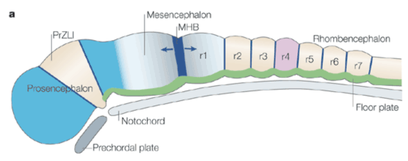
Understanding neural progenitor dynamics in the zebrafish hindbrain

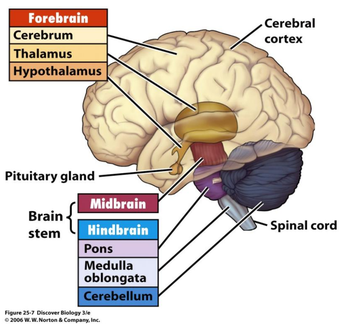
Ferran Capell Pascual, Polina Krivykh, Júlia Urgel i Solas. Sylvia Dyballa, Cristina Pujades.
School of Molecular and Theoretical Biology-2016

Introduction

The **hindbrain** is the third vesicle of the developing brain

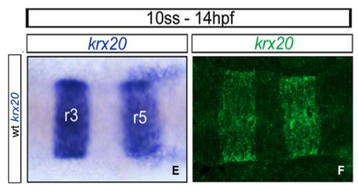


Kiecker and Lumsden, 2005



It will give rise to the pons, the medulla oblongata and the cerebellum. These structures work together controlling the autonomic body systems.

Rhombomeres are units of gene expression

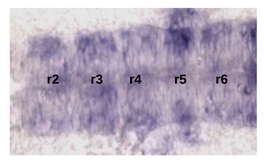


Calzolari et al., 2014

Early in development the hindbrain is transiently segmented into metameres, the socalled rhombomeres. Each rhombomere has a specific **molecular identity**.

Neurogenesis and morphogenesis take place at the same time

neurog1



Mu4127 HuC:Kaede

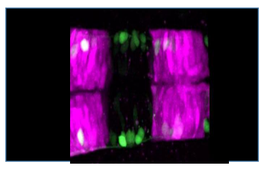
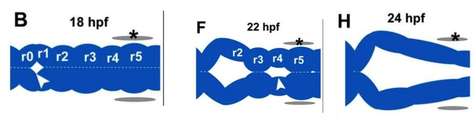


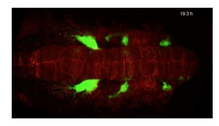
Image cortesy of Adrià Voltes

Neuronal progenitors express proneural genes. Neuronal progenitors can then differentiate to give rise to the neurons that build up the neuronal circuits.



Sive et al., 2010

Tg[neuroD:GFP]



look at hindbrain development

At the same time that neuronal progenitors specify and differentiate, the hindbrain undergoes morphogenesis.

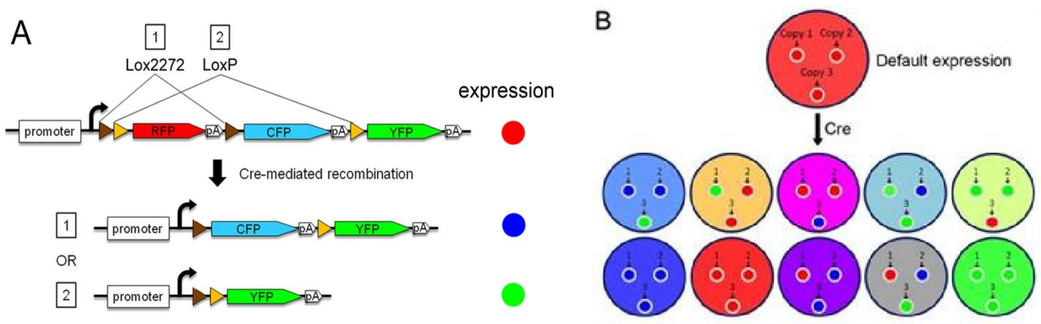
How the wide diversity of neurons is generated and how these neurons establish the neuronal circuits is to this day not well understood. The hindbrain is very conserved across the vertebrate species. Therefore, the zebrafish hindbrain is a good model to study this question. In this model system we can combine genetic tools and in vivo imaging.

Project 1. Clonal relationship and cell behavior of neuronal progenitors in the hindbrain

Introduction and **Goals**

We want the understand the lineage and the cell behavior of hindbrain neural progenitors. For this we use the *Zebrabow* tool because it allows us to label progenitor cells and in a specific color. As the labeling is genetic also all daughter cells will be labelled in the same color. By obtaining a low frequency of labelling we can investigate cell lineage, progenitor divisions (symmetric vs. asymmetric) and distribution or progenitor cells upon hindbrain morphogenesis.

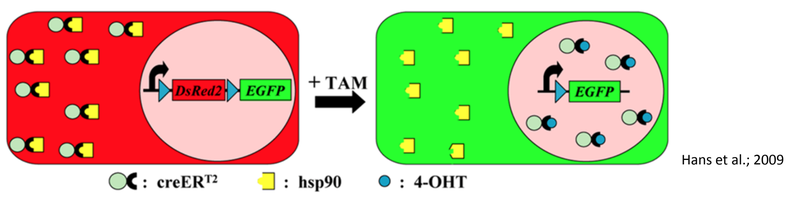
In the zebrabow transgenic line cells can express a broad range of colors upon recombination



Pan et al.; 2013

The zebrabow transgenic fish line carries multizebrabow casette, consisting of coding sequences for three distinct fluorescent proteins: RFP, CFP, YFP.

Recombination can be controlled temporally by using the Cre-ERT2 line

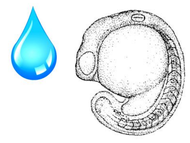


Hans et al.; 2009

The ubi:CreERT2 transgenic line contains the coding sequence of the Cre-recombinase fused to the Estrogen receptor under the control of ubiquitin promotor. Only upon presence of the ligand (tamoxifen) the fusion protein can translocate to the nucleus.

To create the double transgenic embryo we cross the Zebrabow line with the ubi:Cre-ERT2 line

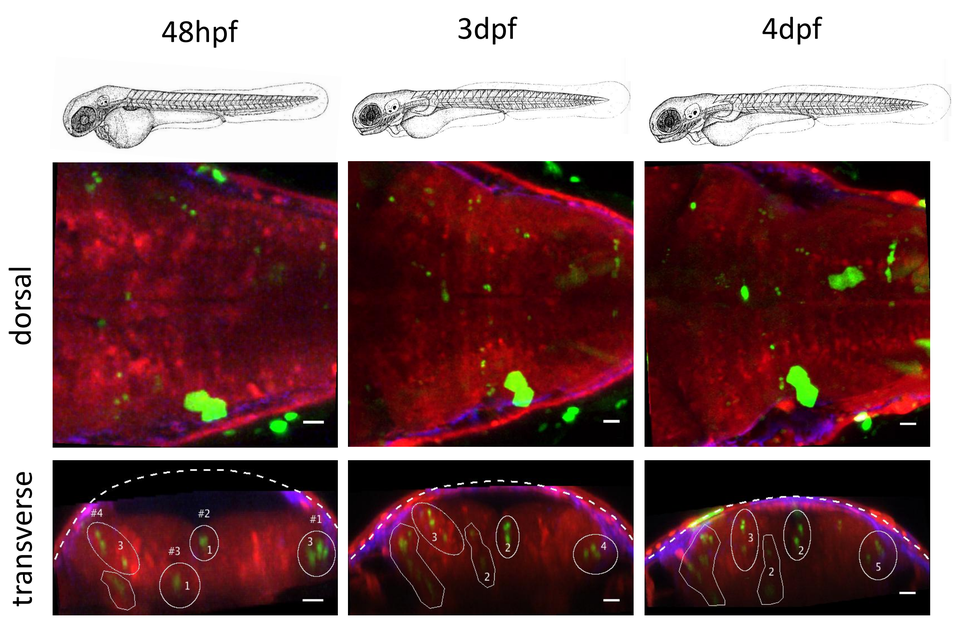
look at the two transgenic fish mating

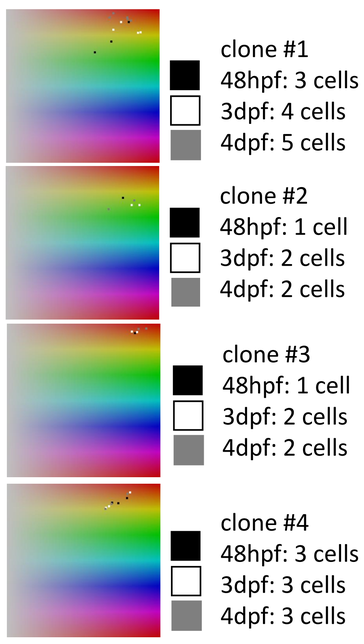


18hpf

We grow the embryos to the desired stage and incubate with tamoxifen.

We image the entire hinbrain each day by confocal microscopy





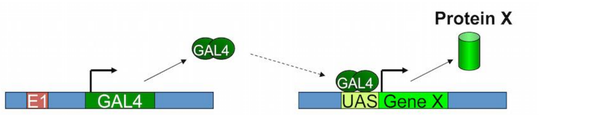
Conclusion: with the (late) incubation (18hpf) of 5µM tamoxifen for 3 hours at room temperature, we are **able to generate a low frequency of recombination**. However, we obtain only a **limited color diversity** (some representative clones shown). Moreover **expression was generally very weak** in comparison to previous experiments (complicated to analyze…).

Project 2. Generation of a transgenic fish line for conditional gene expression

Introduction and **Goals**

The GAL4-UAS system is a versatile tool that permits conditional gene expression to study cell behavior. Though originally from yeast, this system works as well in other organisms, and we use it and improve it for our purposes. In this project we wanted to generate a stable transgenic line, the socalled Ubi-ERT2-GAL4 ACR line (Gerety et al., 2013).

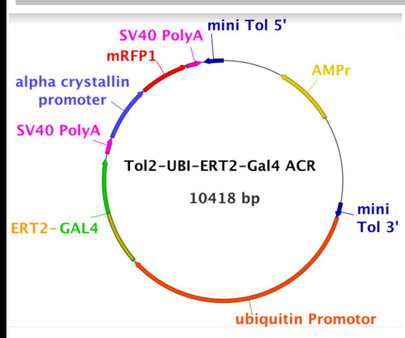
The GAL4 transactivator binds specifically UAS



biophilic.blogspot.com.es

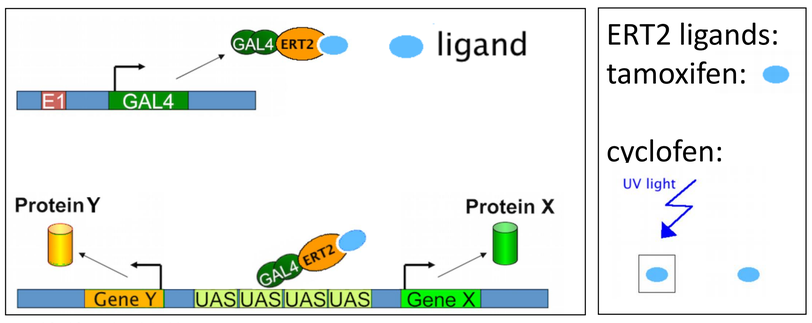
GAL4 is expressed from a specific regulatory element. This can be tissue specific promoter or an ubiquitous promoter for example.

We want to integrate the Ubi:ERT2-GAL4 construct in the zebrafish genome



The vector contains two modules: i) the ubiquitin promoter driving expression of the ERT2-GAL4 fusion protein and ii) the alpha crystallin promoter driving expression of mRFP.
Tol2 sites flank these sequences and will enable plasmid integration via the Tol2 transposase.

The ERT2-GAL4 fusion protein translocates to the nucleus only if an ERT2 ligand is present



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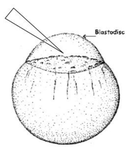
ERT2 ligands:
tamoxifen:
cyclofen:
UV light

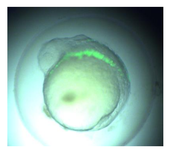
Transcriptional activation can be controlled temporally by incubating with the ligand (4-hydroxy-tamoxifen). We can reach also spatial control by using a caged version of tamoxifen: cyclofen-OH. Further, we can activate expression of two genes from UAS.

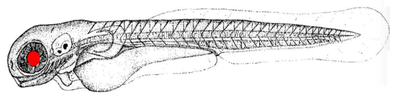
Genomic integration is achieved by microinjection



We inject the vector together with Tol2 mRNA and GFP mRNA.

www.uoneuro.uoregon.edul





Kimmel et al., 1995

GFP expression confirms successful injection. Later in development red eyes confirm successful integration.