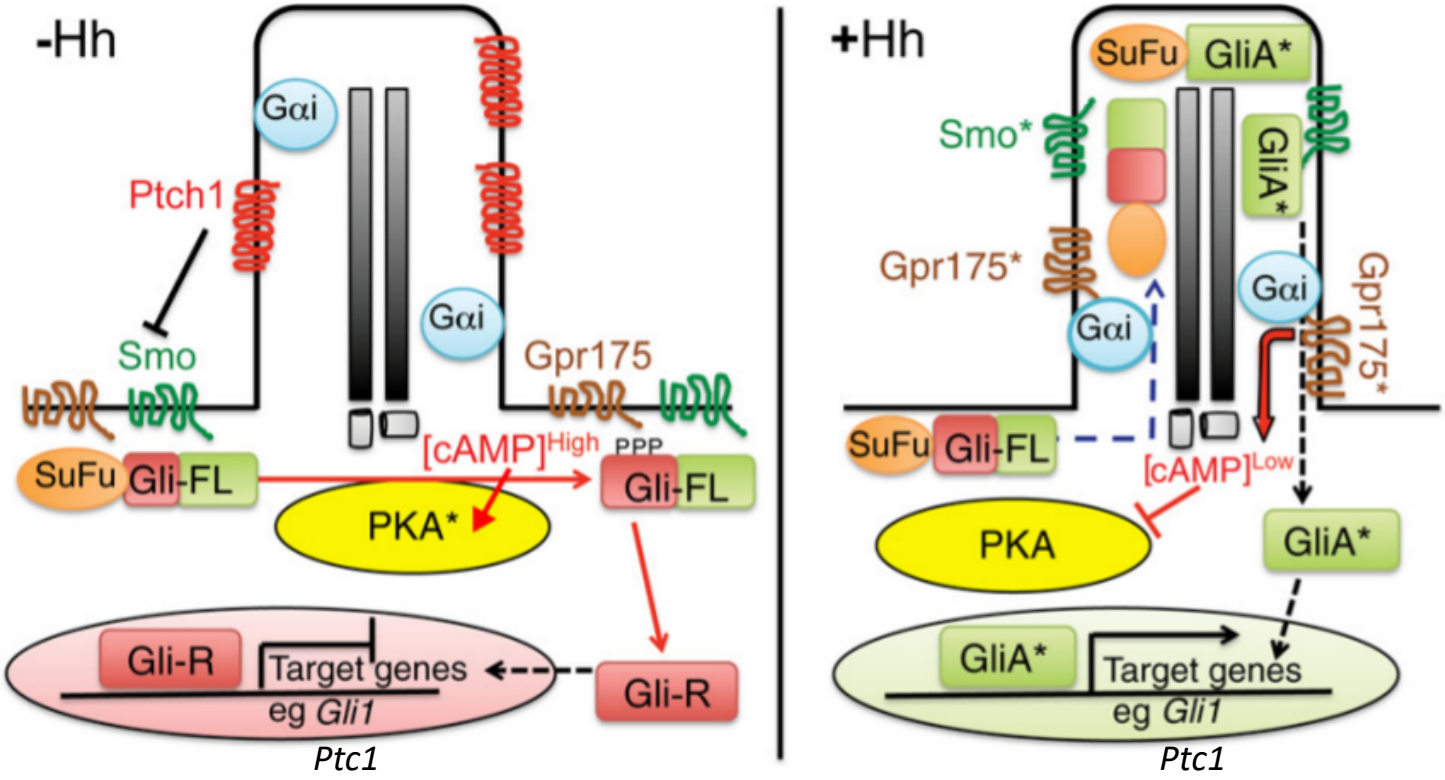
What about Wnt signaling and development?



Examples

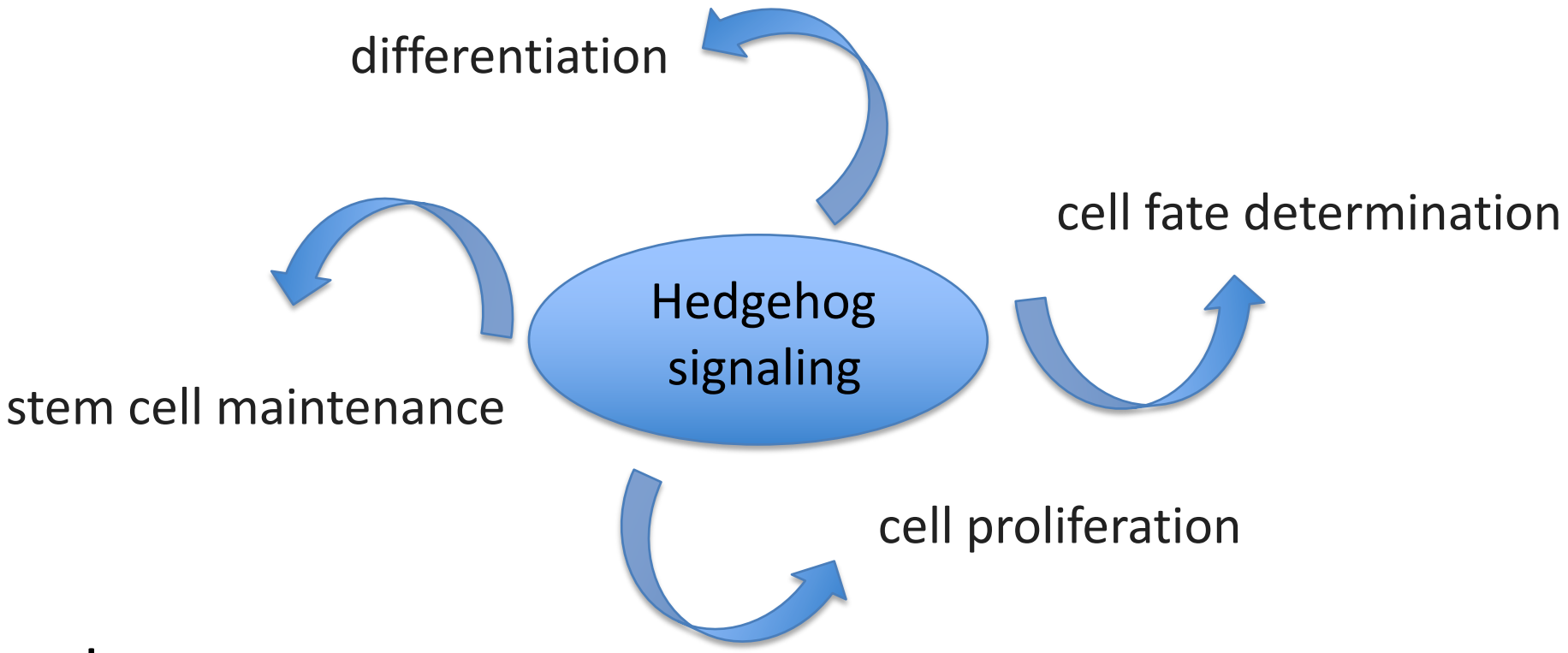
- Formation of the Spemann-Mangold organizer => embryonic body axes
- Antero-posterior regionalisation of the central nervous sytem
- Dorso-ventral regionalisation of the central nervous sytem
- formation of various organ systems including the heart, lungs, kidney, skin and bone

Hedgehog signaling



Adapted from Singh et al., 2015

What about Hedgehog signaling and development?



Examples

- craniofacial development
- Dorso-ventral regionalisation of the central nervous sytem
- Somite differentiation
- Limb bud formation

Here we ask the following question:

Are Hedgehog and/or Wnt signaling pathways involved

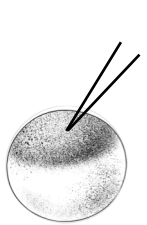
(1) in the context of neural crest cell specification?

And / or

(2) in the context of neural crest cell migration?

Tools available in the lab to design your experiments

✓ Technique for genetic gain/loss of function:
=> microinjection at the 1 cell stage



✓ Tracer for microinjection:
Dextran Fluorescent Lysin (DFL)

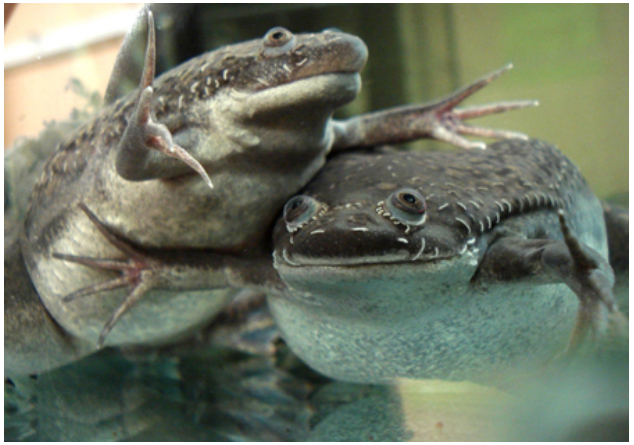
✓ Technique for phenotype analysis: *in situ* hybridization

✓ Plasmid available to synthesize RNA for microinjection:
- *pCS2-lacZ (ßGal)*
- *pCDNA-dnTCFGR* (dn: dominant negative; inducible activity thanks to the fusion with the glucocorticoid receptor GR)

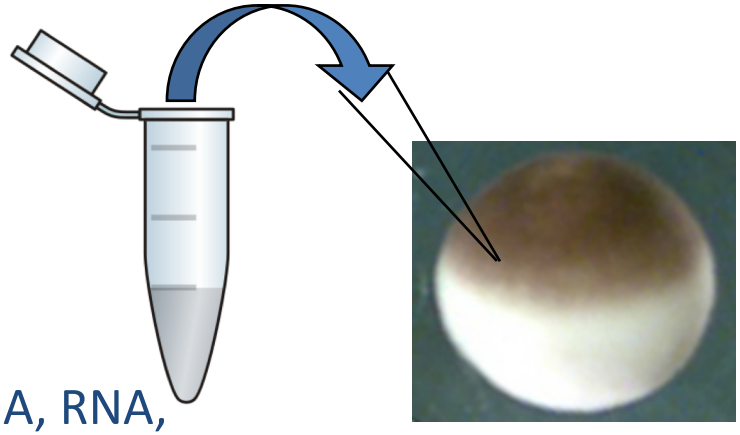
✓ pharmacological agent available:
- Cyclopamine: Hedgehog signaling inhibitor
- Dexamethasone: glucocorticoid receptor (GR) activity inducer

✓ Plasmid available to design antisens RNA probes for *in situ* hybridization:
- *pBS-Twist*
- *pCS2-Ptc1* (*Ptc1*=Hedgehog target gene)
- *pBS-CyclinD1* (*CyclindD1*=Wnt target gene)

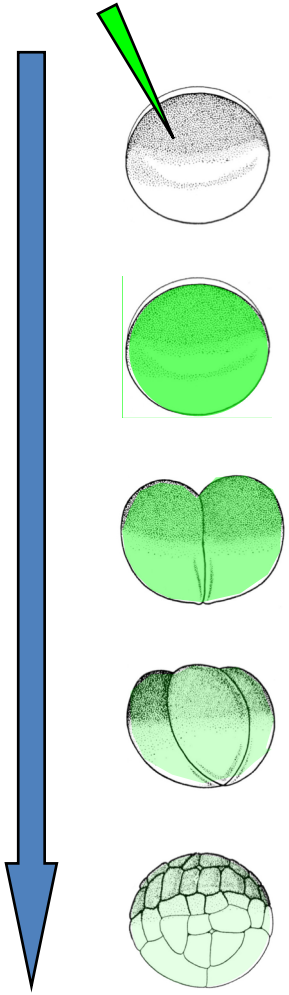
Xenopus laevis, an ideal model to study the precocious steps of the development



Microinjection experiments

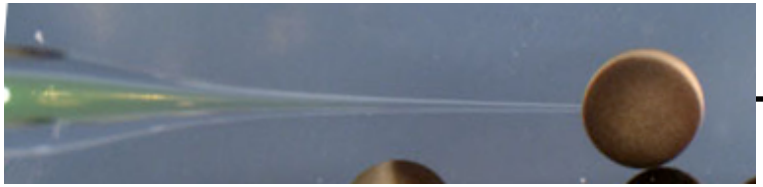


DNA, RNA,
morpholino



Xenopus laevis, an ideal model to study the precocious steps of the development

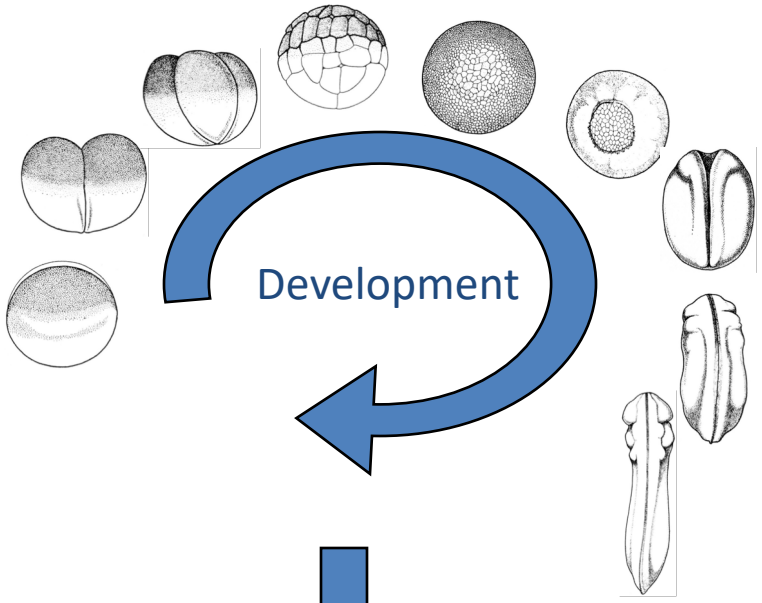
Step 1



incubation

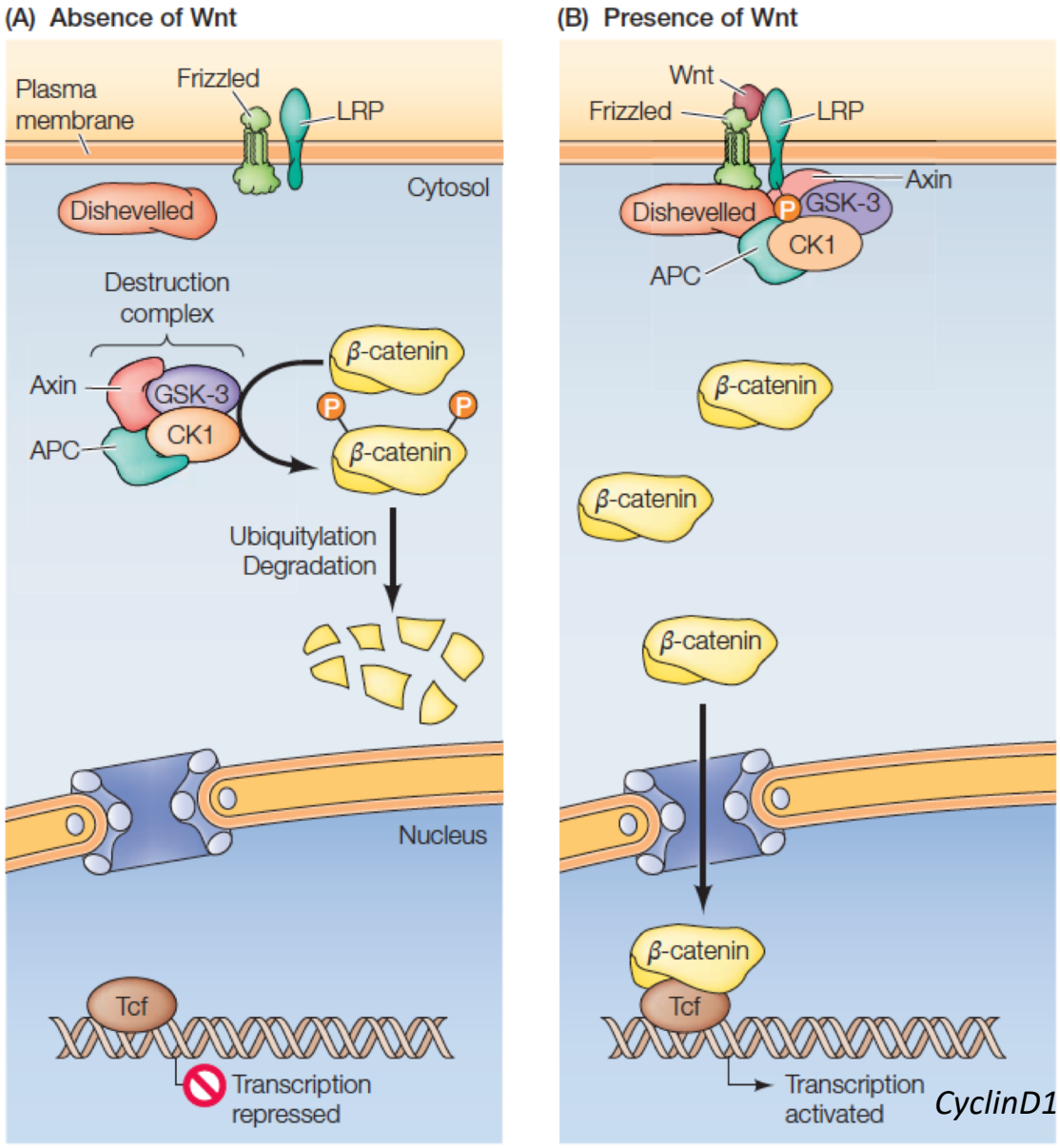
15-21 ° C

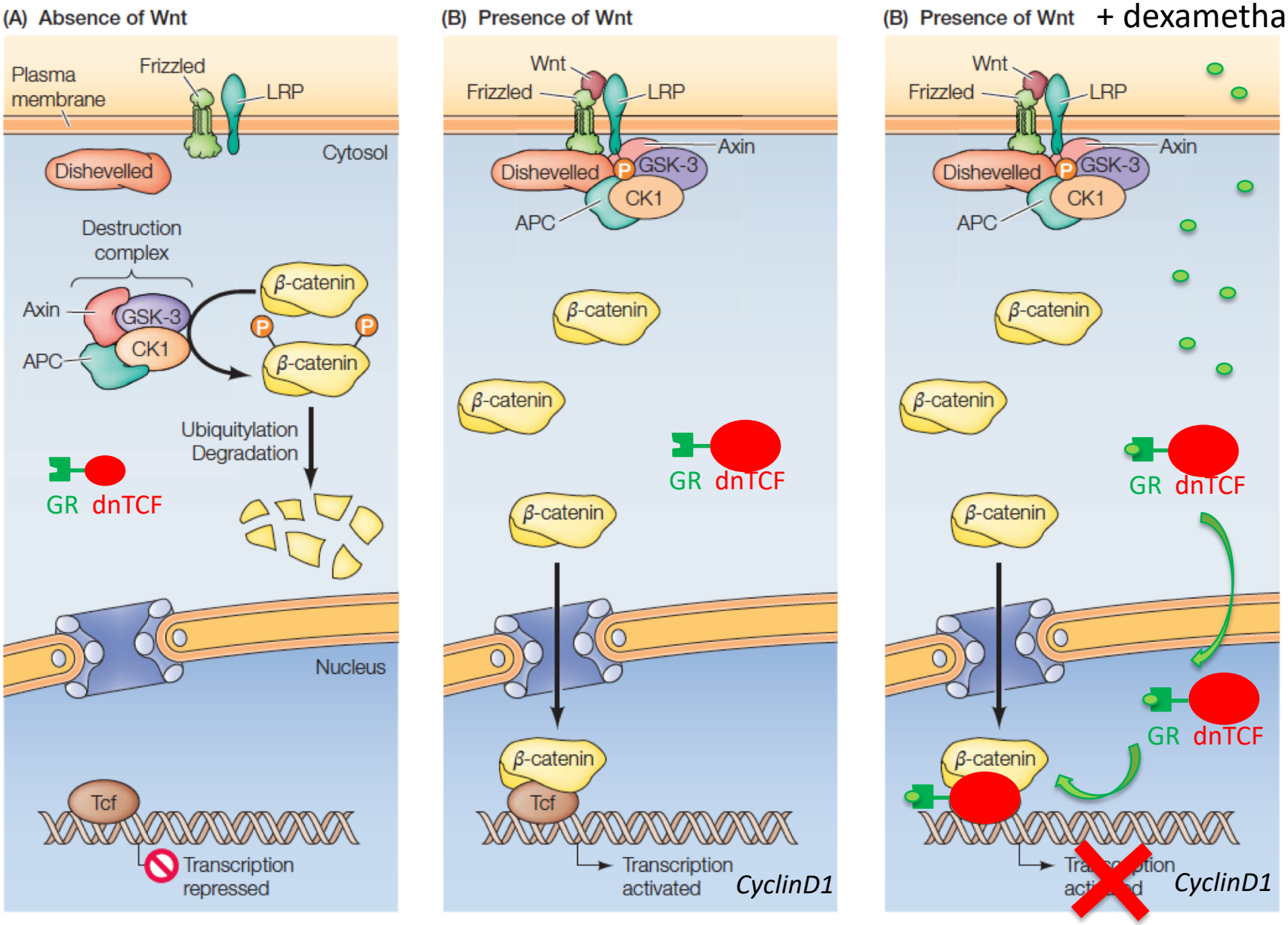
Step 2



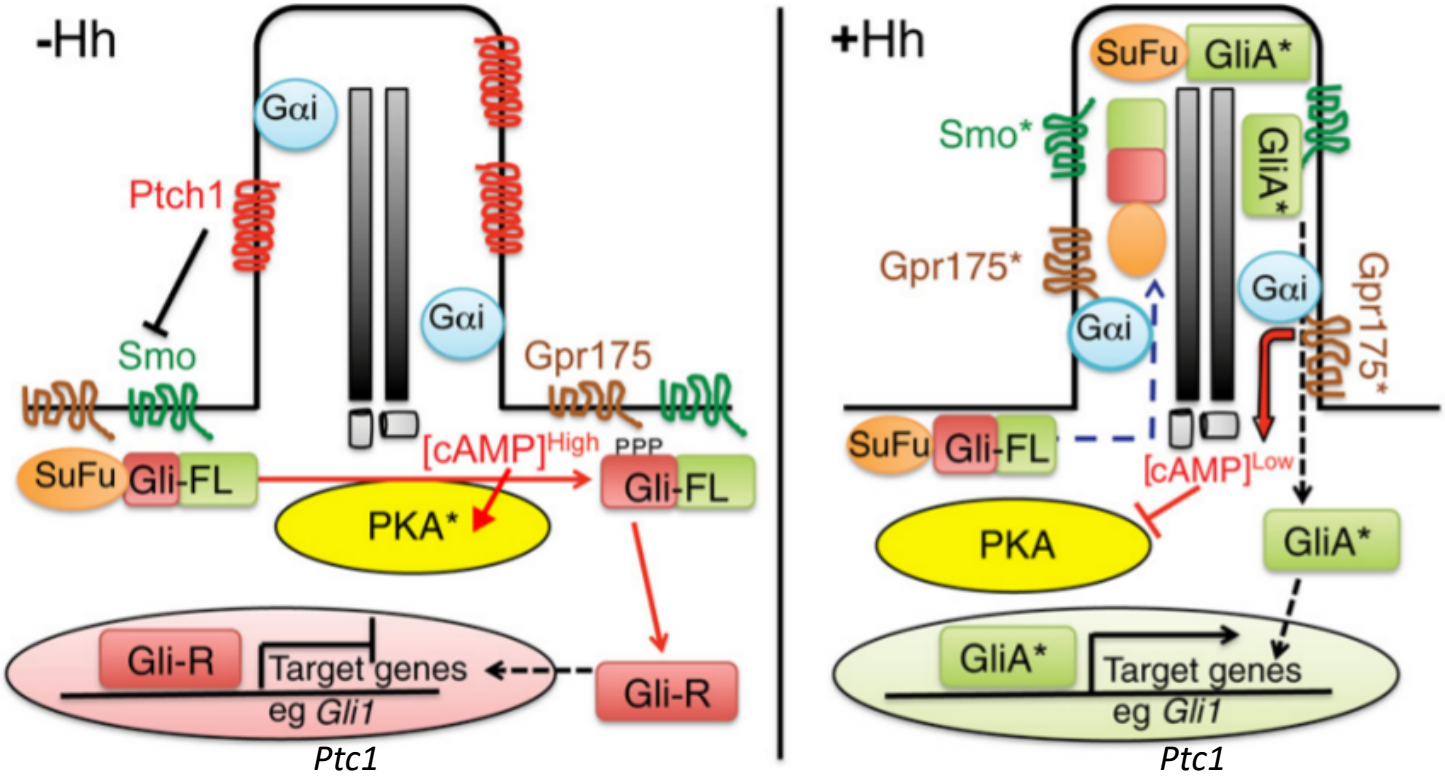
Analysis

Wnt signaling



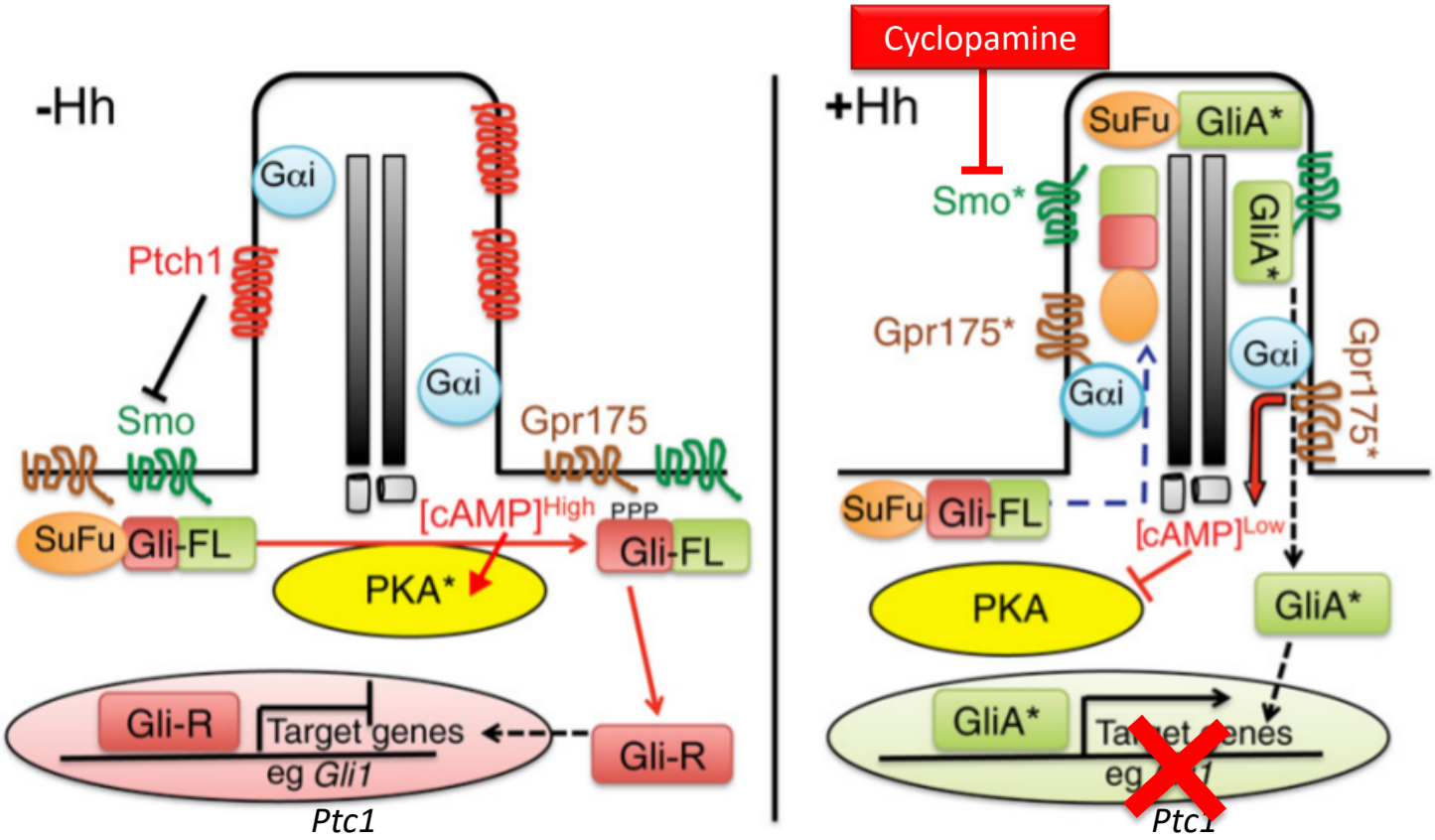

(A) Absence of Wnt
Plasma membrane
Frizzled
LRP
Cytosol
Dishevelled
Destruction complex
Axin
GSK-3
CK1
APC
β-catenin
P
β-catenin
Ubiquitylation
Degradation
GR dnTCF
Nucleus
Tcf
Transcription repressed
(B) Presence of Wnt
Wnt
Frizzled
LRP
Axin
Dishevelled
P
GSK-3
CK1
APC
β-catenin
β-catenin
GR dnTCF
β-catenin
β-catenin
Tcf
Transcription activated
CyclinD1
(B) Presence of Wnt + dexamethasone
Wnt
Frizzled
LRP
Axin
Dishevelled
P
GSK-3
CK1
APC
β-catenin
β-catenin
GR dnTCF
β-catenin
GR dnTCF
β-catenin
CyclinD1

Hedgehog signaling



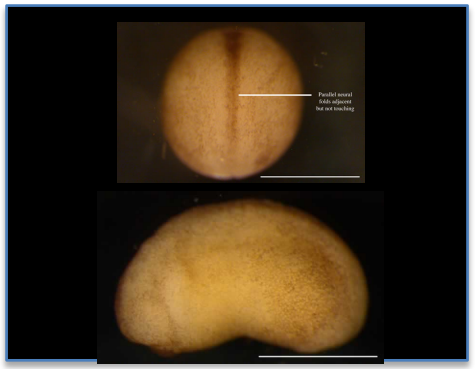
Adapted from Singh et al., 2015

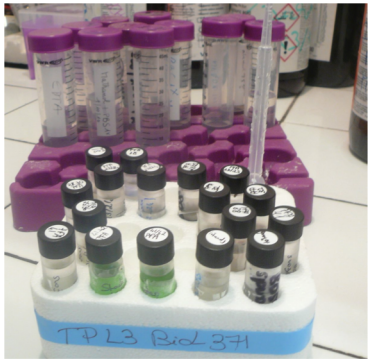
Hedgehog signaling

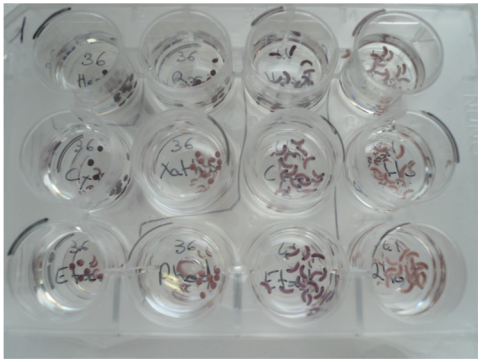


Adapted from Singh et al., 2015

From genetic/pharmacological perturbations to phenotypic analysis





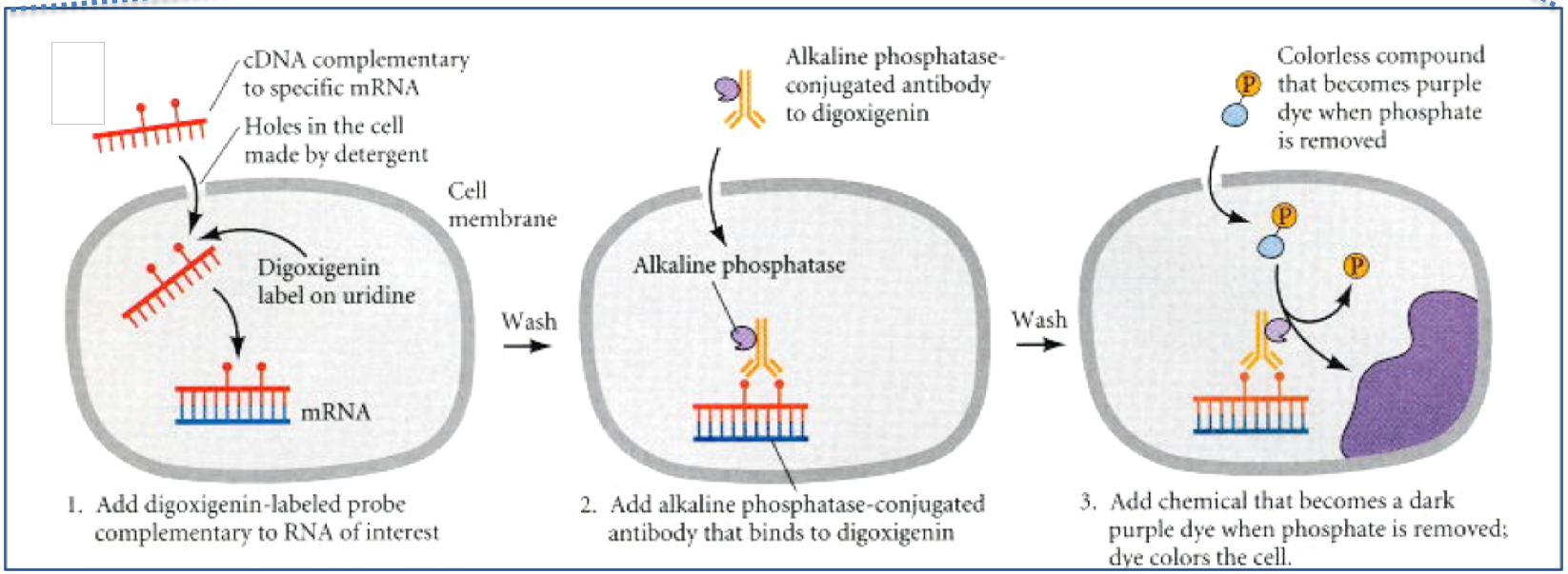


Tuesday
FIV and mRNA injections

Wednesday, thursday
Dex/cyclopamine treatments

Thursday, Friday
Embryo fixation

Next week
WISH

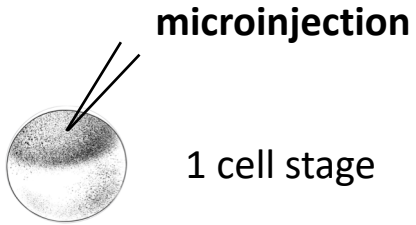


Group work: Design of the experimental plan

Don’t forget all the experimental controls!

Tools available in the lab to design your experiments

✓ Technique for genetic gain/loss of function:
=> microinjection at the 1 cell stage



✓ Tracer for microinjection:
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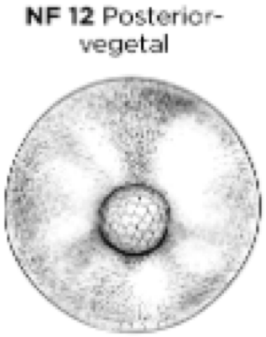
- *pBS-Twist*
- *pCS2-Ptc1* (*Ptc1*=Hedgehog target gene)
- *pBS-CyclinD1* (*CyclindD1*=Wnt target gene)

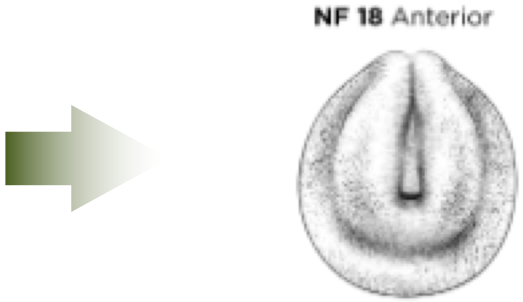
Experimental design

Q1: Is Hedgehog signaling pathway involved in the context of neural crest cell specification?



Cylopamine treatment stage 12

Controls :
- EtOH treated embryos
- WT embryos



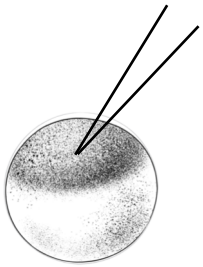
In situ hybridization later (stage 18):

Ptc1: control of the Hh signaling inhibition
Twist: analysis of the NC specification

Experimental design

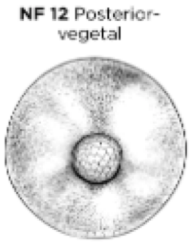
Q2: Is Wnt signaling pathway involved in the context of neural crest cell specification?

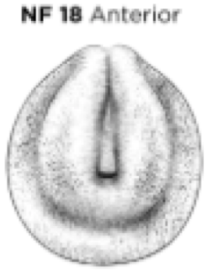
dnTCF-GR mRNA injection
(+ DFL as a tracer)



Controls :
- ß-Gal + DFL injected embryos
- WT embryos

dnTCF activation with dexamethasone at stage 12



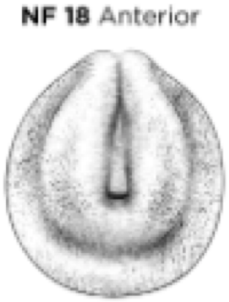


In situ hybridization later (stage 18):

CyclinD1: control of the Wnt signaling inhibition
Twist: analysis of the NC specification

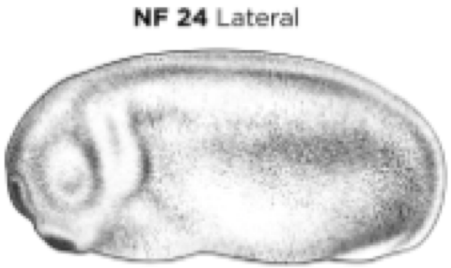
Experimental design

Q3: Is Hedgehog signaling pathway involved in the context of neural crest cell migration?



Cylopamine treatment stage 18

Controls :
- EtOH treated embryos
- WT embryos



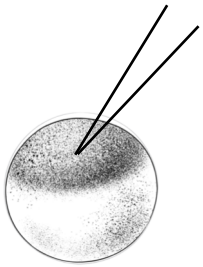
In situ hybridization later (stage 24):

Ptc1: control of the Hh signaling inhibition
Twist: analysis of the NC specification

Experimental design

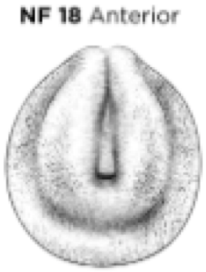
Q4: Is Wnt signaling pathway involved in the context of neural crest cell migration?

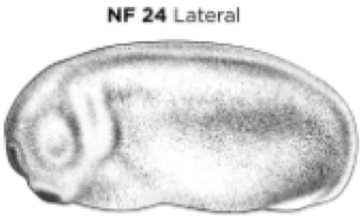
dnTCF-GR mRNA injection
(+ DFL as a tracer)



Controls :
- ß-Gal + DFL injected embryos
- WT embryos

dnTCF activation with dexamethasone at stage 18





In situ hybridization later (stage 24):

CyclinD1: control of the Wnt signaling inhibition
Twist: analysis of the NC specification

To summarize:

- Injection at 1 cell stage :
- **ß-Gal mRNA + DFL**
- **dnTCF-GR mRNA + DFL**

- **+ dex at stage 12 or stage 18**

- **cyclopamine or EtOH at stage 12 or stage 18**

Don’t forget WT embryos!

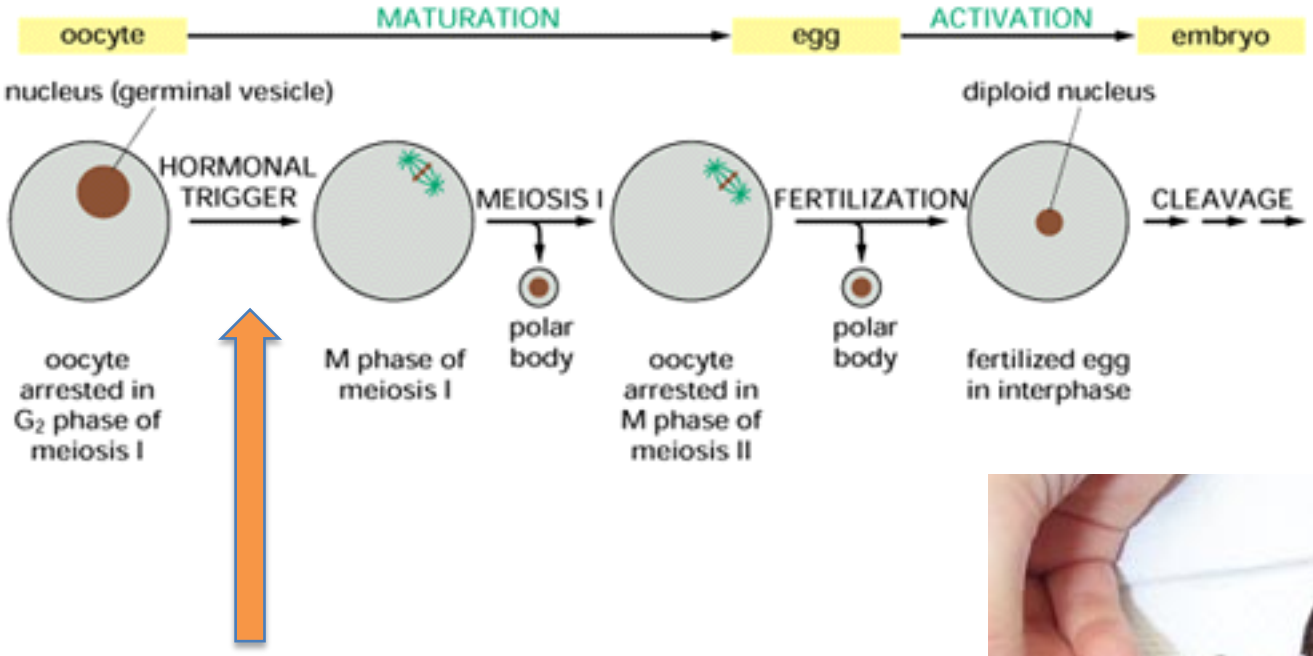
Schedule of the course

	Monday oct 13rd **UVSQ**		*Tuesday oct 14th* **NeuroPSI**	*Wednesday oct 15th* **UVSQ**		*Thursday oct 16th* **NeuroPSI**	*Friday oct 17th* **UVSQ**
9h-10h	General introduction		Xenopus FIV and injection training	personal work		Fixation of the first batch of embryos at stage 18 / induction by DEX treatment of the second batch of embryos at stage 18 / cyclopamine treatment at stage 18	Cell culture & observation
10-11h	Experimental design of the Xenopus project			Cell culture & observation	dissection		
11h-12h	Experimental design of the Drosophila project						
12h-13h	*lunch*		*lunch*		dissociation		
13h-14h	Design of the cell culture projects & Cell Culture	*Drosophila* dissection training	Xenopus FIV and mRNA injection	*lunch*		*lunch*	*lunch*
14h-15h				cytometry acquisition		WISH probe synthesis	cytometry analysis
15h-16h							
16h-17h							
17h-18h							personal work
made by teachers	Inducing Ovulation / *Drosophila* infection		*Drosophila* infection	checking of the embryos / induction by DEX treatment at stage 12,5 / cyclopamine treatment at stage 12,5			fixation of the second batch at stage 24

	Monday oct 20th **NeuroPSI**	*Tuesday oct 21st* **NeuroPSI**		*Wednesday oct 22nd* **UVSQ**		*Thursday oct 23rd* **NeuroPSI**	*Friday oct 24th* **UVSQ**	
9h-10h	WISH day 1: pre-treatments	WISH day 2: probe washes and incubation with anti-DIG antibody		Cell culture & observation	Dissection fixation mounting	WISH day 4: Post-fixation, pictures of whole mount embryos	Cell Culture & observation	
10-11h								
11h-12h							Cell Culture analysis	Drosophila analysis
12h-13h							*lunch*	
13h-14h	*lunch*	*lunch*		*lunch*		*lunch*	Perconal work, Cell Culture analysis	Perconal work, Drosophila analysis
14h-15h	WISH day 1: pre-treatments, personal work, **QUIZZ**		WISH day 2: probe washes and incubation with anti-DIG antibody	Cell culture & observation	confocal acquisition/ analysis	personal work, analysis of the results		
15h-16h		Conference on organoids						
16h-17h								
17h-18h	WISH day 1 : o/n hybridization	WISH day 2 : o/n washes						
made by teachers	medium changes / *Drosophila* infection	*Drosophila* infection		WISH day 3: start NBT/BCIP coloration				

X. laevis female hormonal stimulation

- complete meiosis 1
- induce ovulation and laying



HCG

Eggs laying

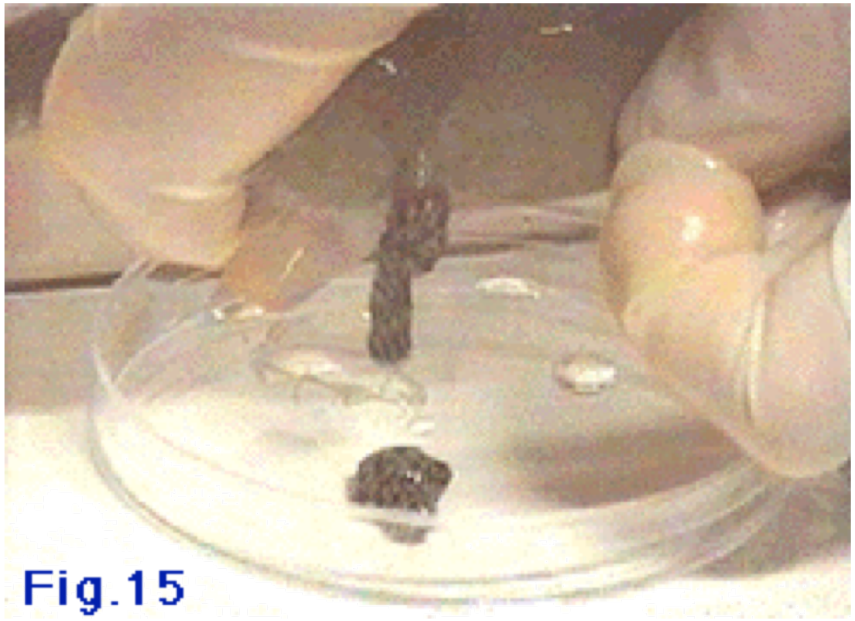


Fig.15

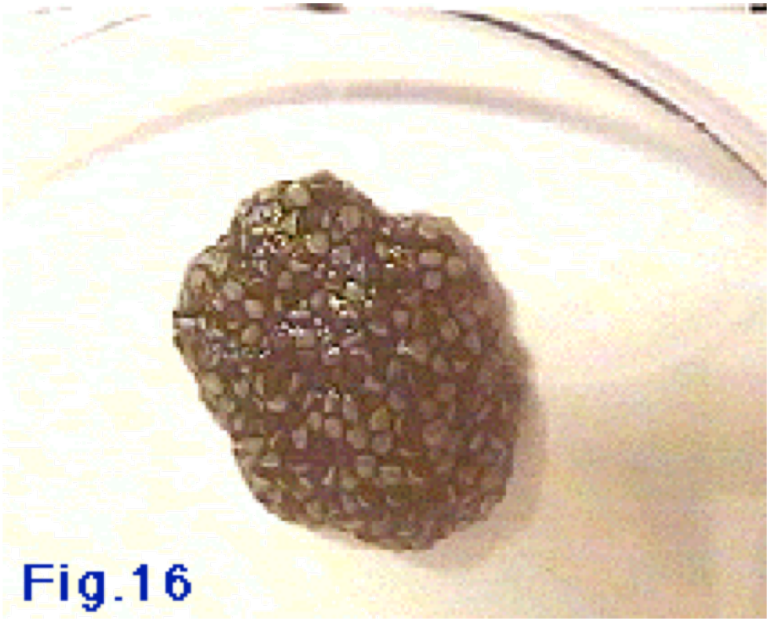


Fig.16

=> *In vitro* fertilization

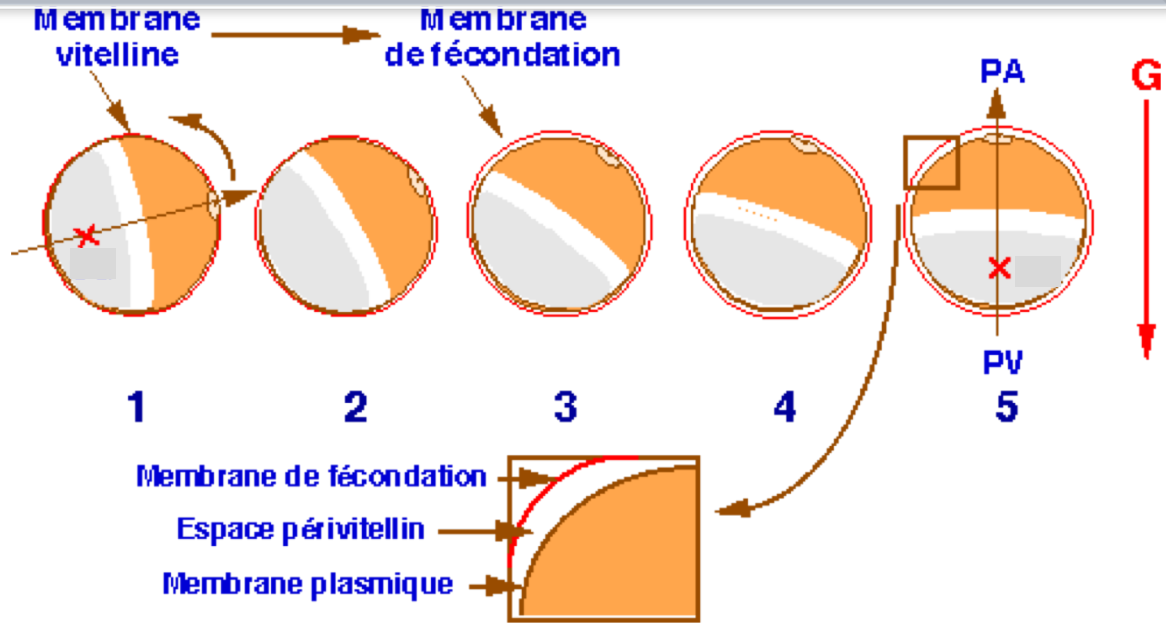
Fertilization

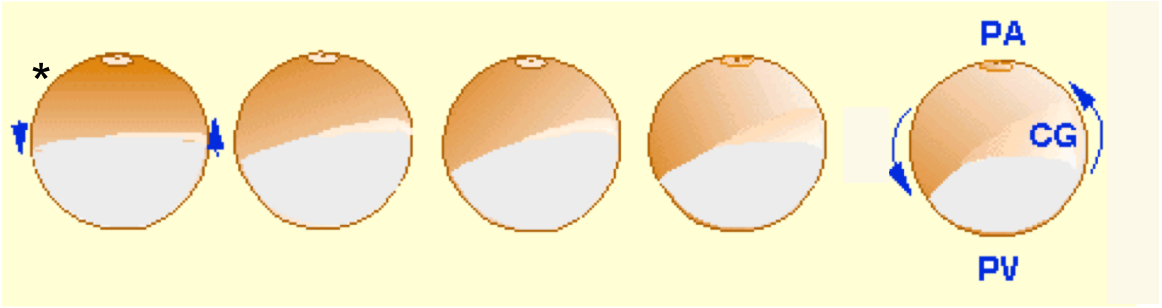
Steps upon fertilization

1. Déblocage de la méiose dans l'ovocyte II :
- émission du GP2
- fusion des pronuclei : restauration de la diploïdie

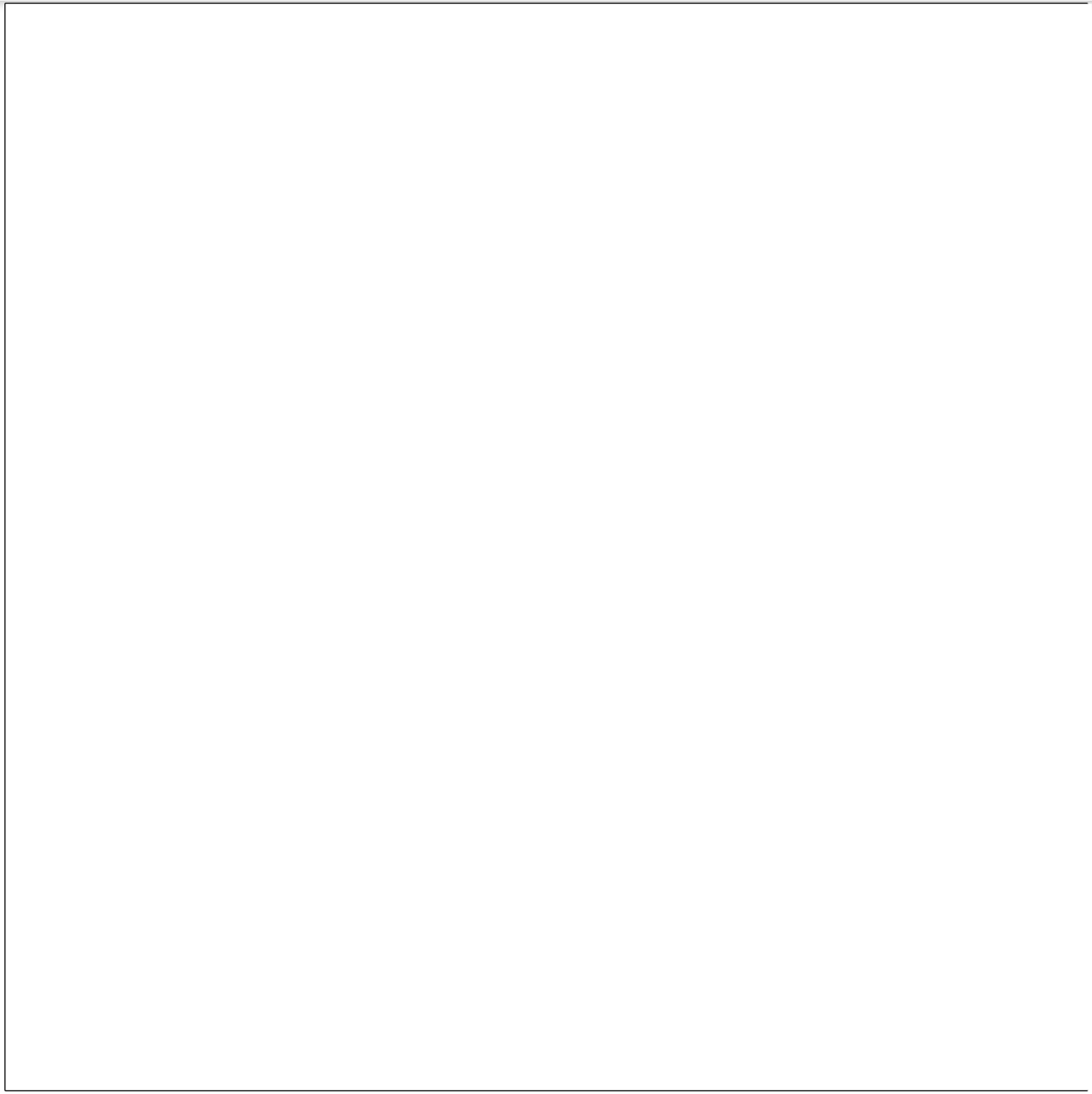
2. Formation de la **membrane de fécondation**
Rotation d'**équilibration** de la cellule œuf

3. **Rotation corticale** et formation du croissant gris





Cleavage



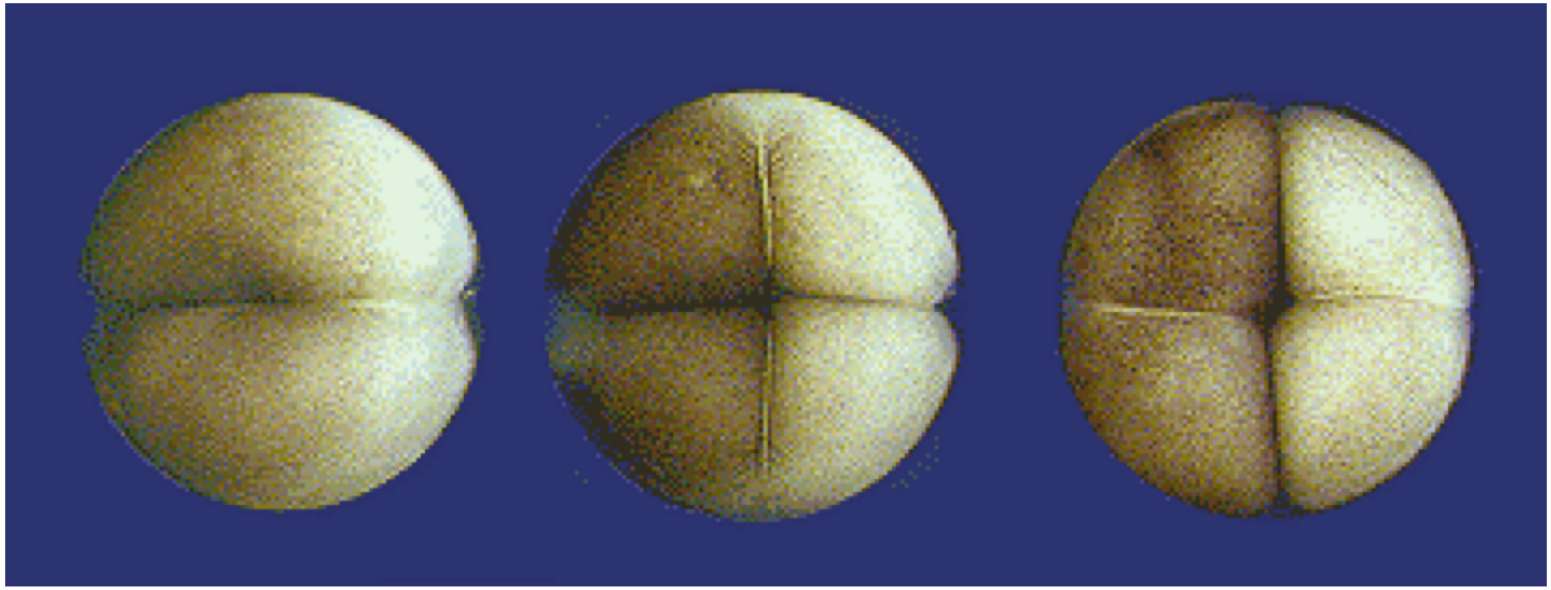
Gastrulation and neurulation

Injection needle calibration

Electrophysiology glass capillaries, with inner filament (capillarity).

- Keep diameter as small as possible
- Keep flow as gentle as possible (long injection time)
- calibrate for 10nl injection volume

Microinjection at two or four cell stage



Microinjection at two and four cell stage