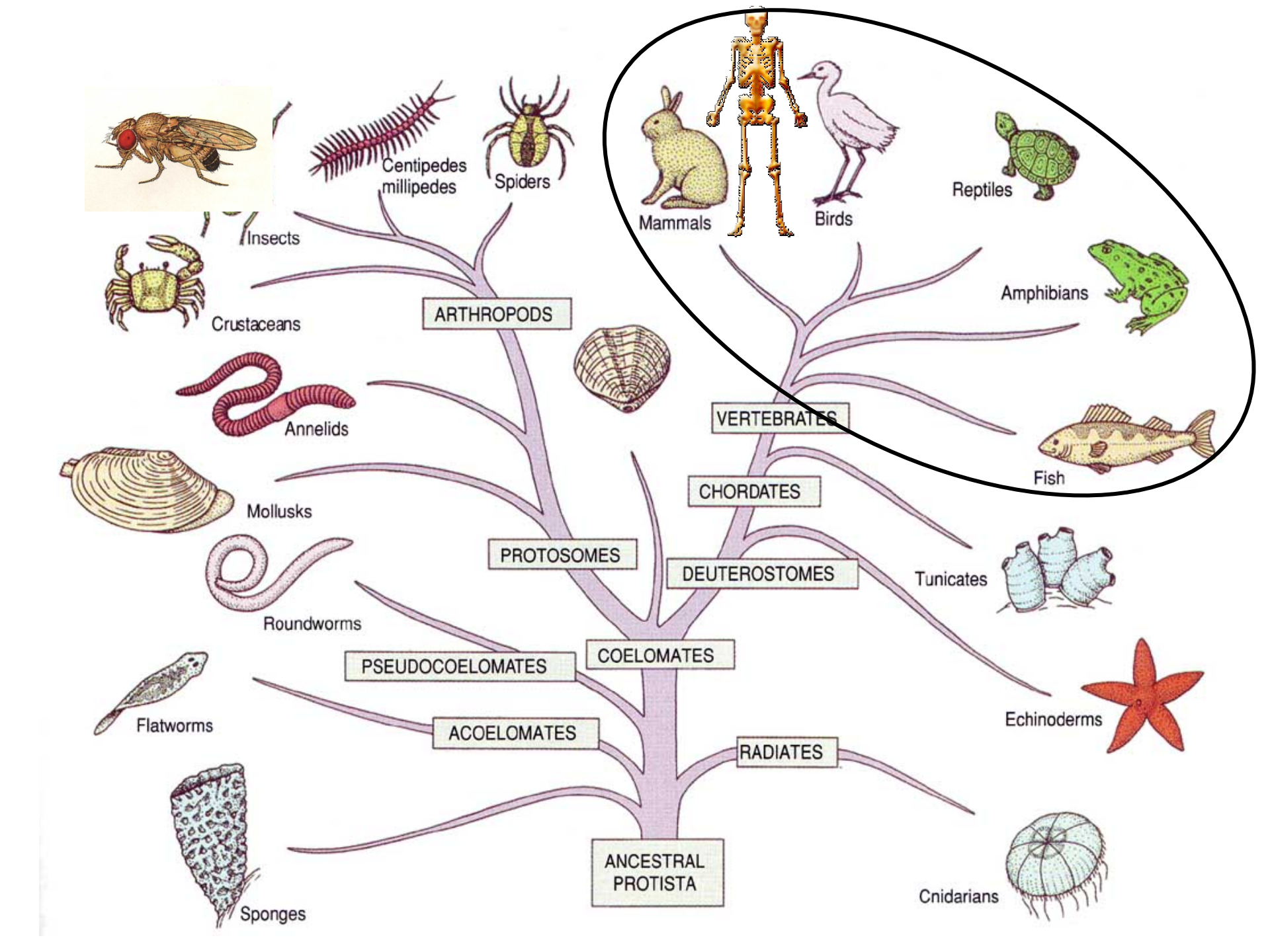
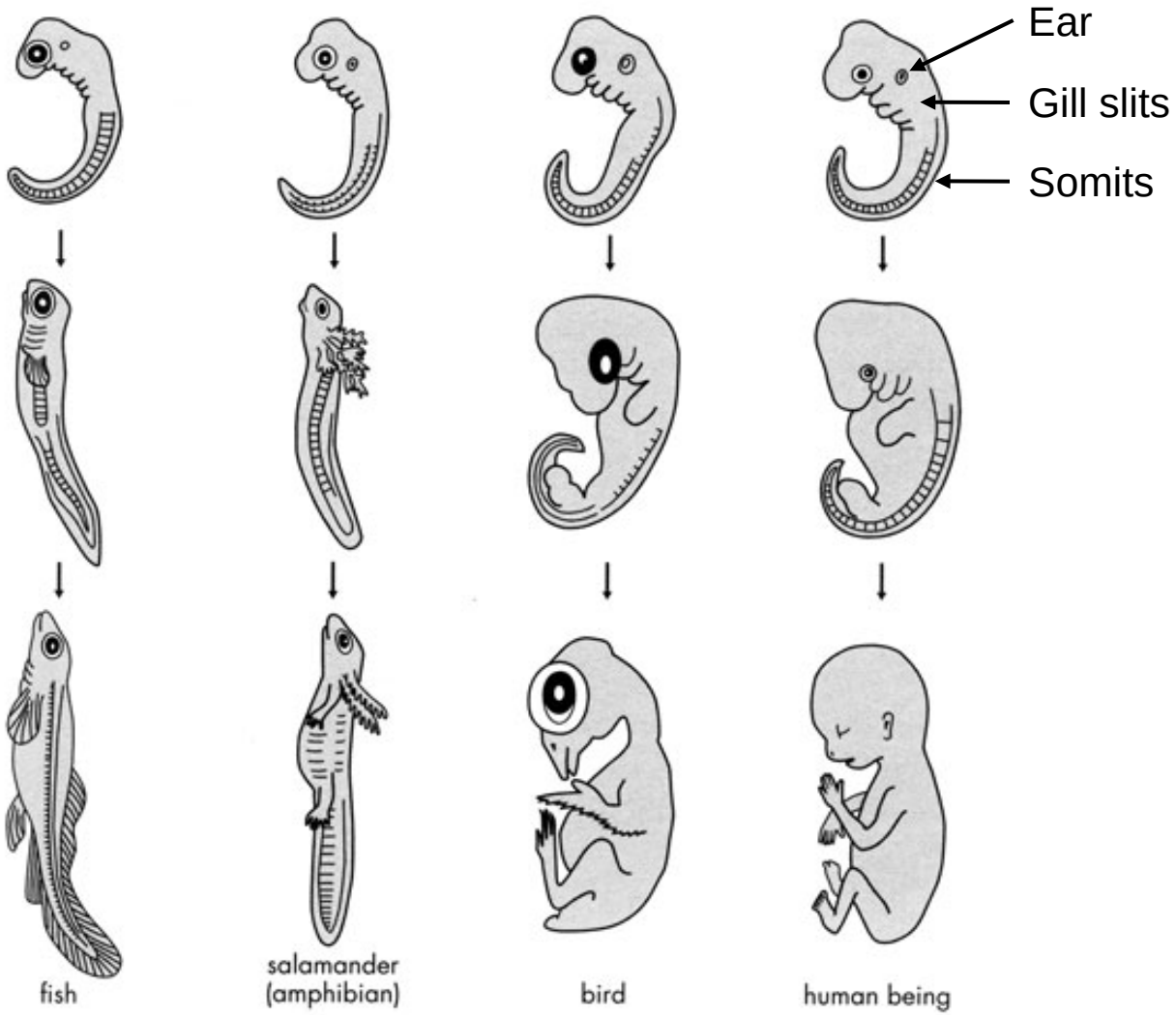
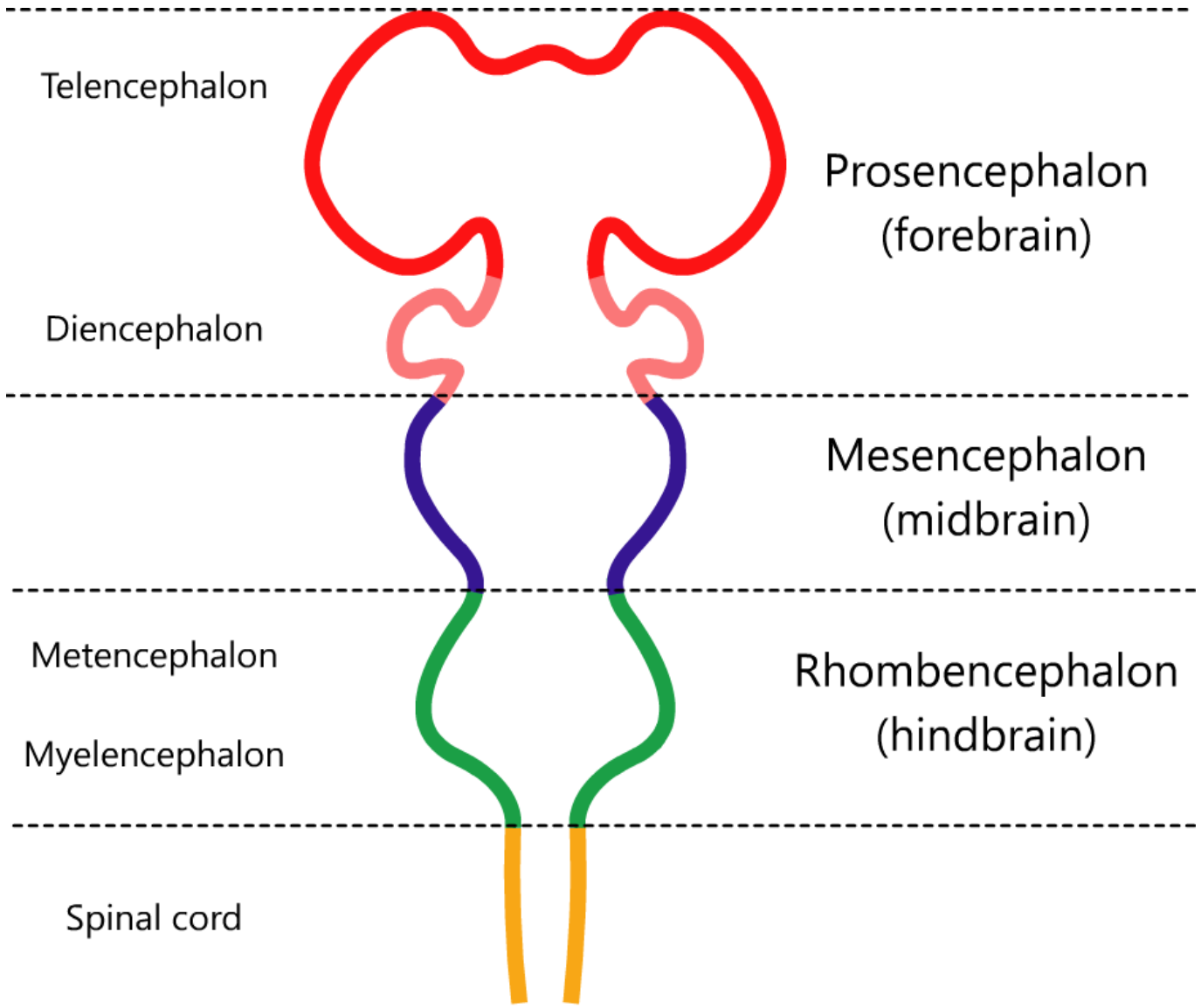
Zebrafish, a model of choice for biomedical research

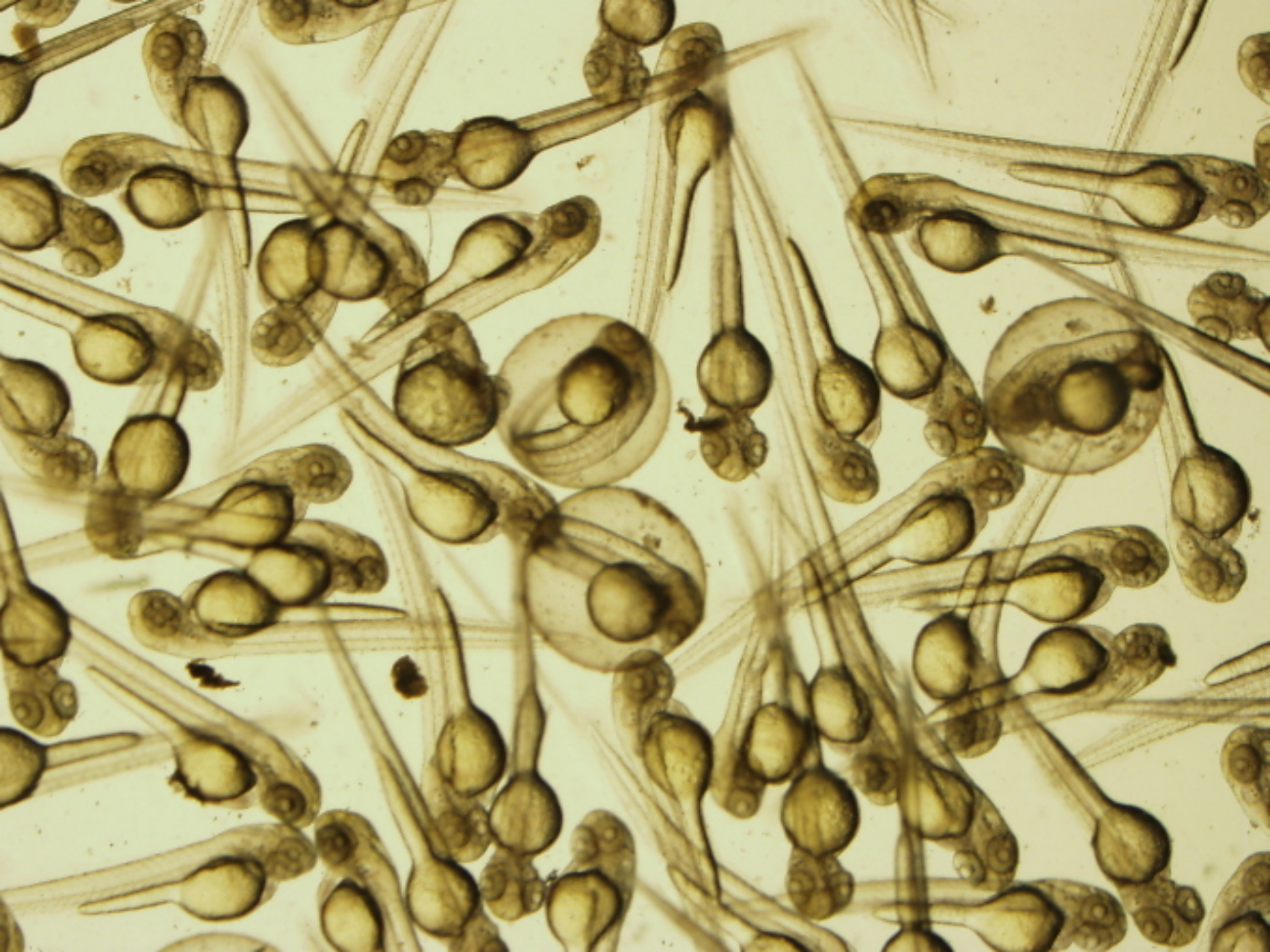
Yoav Gothilf
Dept. Neurobiology, Tel Aviv University
yoavgothilf@gmail.com

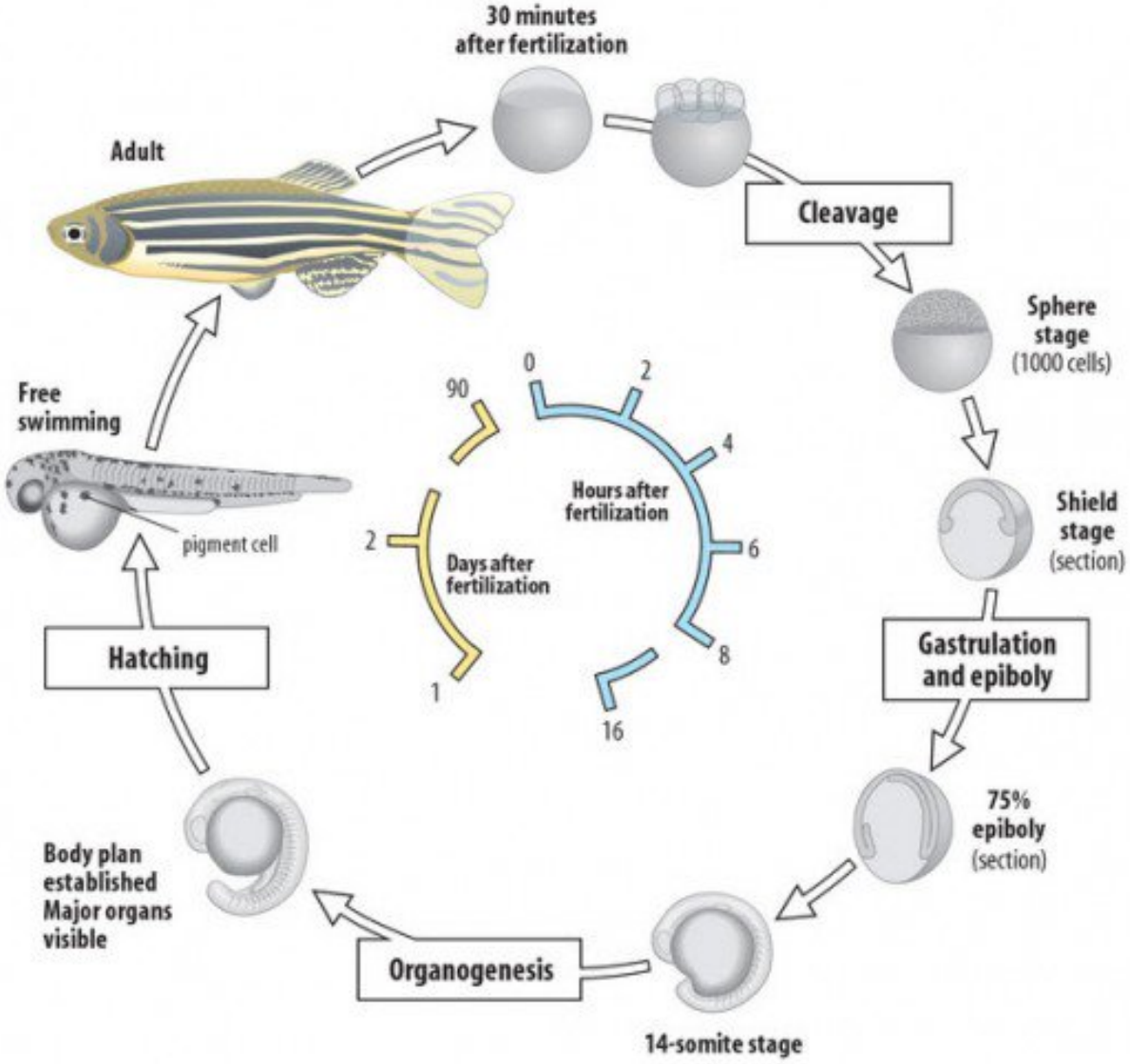

Insects
Centipedes
millipedes
Spiders
Crustaceans
ARTHROPODS
Annelids
Mollusks
Roundworms
PROTOSOMES
Flatworms
PSEUDOCOELOMATES
ACOELOMATES
Sponges
ANCESTRAL
PROTISTA
Cnidarians
RADIATES
COELOMATES
Echinoderms
DEUTEROSTOMES
Tunicates
CHORDATES
Fish
VERTEBRATES
Amphibians
Reptiles
Birds
Mammals

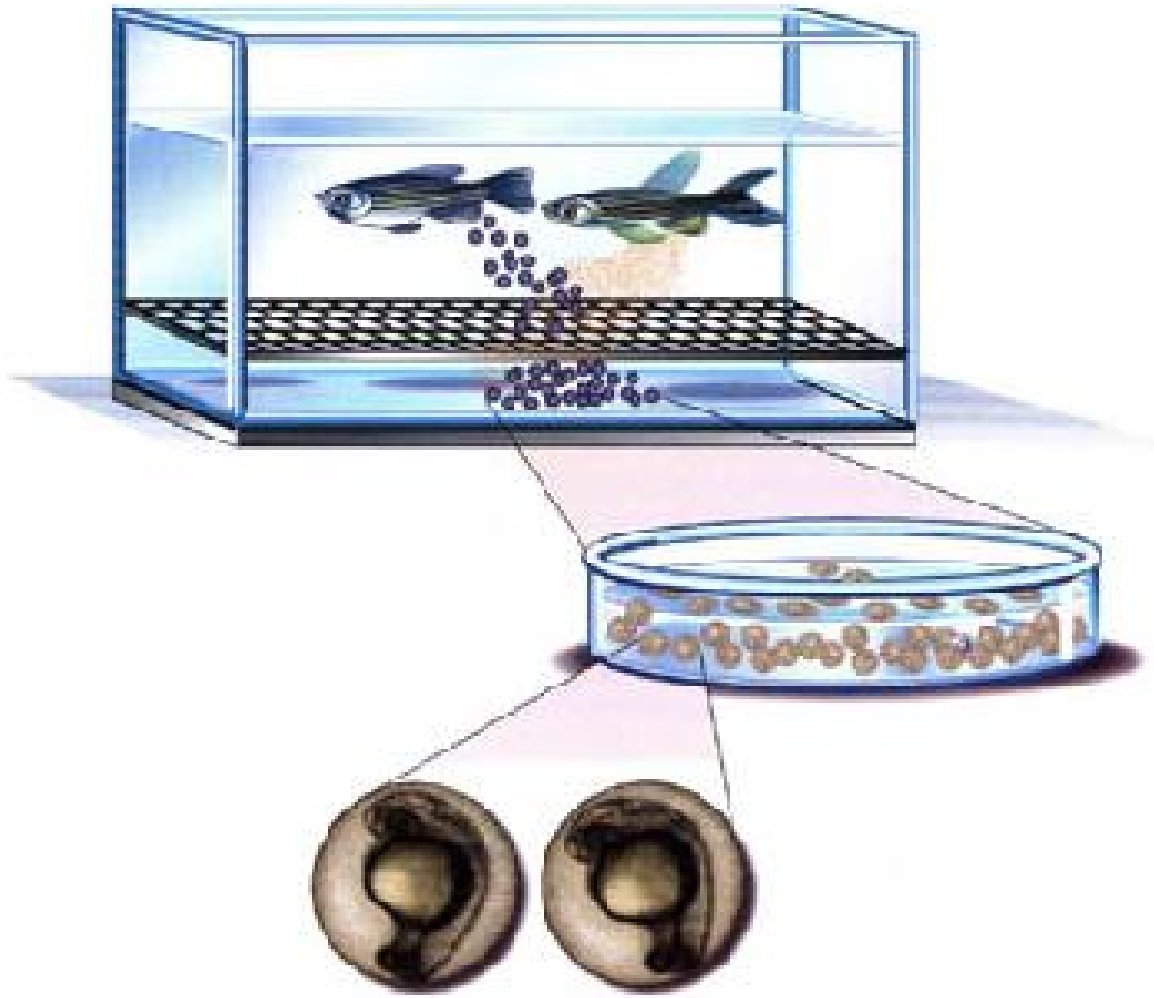


The embryos of animals often reflect their evolutionary past. This famous picture, drawn by the German biologist, Ernst Haeckel (1834–1919), shows the similarity of all early vertebrate embryos (top row). The resemblance is explained by descent from a common ancestor.


Telencephalon
Diencephalon
Prosencephalon
(forebrain)
Mesencephalon
(midbrain)
Metencephalon
Myelencephalon
Rhombencephalon
(hindbrain)
Spinal cord



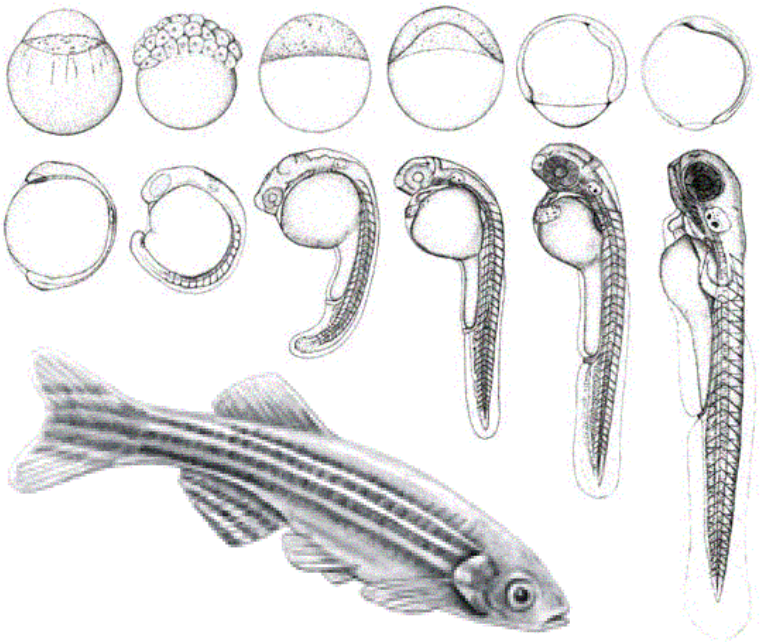

30 minutes
after fertilization
Cleavage
Sphere
stage
(1000 cells)
Shield
stage
(section)
Gastrulation
and epiboly
75%
epiboly
(section)
14-somite stage
Organogenesis
Body plan
established
Major organs
visible
Hatching
Free
swimming
pigment cell
Adult
0
2
4
6
8
16
90
2
1
Hours after
fertilization
Days after
fertilization



https://youtu.be/4c-Kw4timVA

Zebrafish

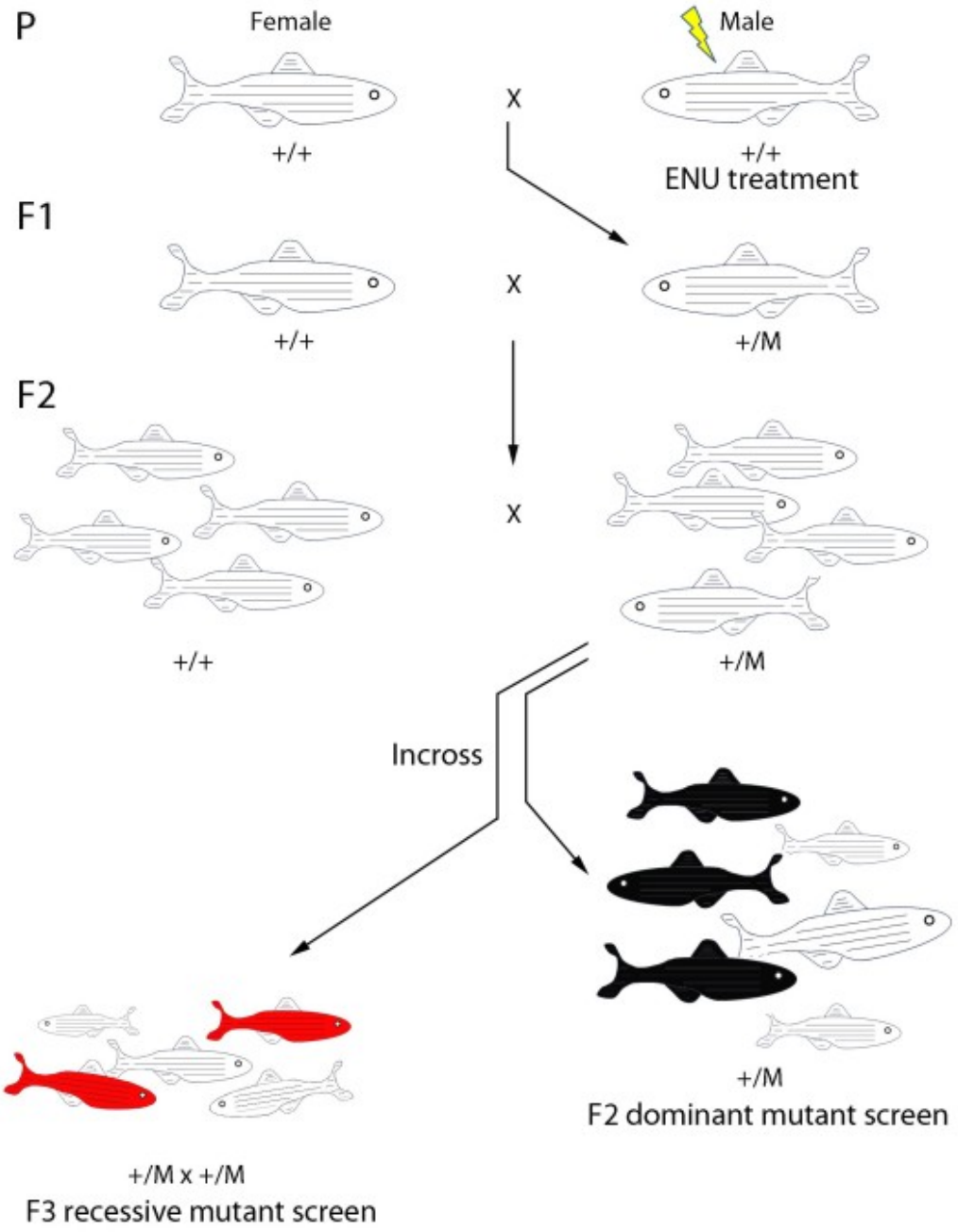
- Vertebrate
- External fertilization
- Many embryos
- Transparent embryos
- Fast development
- Short generation time
- Easy to maintain

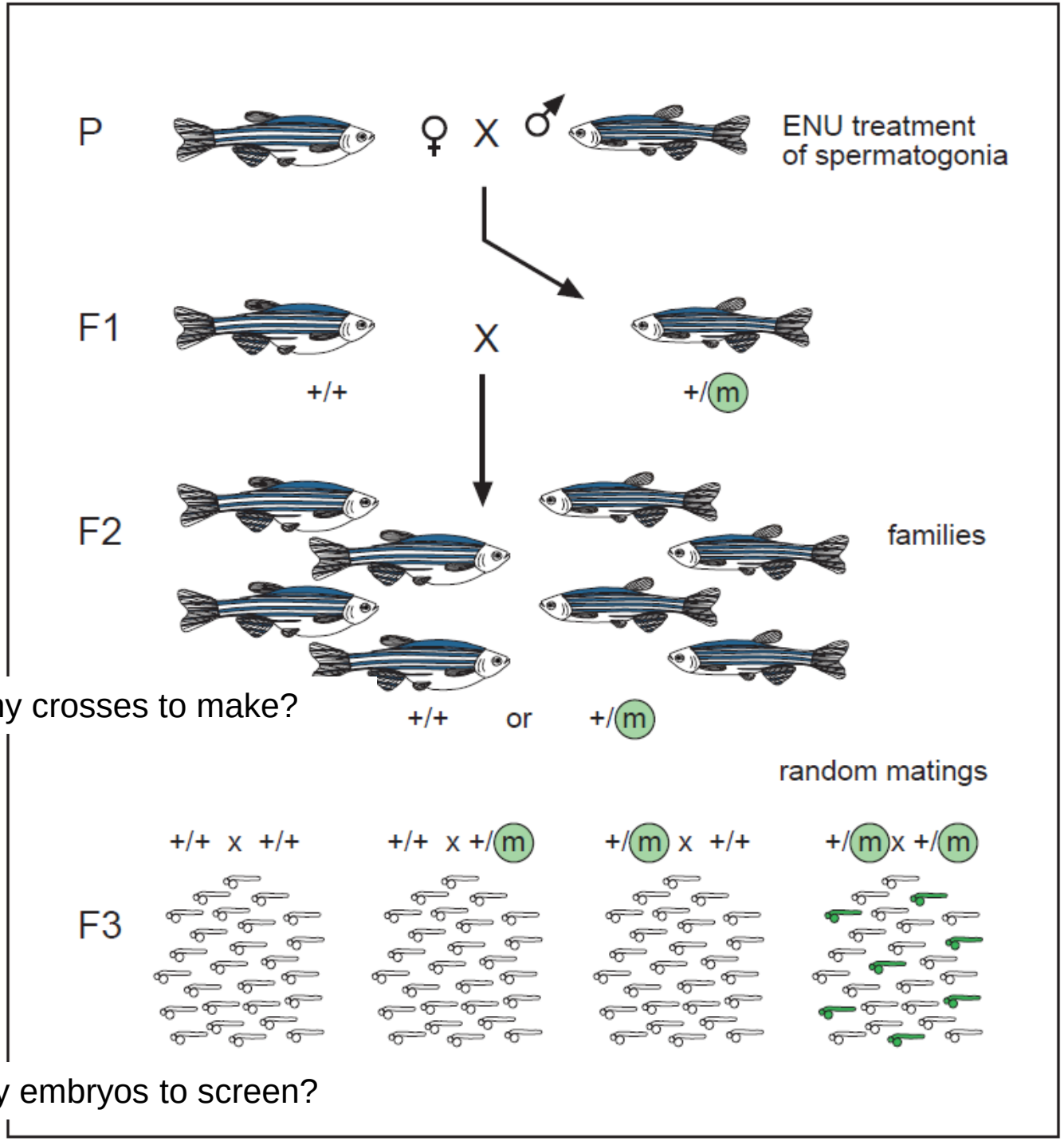


How do multicellular organisms develop from a single cells to morphologically complex forms?

Christiane Nüsslein-Volhard

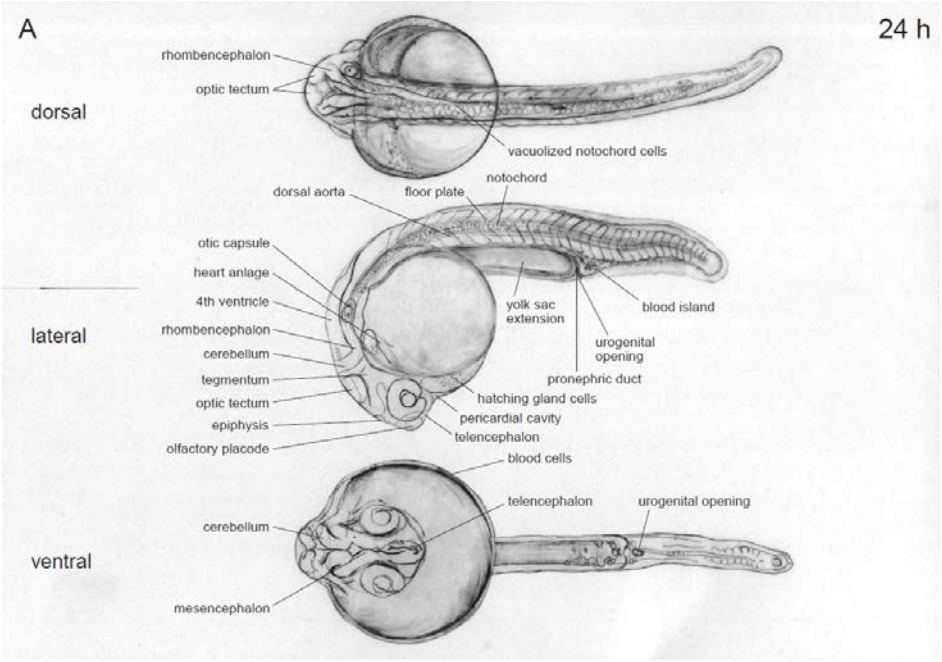
1995 Nobel Prize in Physiology or Medicine for her research on the genetic control of embryonic development in the fruit fly

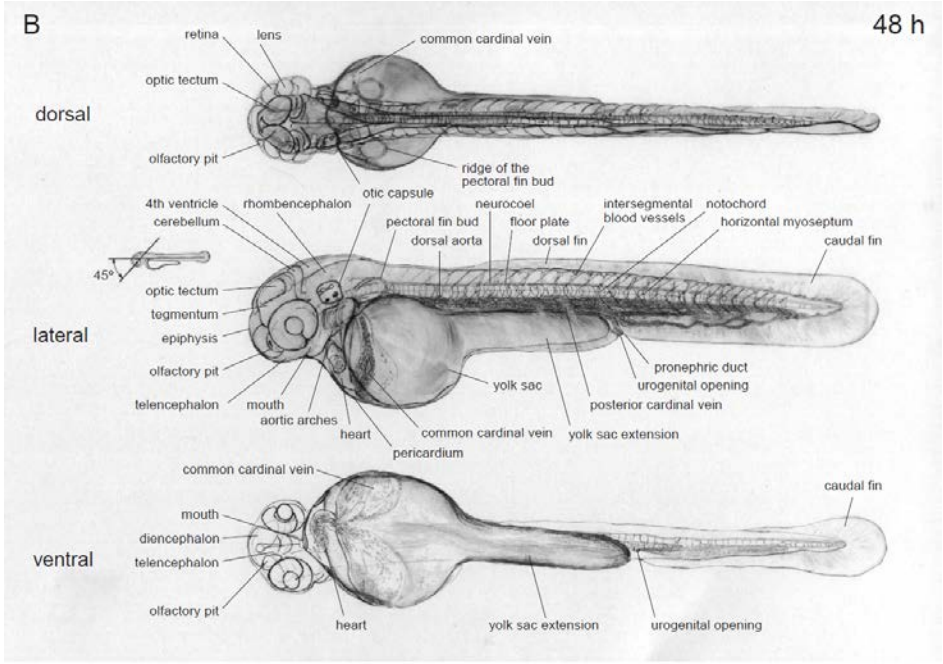

P
Female
Male
X
+/+
+/+
ENU treatment
F1
X
+/+
+/M
F2
X
+/+
+/M
Incross
+/M
F2 dominant mutant screen
+/M x +/M
F3 recessive mutant screen

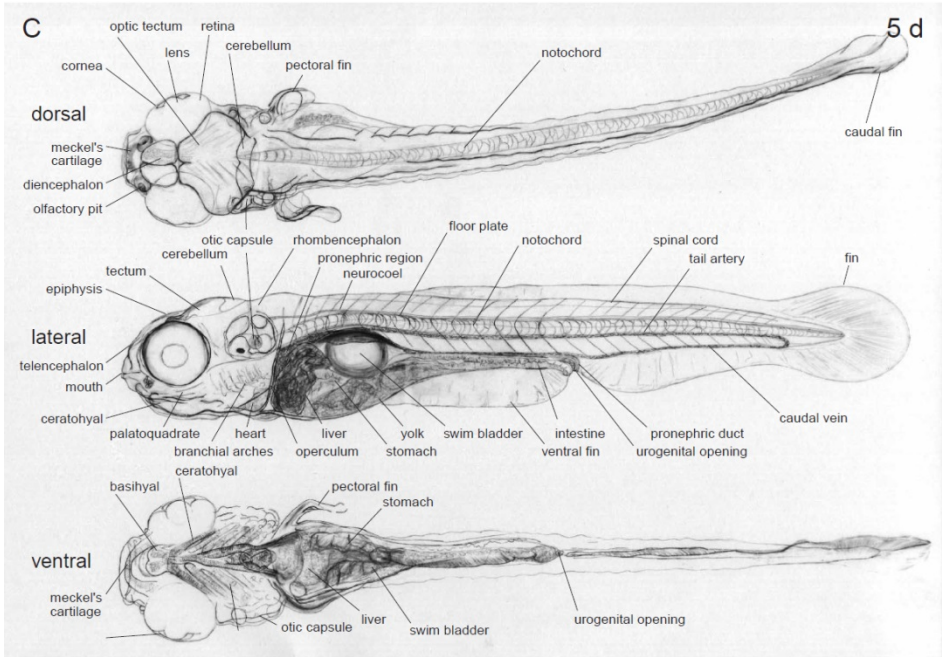


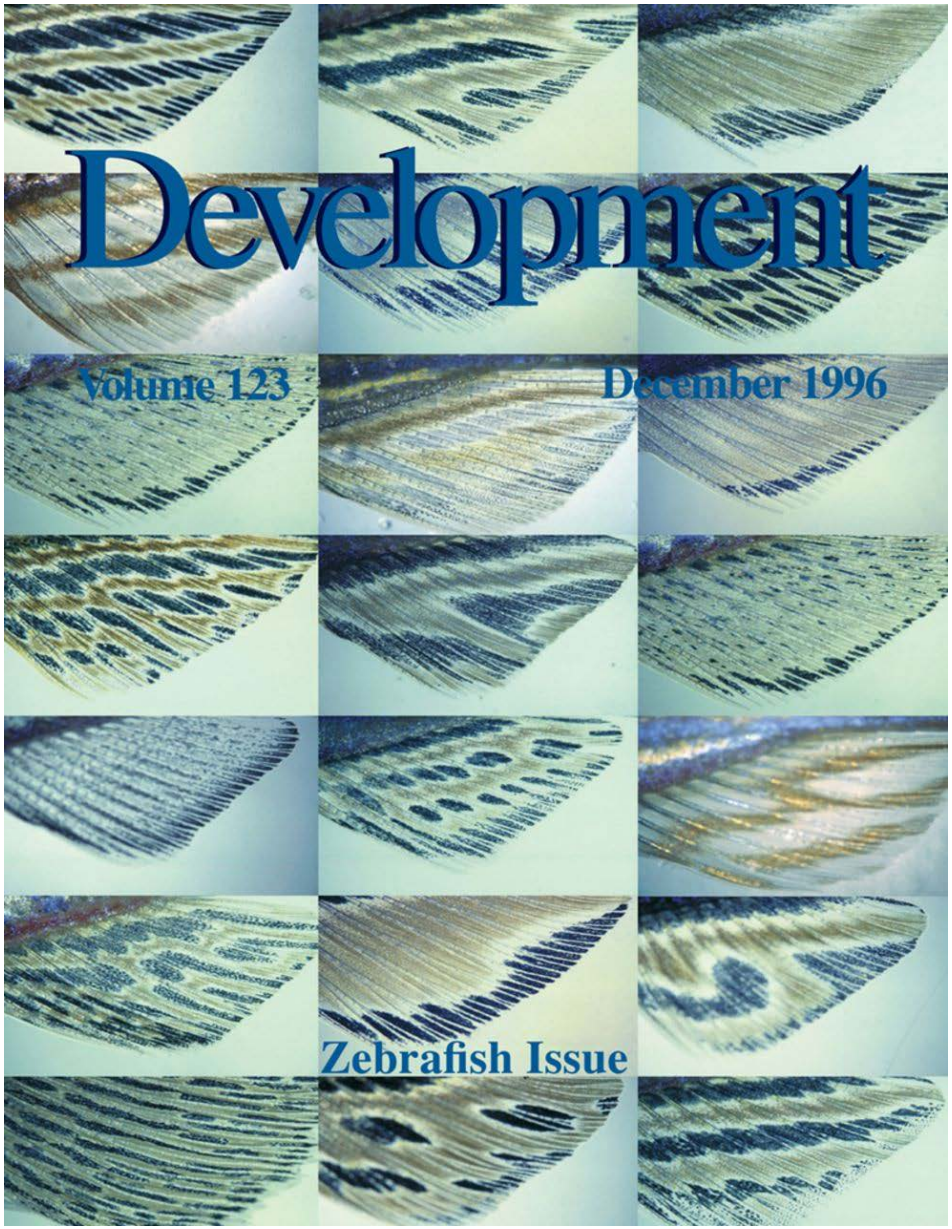
How many crosses to make?

How many embryos to screen?


A
24 h
dorsal
rhombencephalon
optic tectum
vacuolized notochord cells
lateral
dorsal aorta
floor plate
notochord
otic capsule
heart anlage
4th ventricle
rhombencephalon
cerebellum
tegmentum
optic tectum
epiphysis
olfactory placode
yolk sac extension
blood island
urogenital opening
pronephric duct
hatching gland cells
pericardial cavity
telencephalon
ventral
blood cells
telencephalon
urogenital opening
cerebellum
mesencephalon


B
48 h
dorsal
retina
lens
common cardinal vein
optic tectum
olfactory pit
ridge of the pectoral fin bud
otic capsule
4th ventricle
cerebellum
rhombencephalon
neurocoel
intersegmental blood vessels
notochord
pectoral fin bud
floor plate
horizontal myoseptum
dorsal aorta
dorsal fin
caudal fin
45°
lateral
optic tectum
tegmentum
epiphysis
olfactory pit
telencephalon
mouth
aortic arches
heart
pericardium
common cardinal vein
yolk sac
yolk sac extension
pronephric duct
urogenital opening
posterior cardinal vein
ventral
common cardinal vein
caudal fin
mouth
diencephalon
telencephalon
olfactory pit
heart
yolk sac extension
urogenital opening


C
5 d
optic tectum
retina
lens
cerebellum
cornea
pectoral fin
notochord
caudal fin
dorsal
meckel's cartilage
diencephalon
olfactory pit
otic capsule
rhombencephalon
floor plate
notochord
spinal cord
cerebellum
pronephric region
neurocoel
tail artery
fin
tectum
epiphysis
lateral
telencephalon
mouth
ceratohyal
palatoquadrate
heart
liver
yolk
swim bladder
intestine
pronephric duct
caudal vein
branchial arches
operculum
stomach
ventral fin
urogenital opening
ceratohyal
basihyal
pectoral fin
stomach
ventral
meckel's cartilage
otic capsule
liver
swim bladder
urogenital opening



https://dev.biologists.org/content/123/1

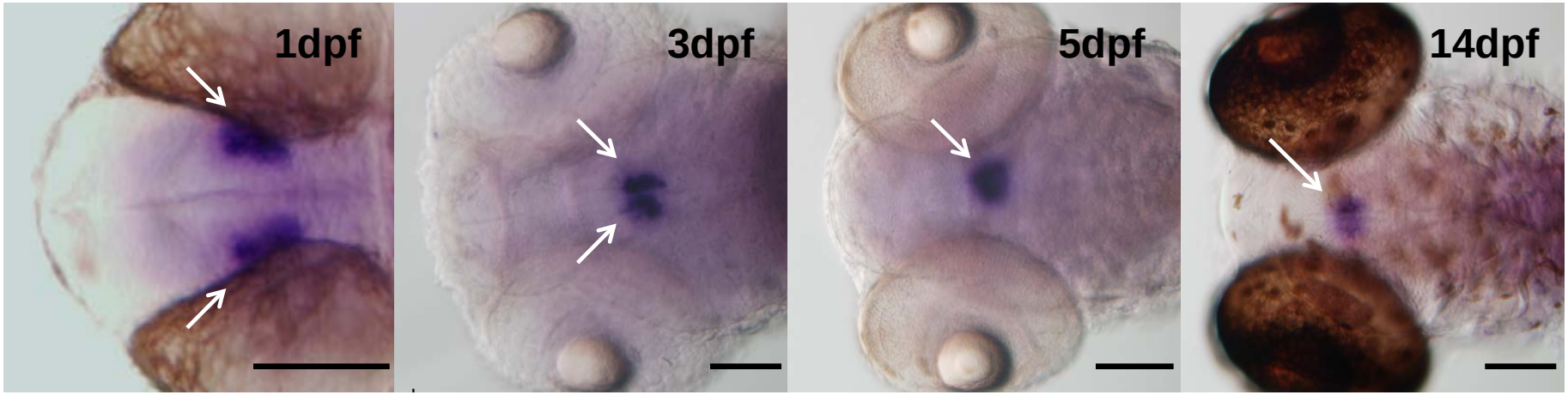
Zebrafish, a model of Choice

- Vertebrate
- Large number of accessible transparent embryos
- Fast development and short generation time
- Developmental mutants
- Gene expression analysis
- Transgenesis
- Gene knockdown, knockout and knockin
- Behavioral tests
- Imaging capabilities

Spatial and temporal gene expression patterns

Gene expression analysis using whole mount *in situ* hybridization

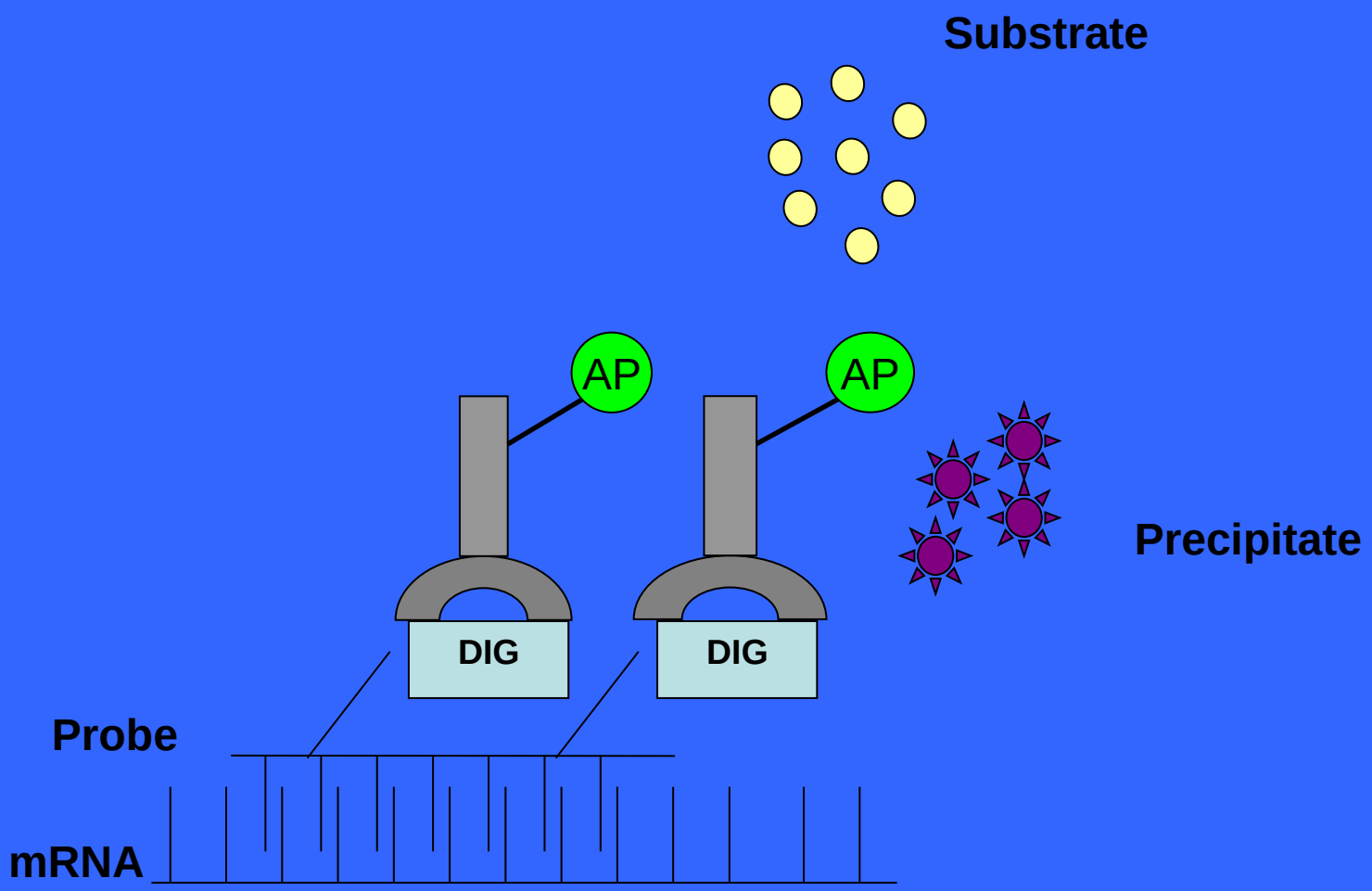
GnRH2 mRNA localization during zebrafish development



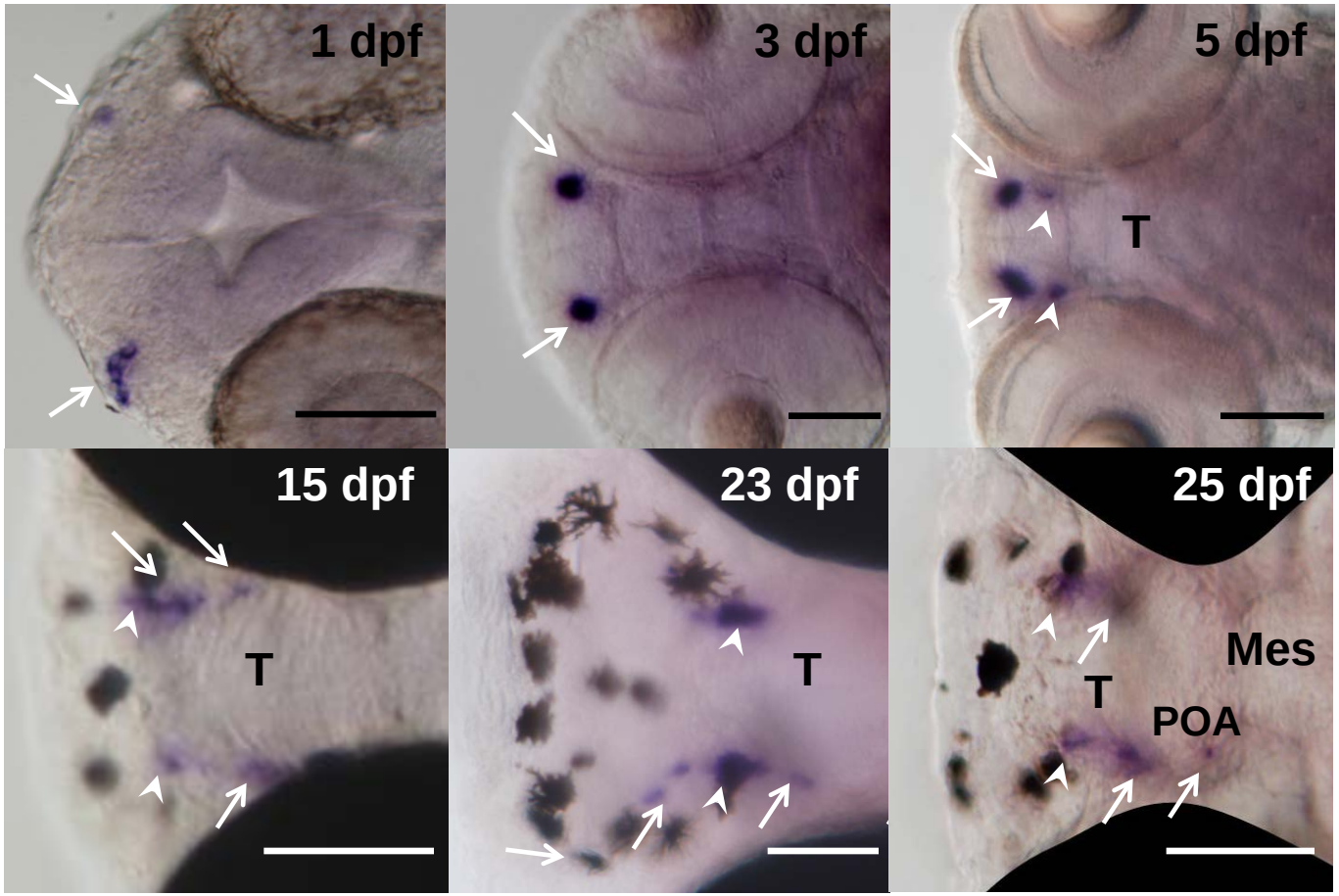
https://www.jove.com/video/50644/high-resolution-whole-mount-situ-hybridization-within-zebrafish

Ori Palevitch 2005

Whole mount *in situ* hybridization

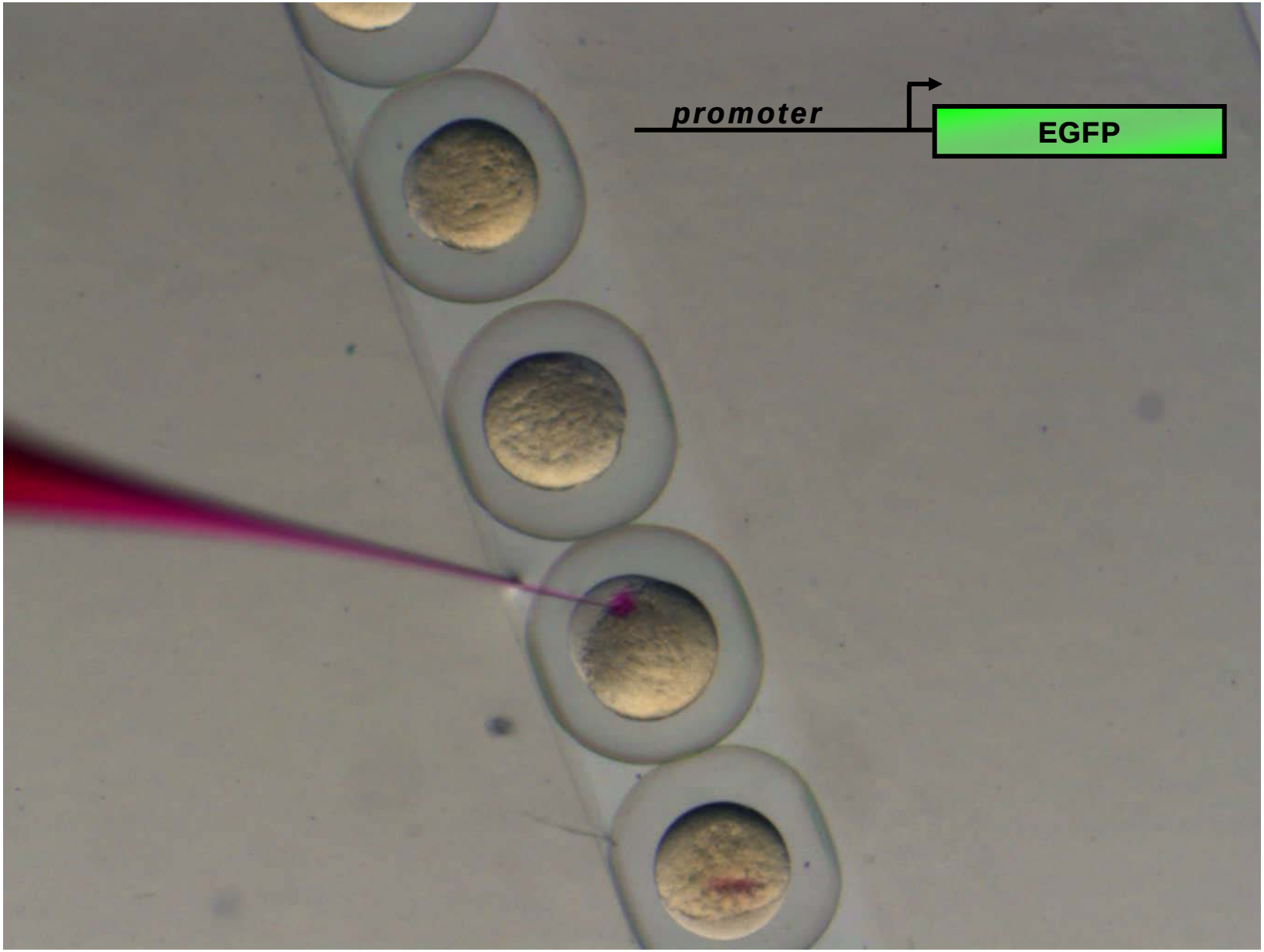


GnRH3 mRNA localization during zebrafish development

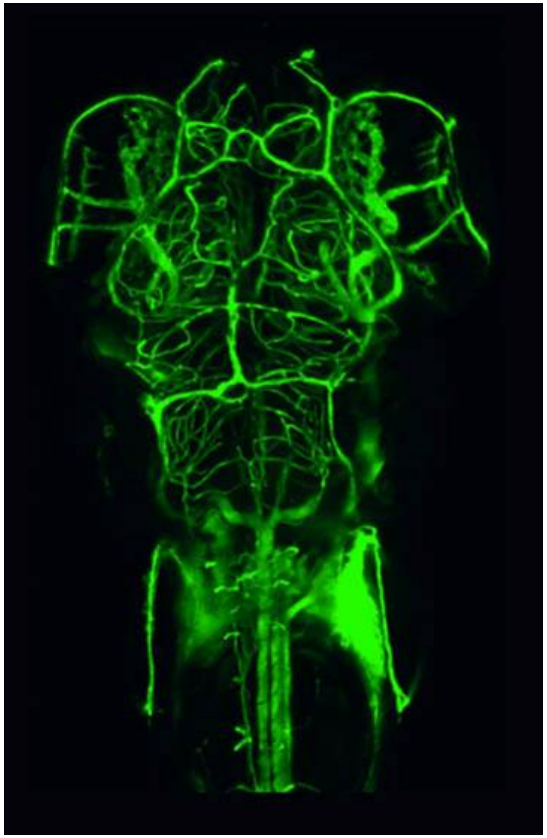


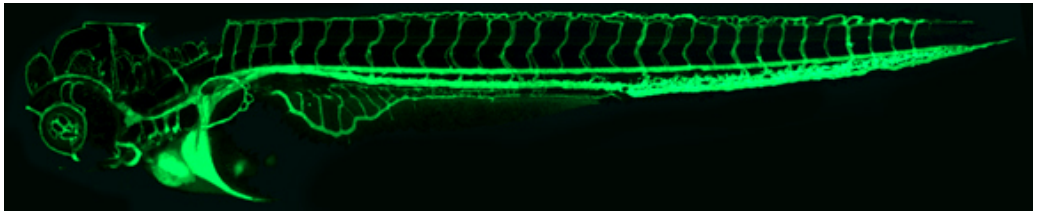
Ori Palevitch 2005

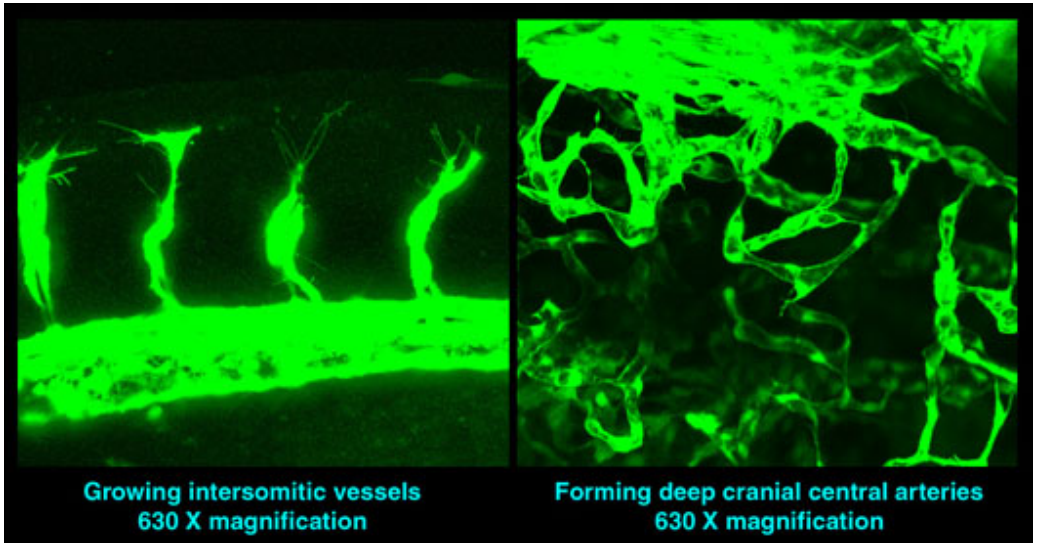
Transgenesis:
Method development and utilization for visualizing and manipulating neuros


promoter
EGFP

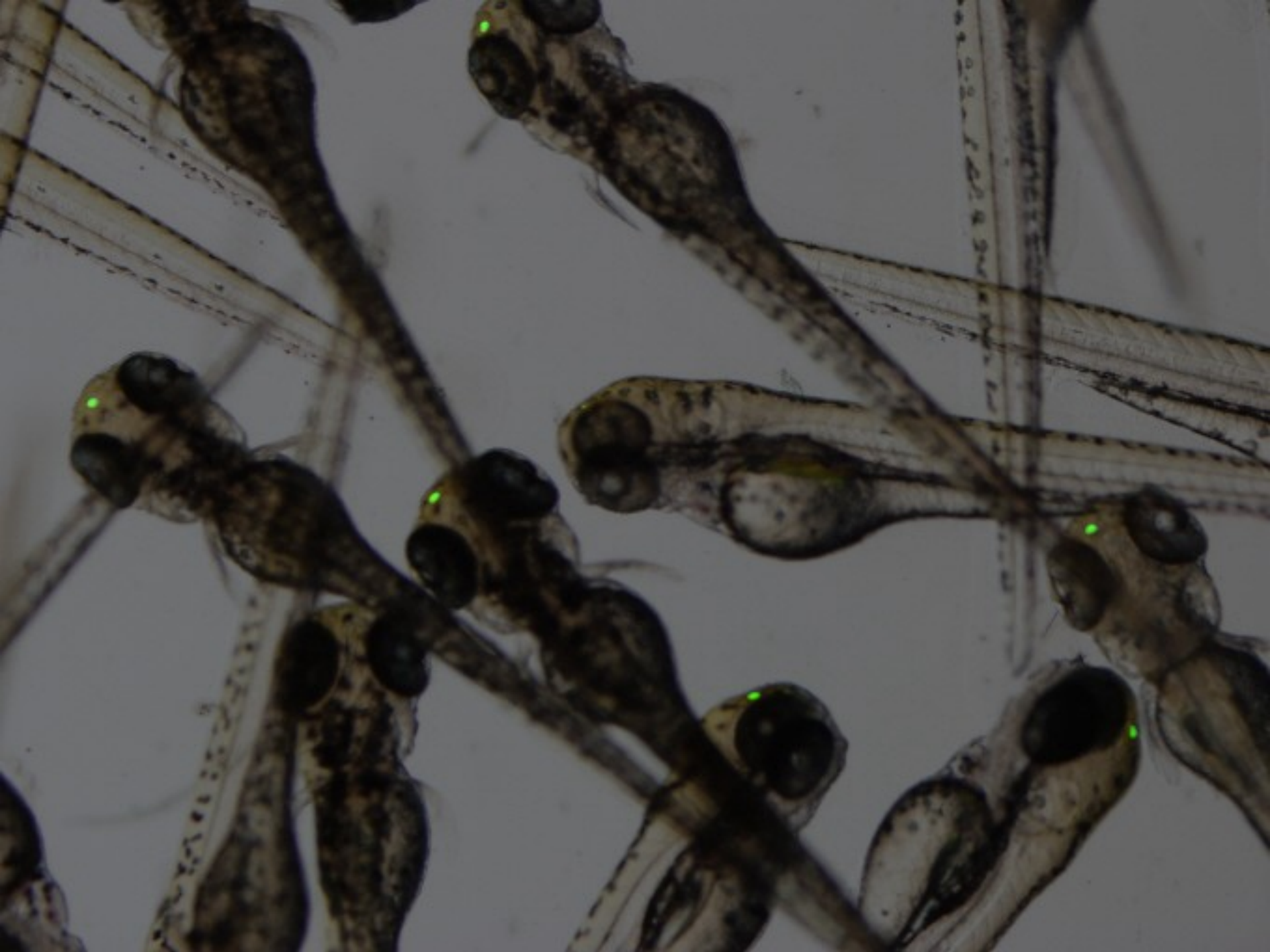
Transgenic fish in which GFP is driven by vascular-specific promoters

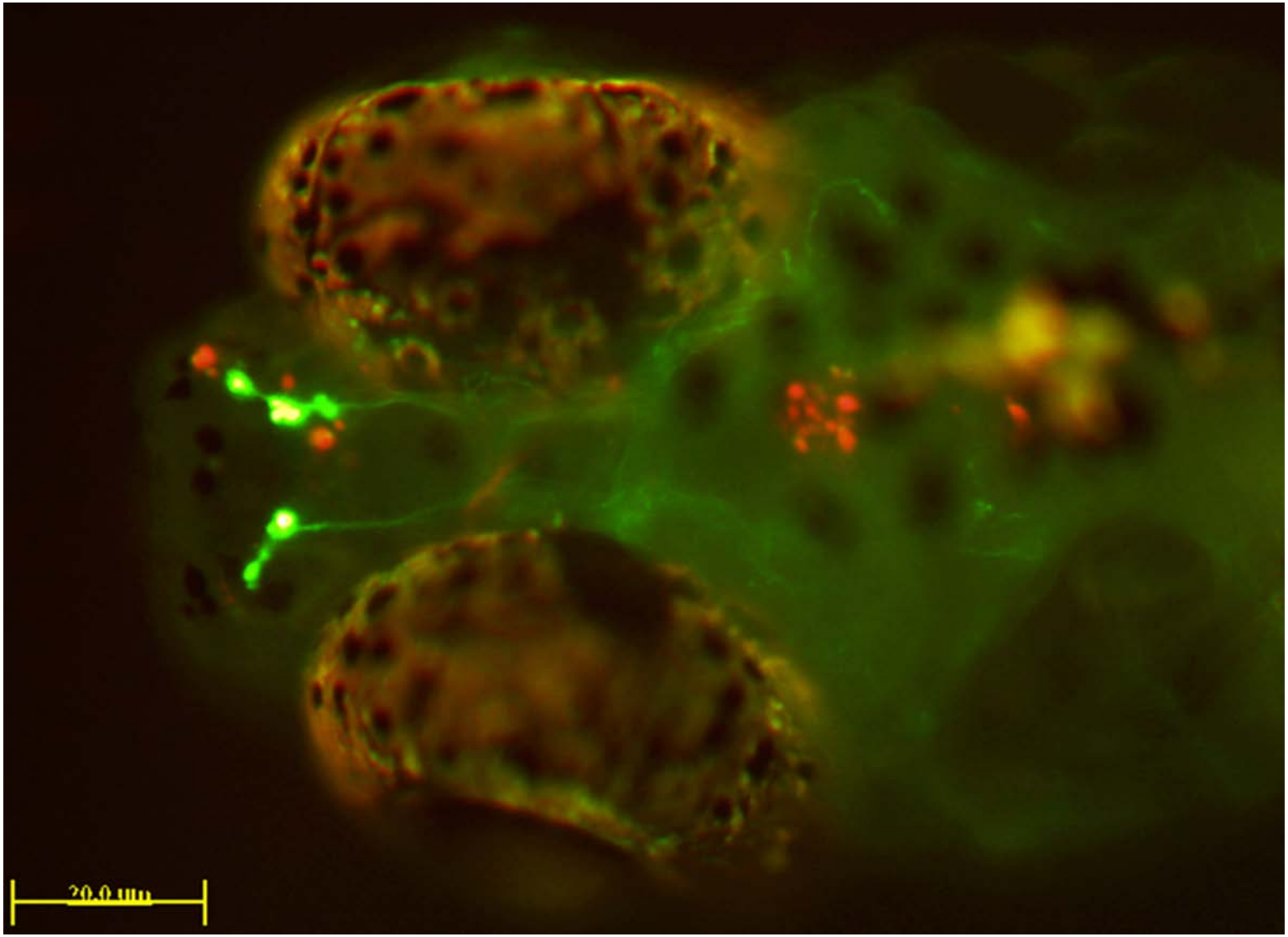


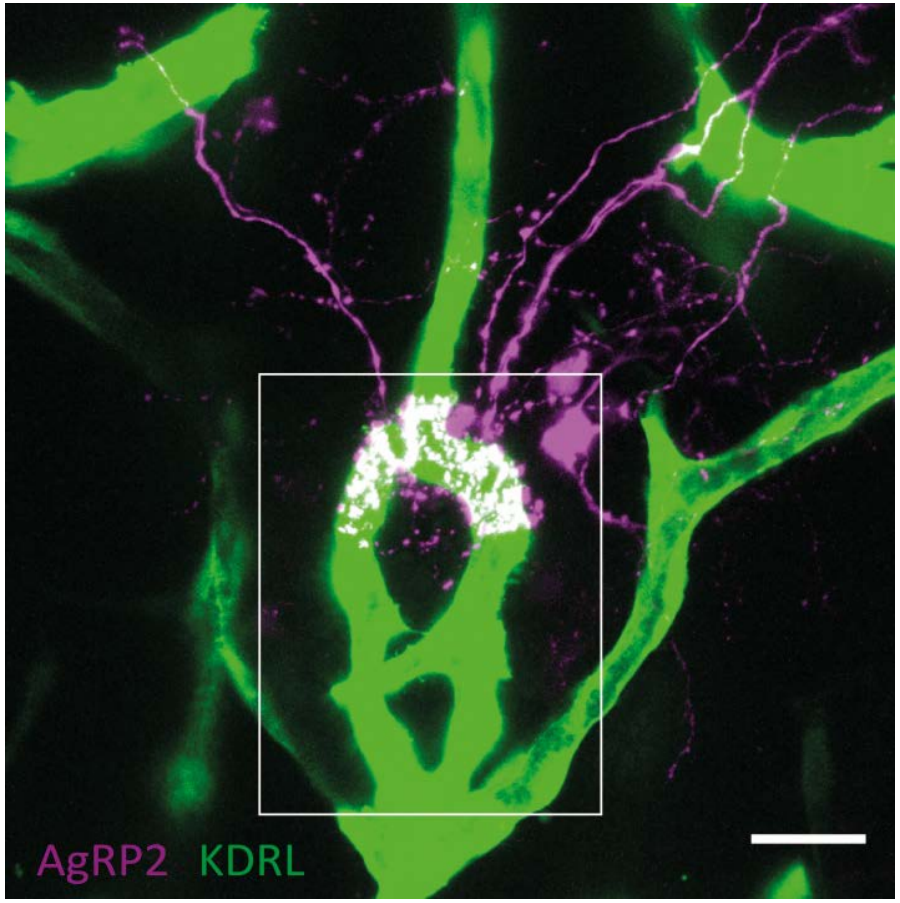




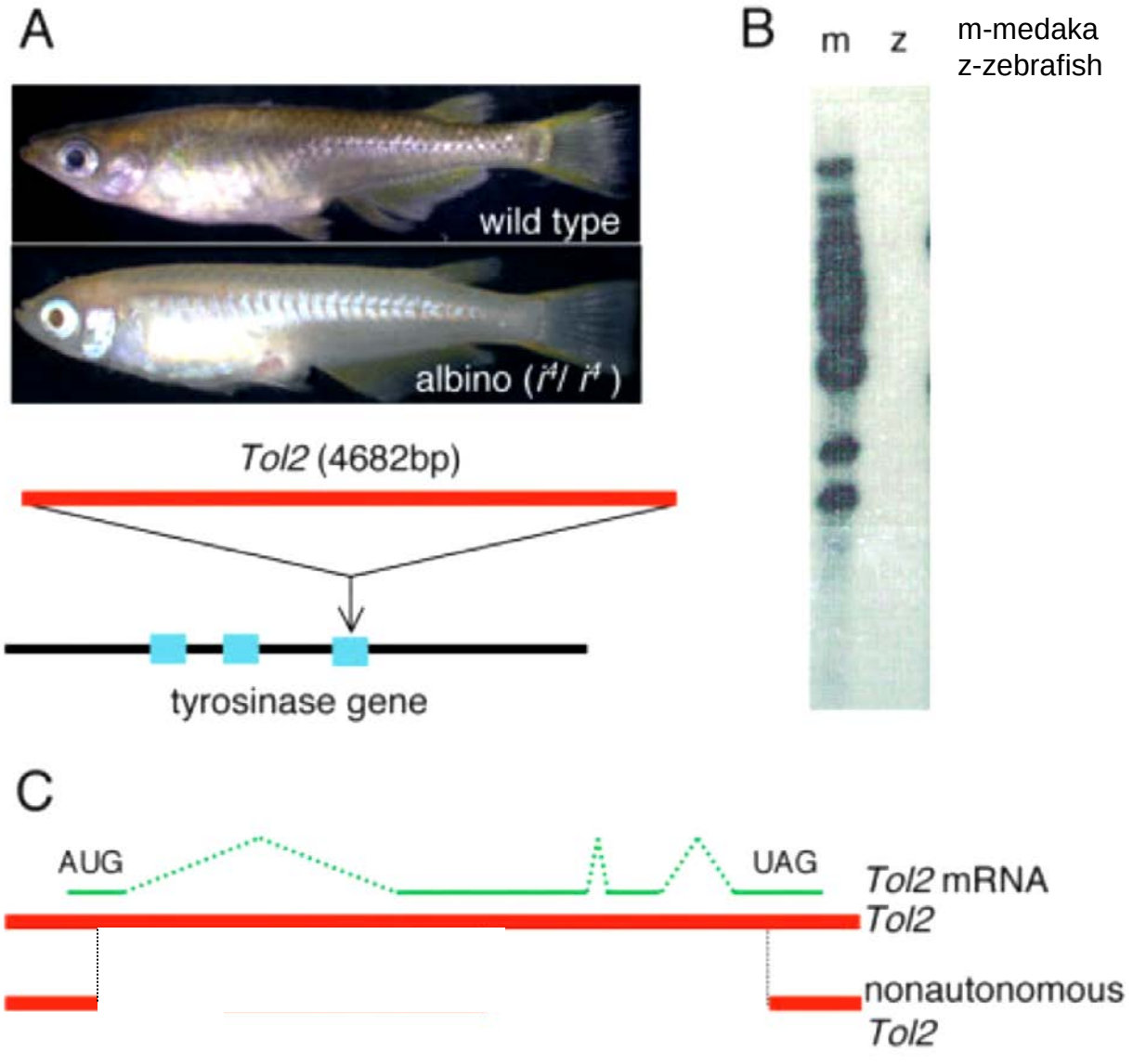
https://youtu.be/yk7TWOtrphM



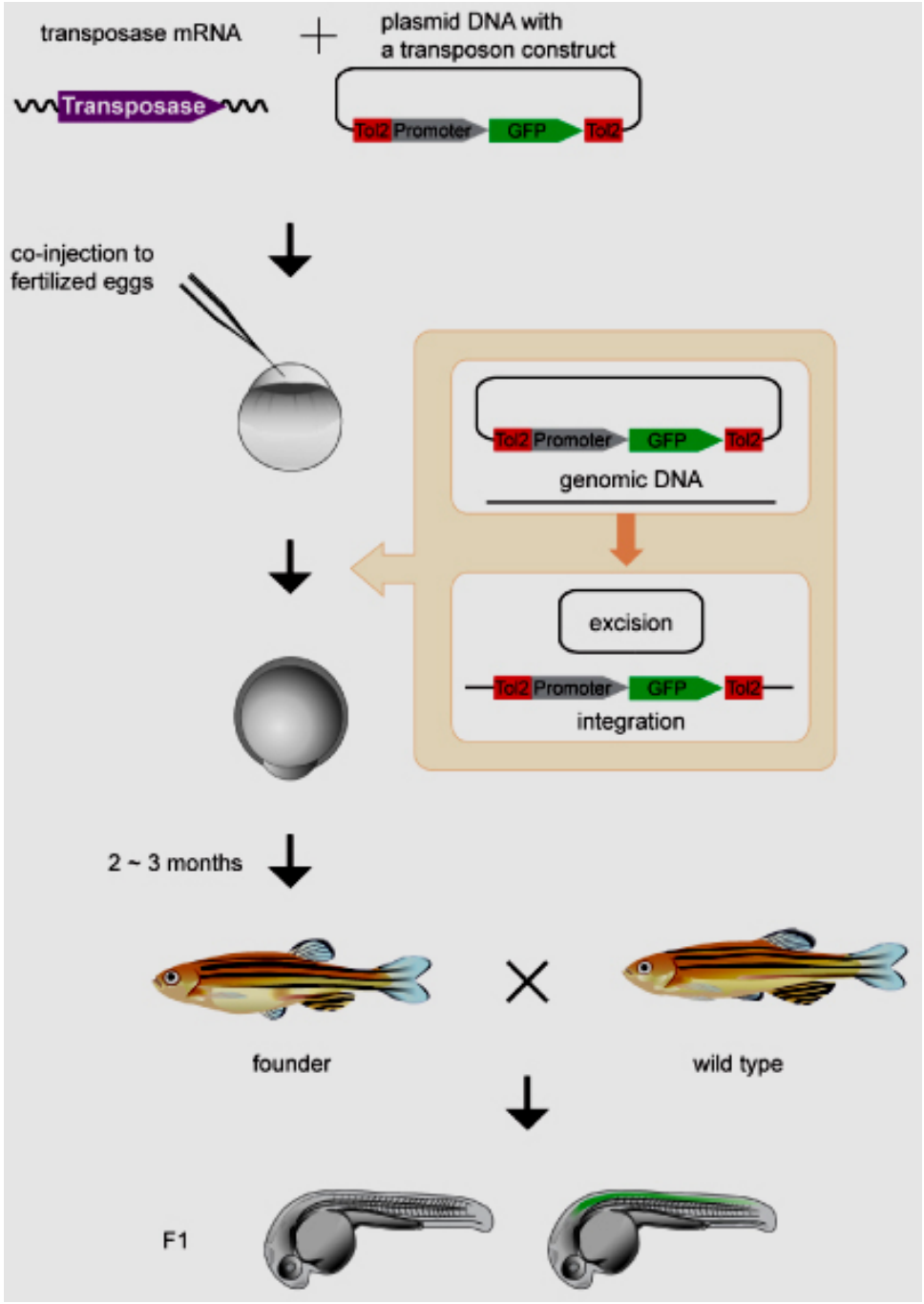

20.0 um


AgRP2
KDRL

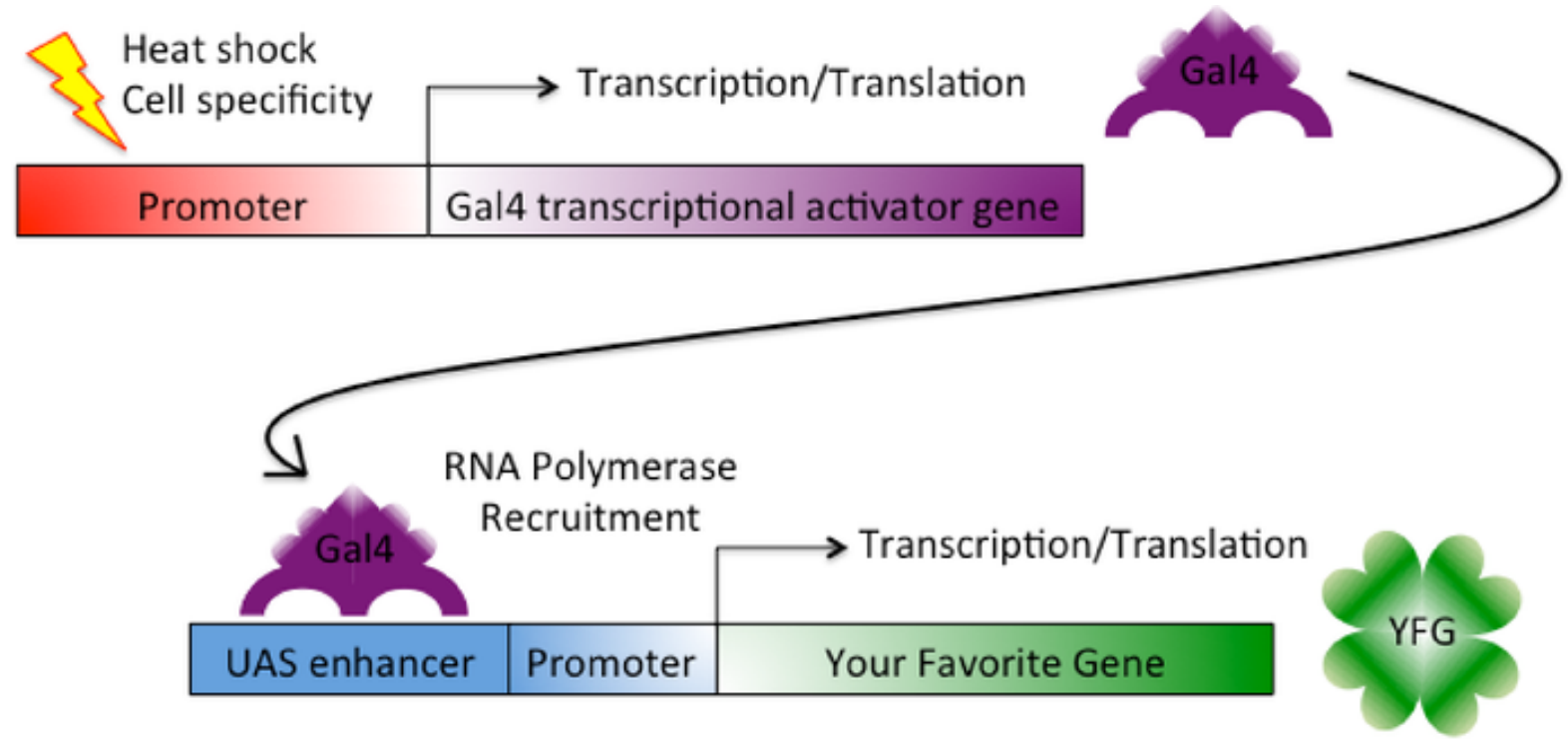
Discovering the Tol2 transposase system



Kawakami 2005

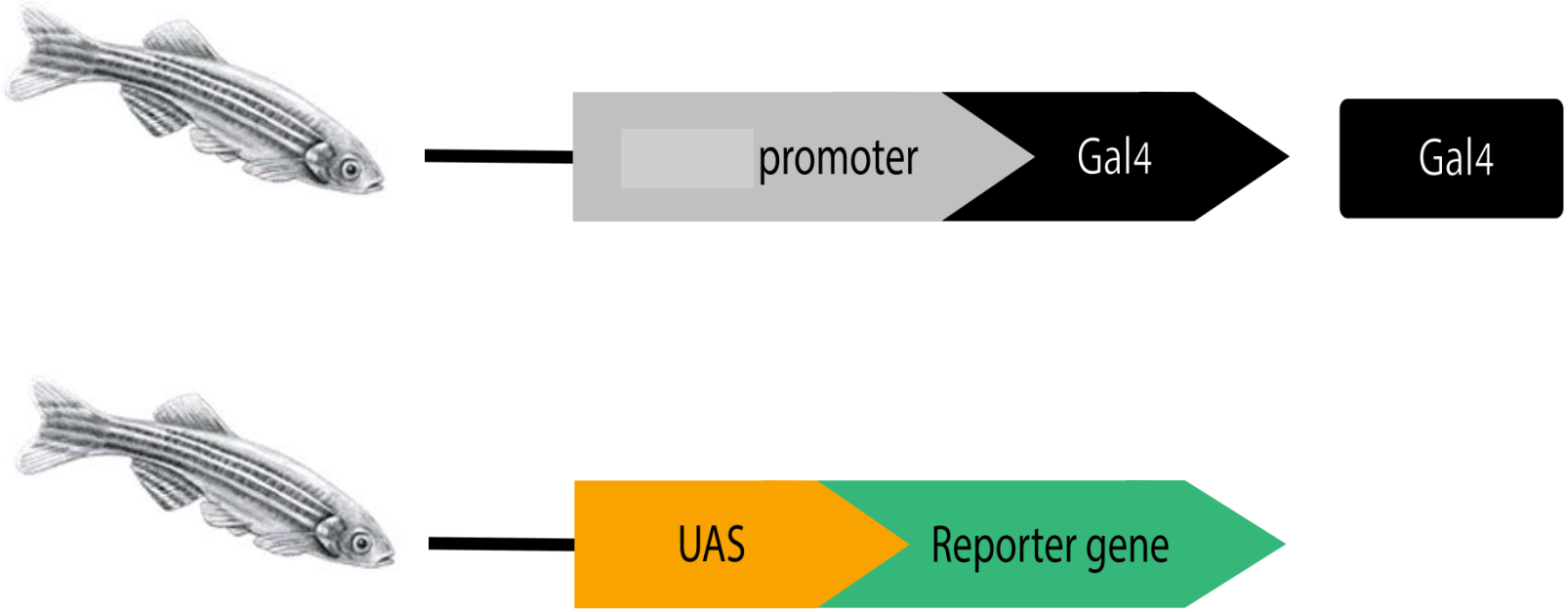

transposase mRNA
plasmid DNA with
a transposon construct
Transposase
Tol2 Promoter GFP Tol2
co-injection to
fertilized eggs
Tol2 Promoter GFP Tol2
genomic DNA
excision
Tol2 Promoter GFP Tol2
integration
2 ~ 3 months
founder
wild type
F1

The GAL4-UAS system

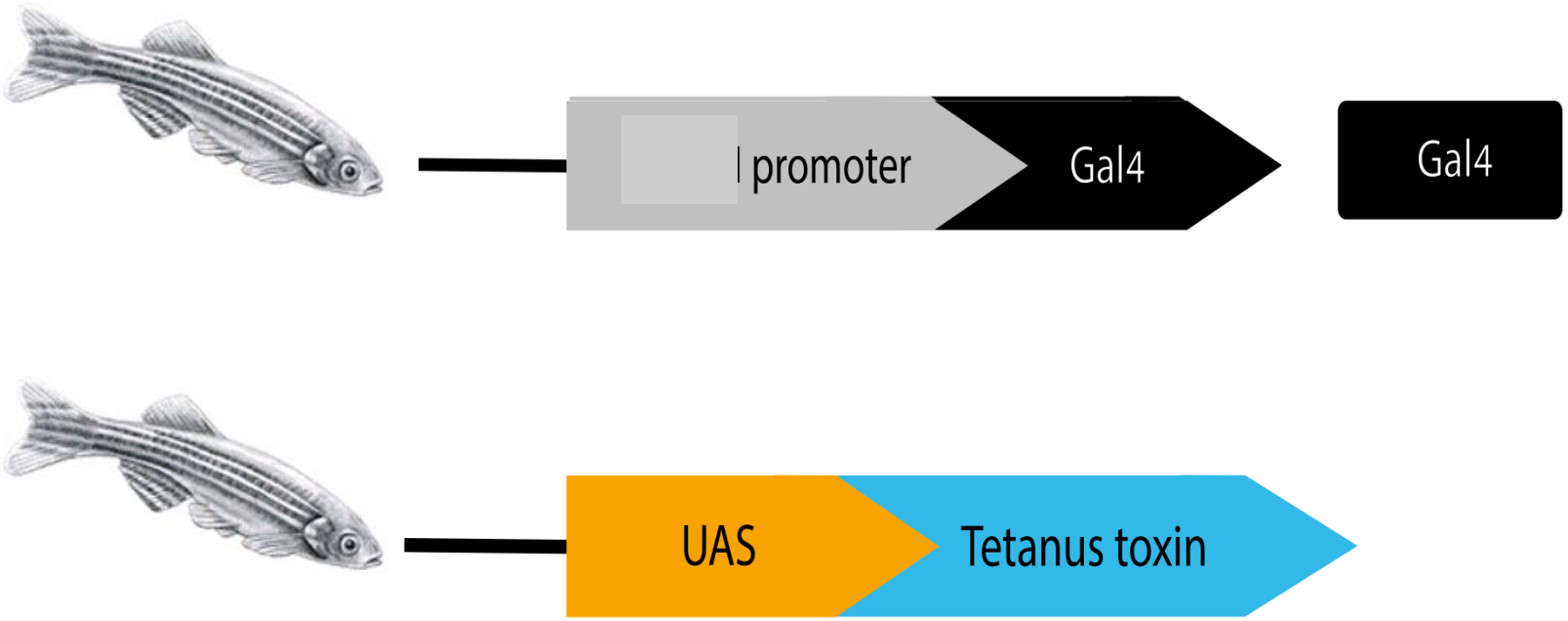


Schematic of the Gal4/UAS System

Utilization of the GAL4-UAS system in zebrafish



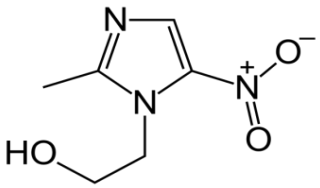
Specific neuronal inhibition



Neuronal ablation

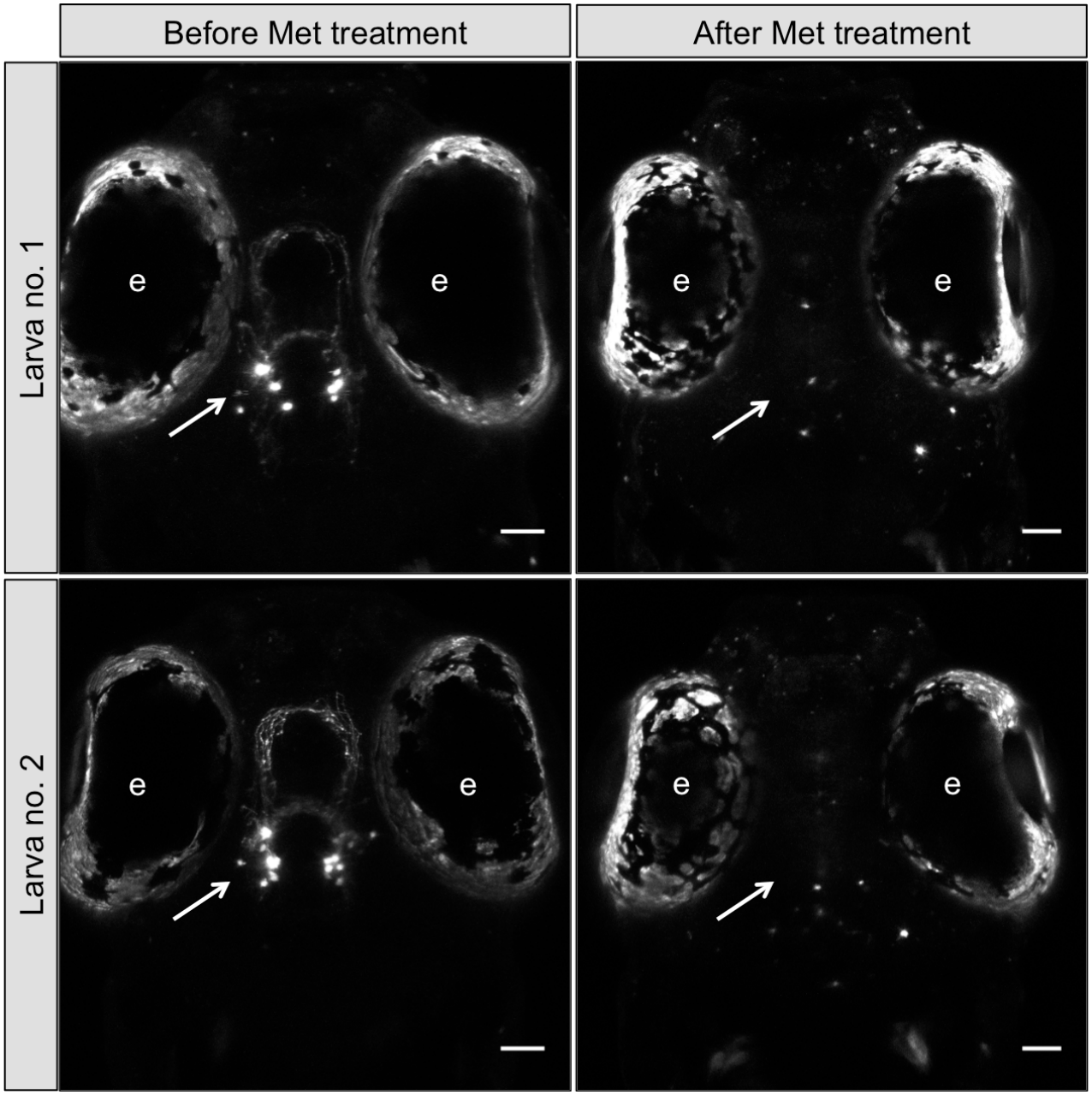
promoter Gal4 → Gal4

UAS nfsB mCherry



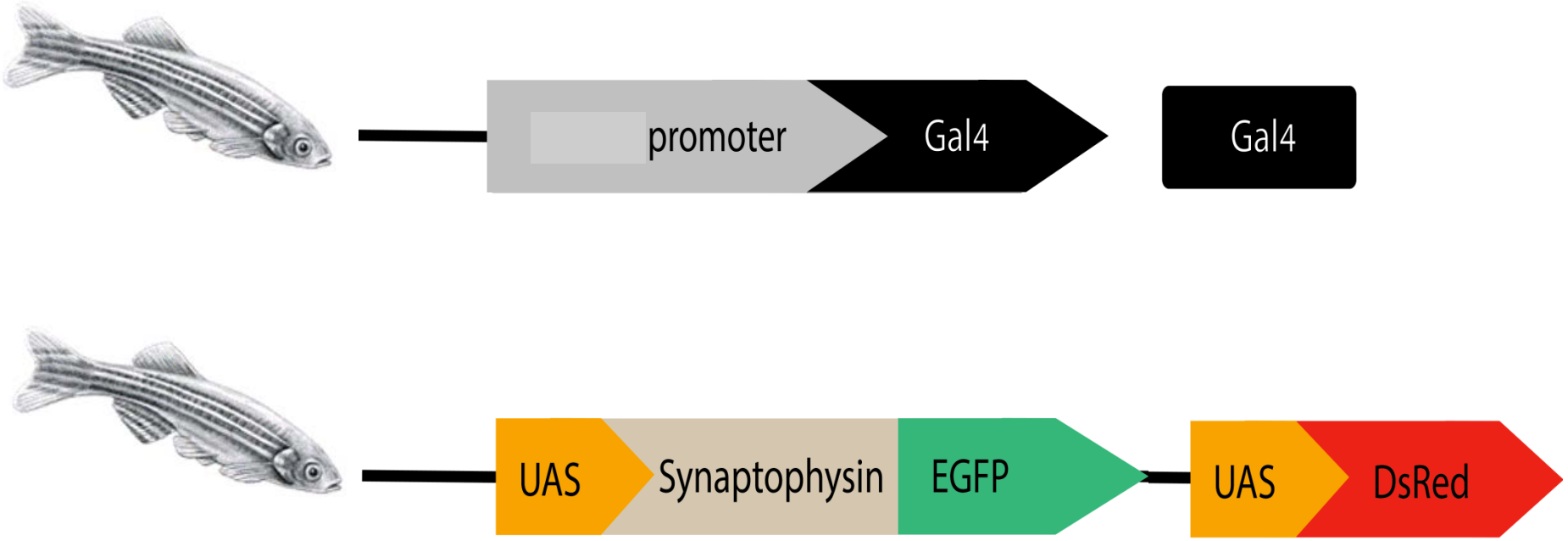
Metronidazole

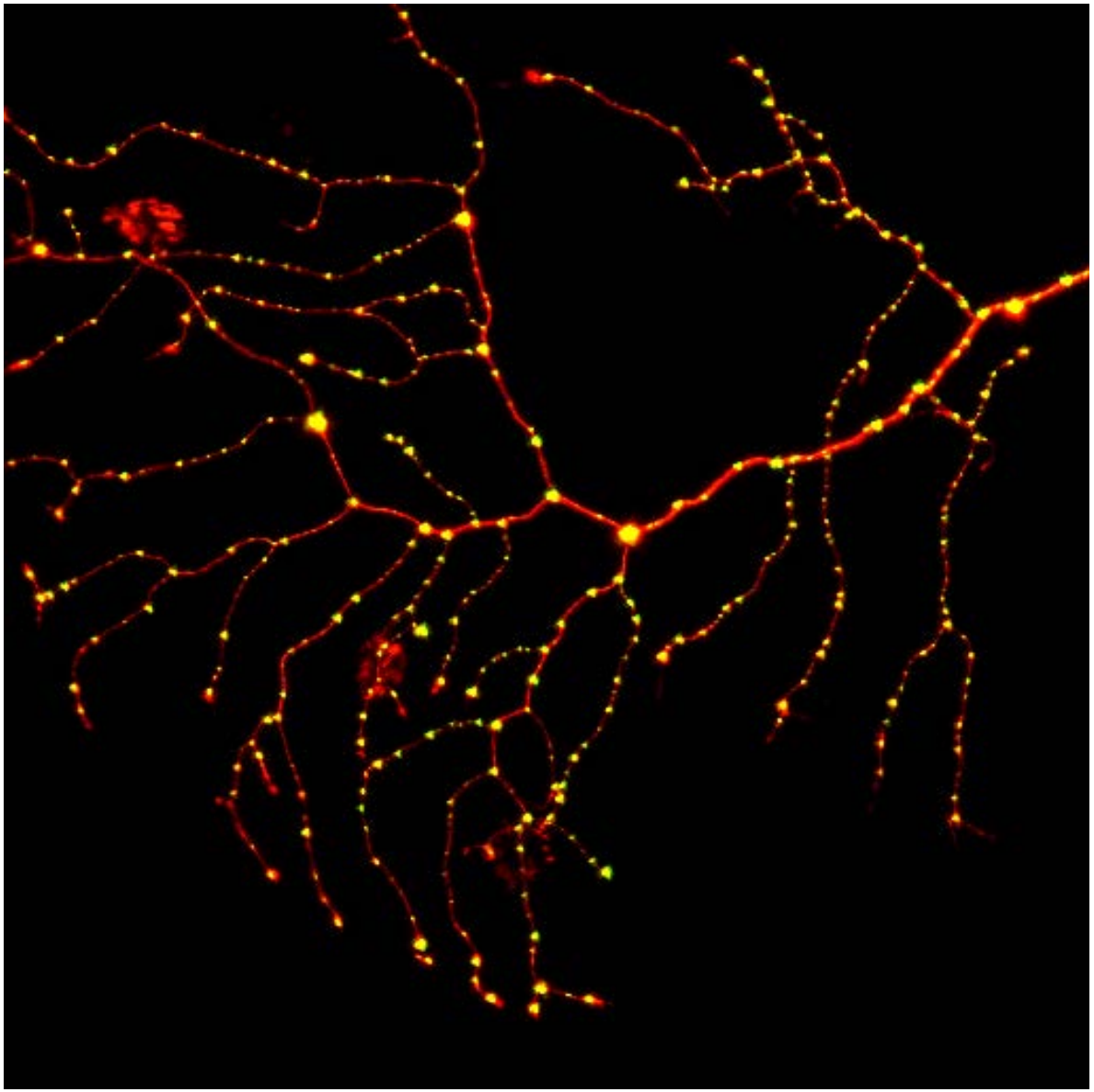
\+ Nitroreducdase (nfsB gene) → Cell death



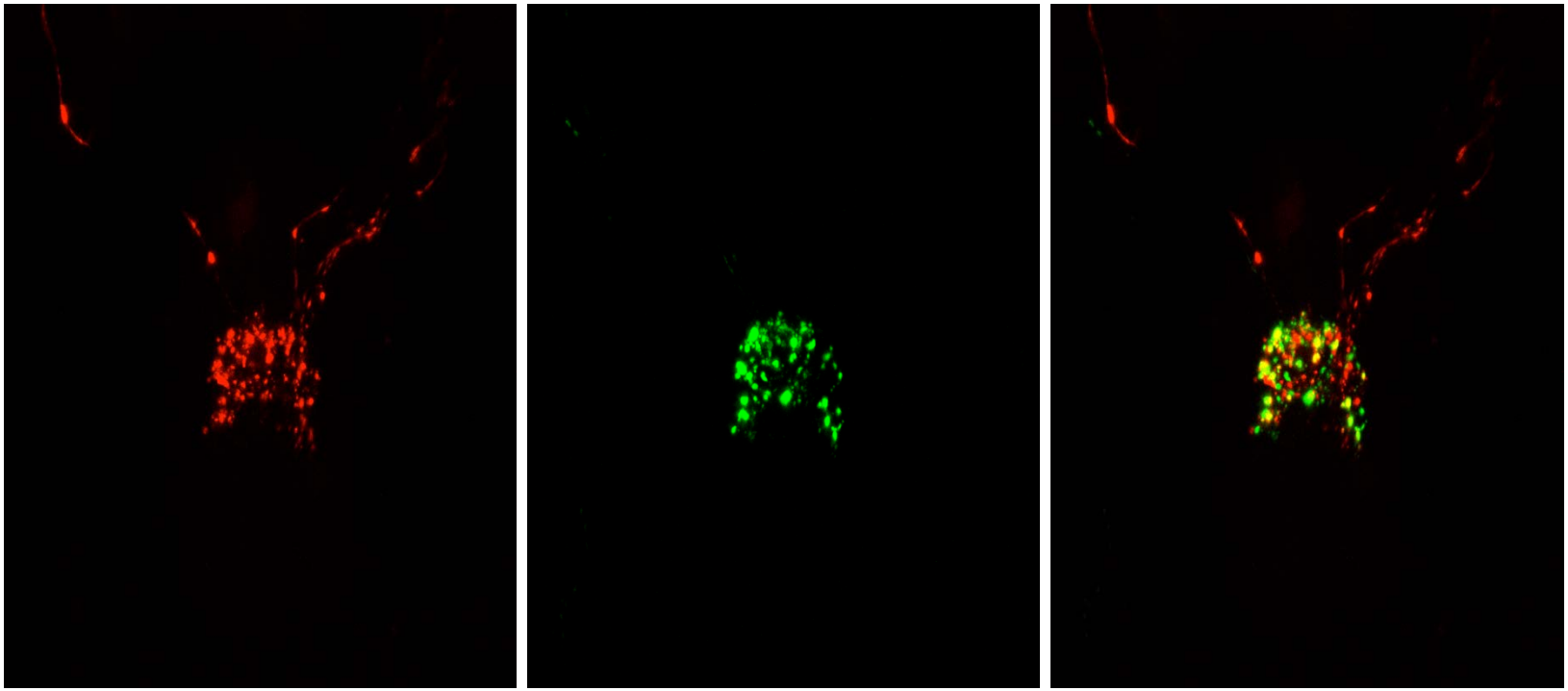
Shainer et al., 2019

Synaptic plasticity



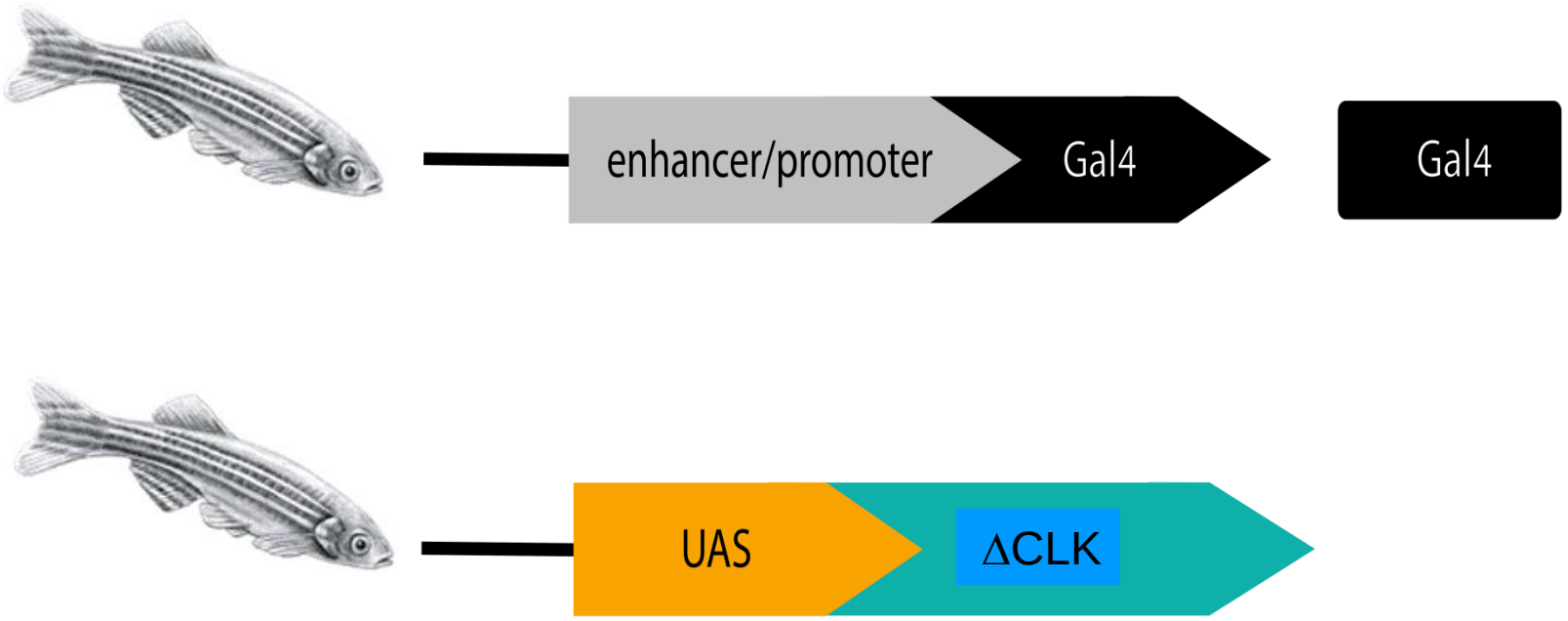


Lior Appelbaum, BIU

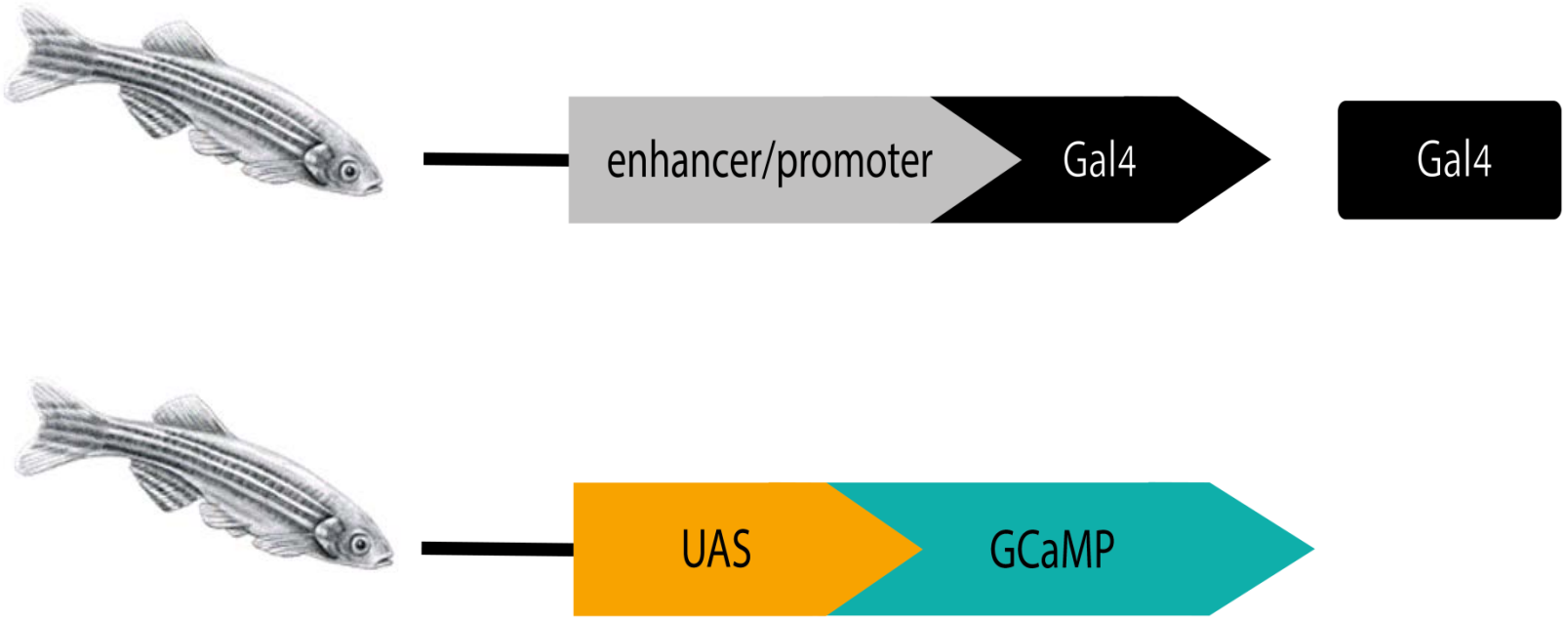


Shainer et al., 2017

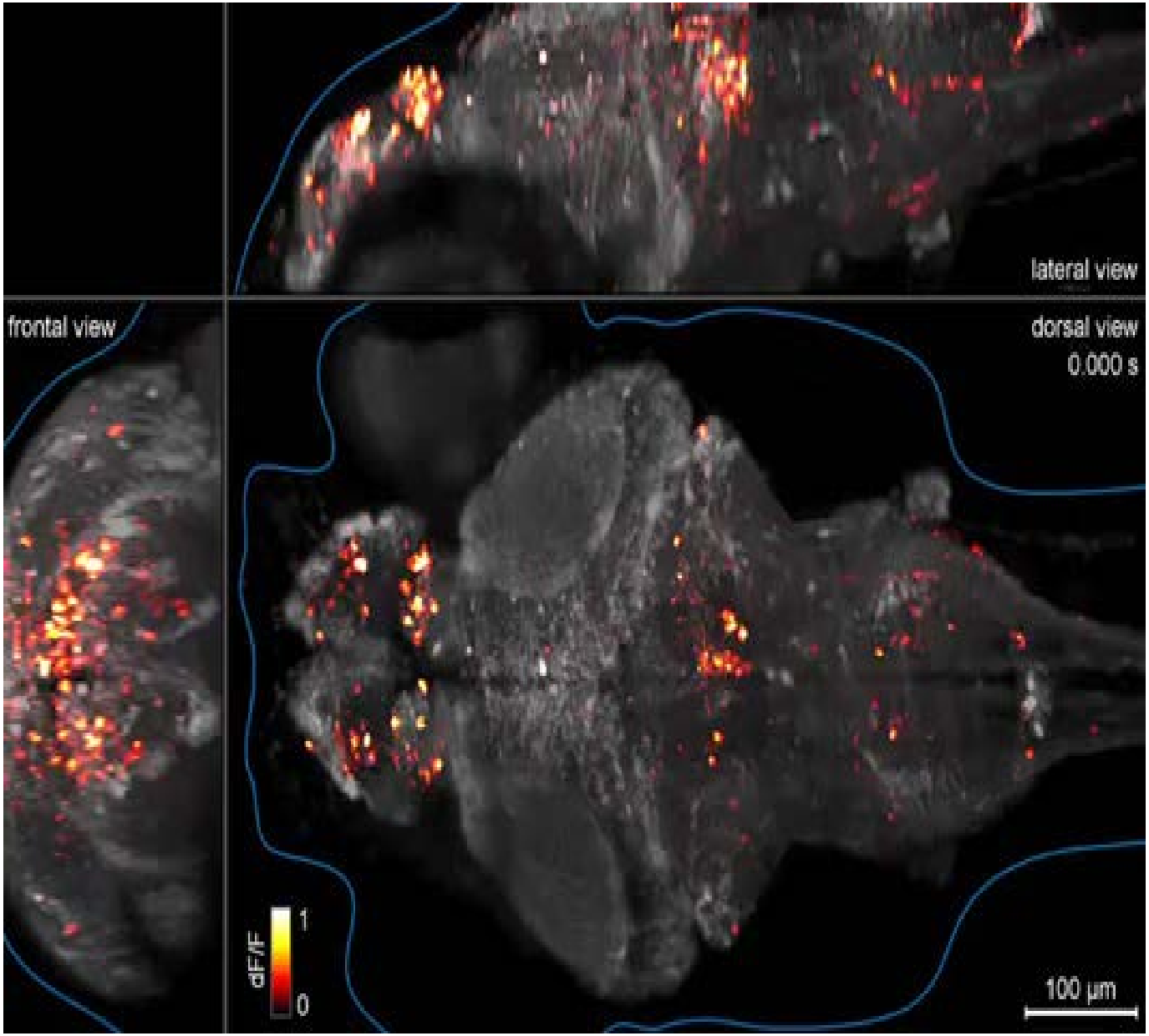
Blocking the clock



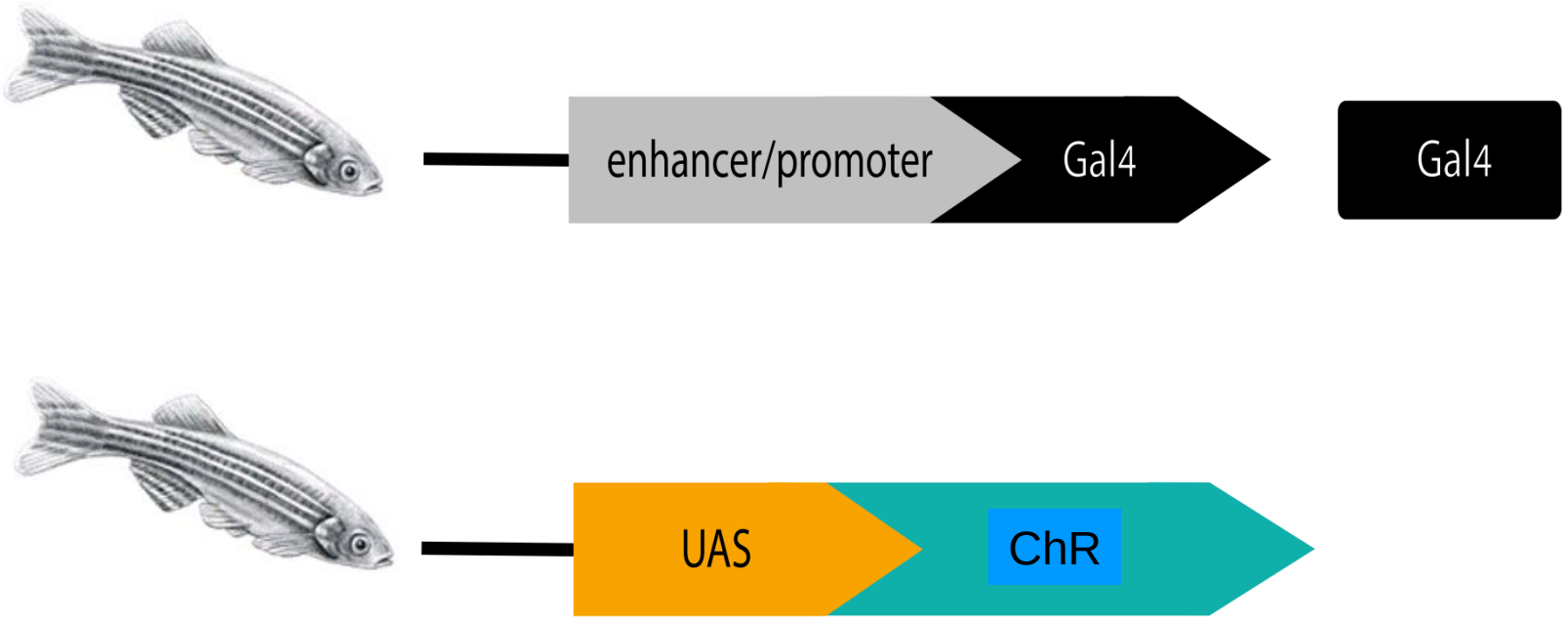
Seeing neurons in action, using Ca^{++} sensor



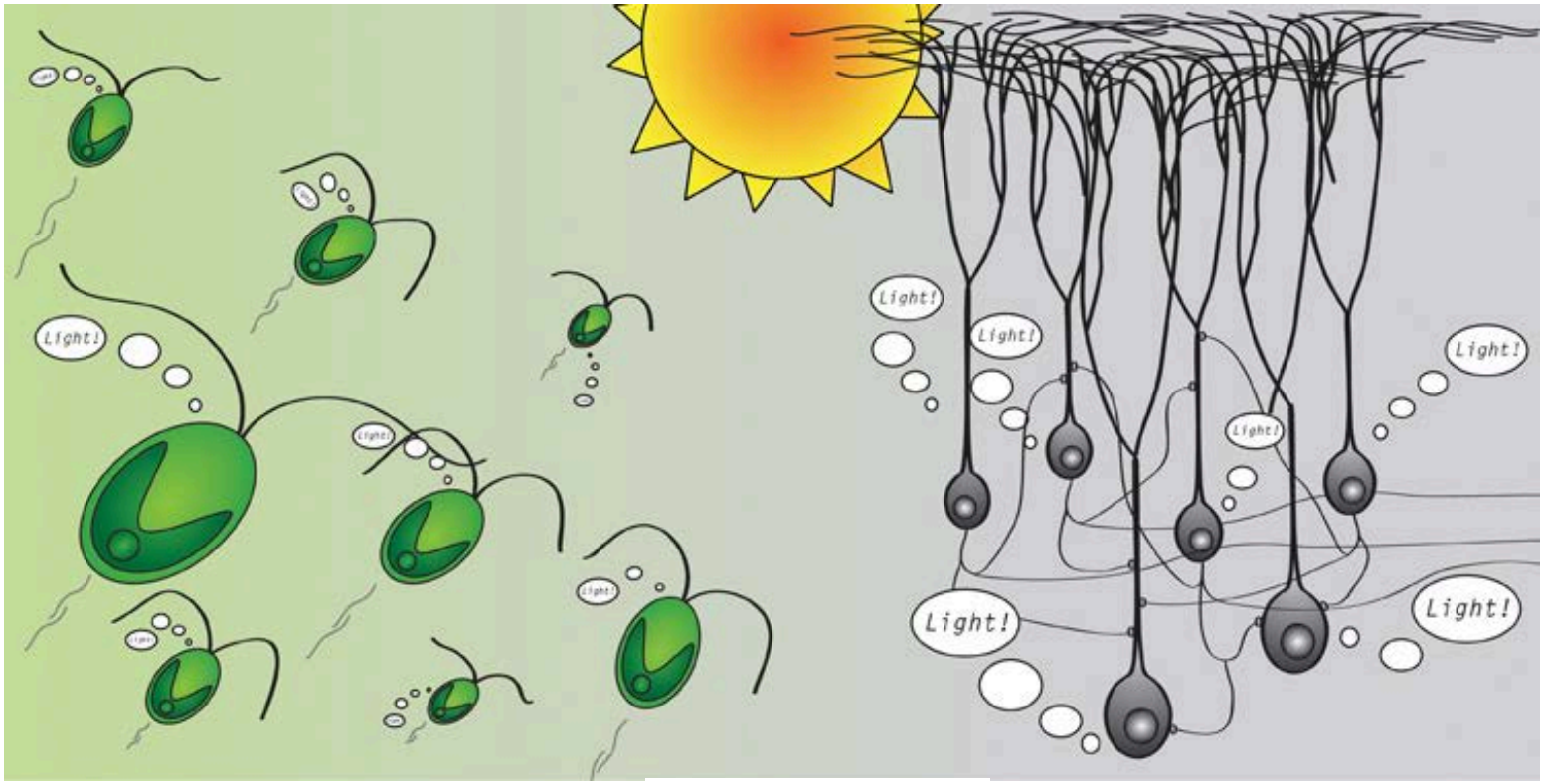
https://www.youtube.com/watch?v=1Q-g1uCvYOA


lateral view
frontal view
dorsal view
0.000 s
dF/F
1
0
100 µm

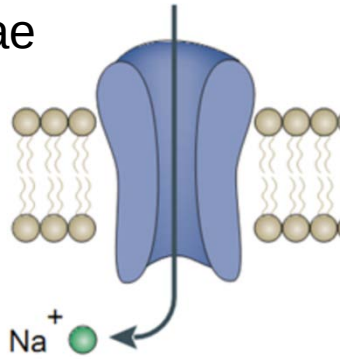
Activating neurons



Optogenetics

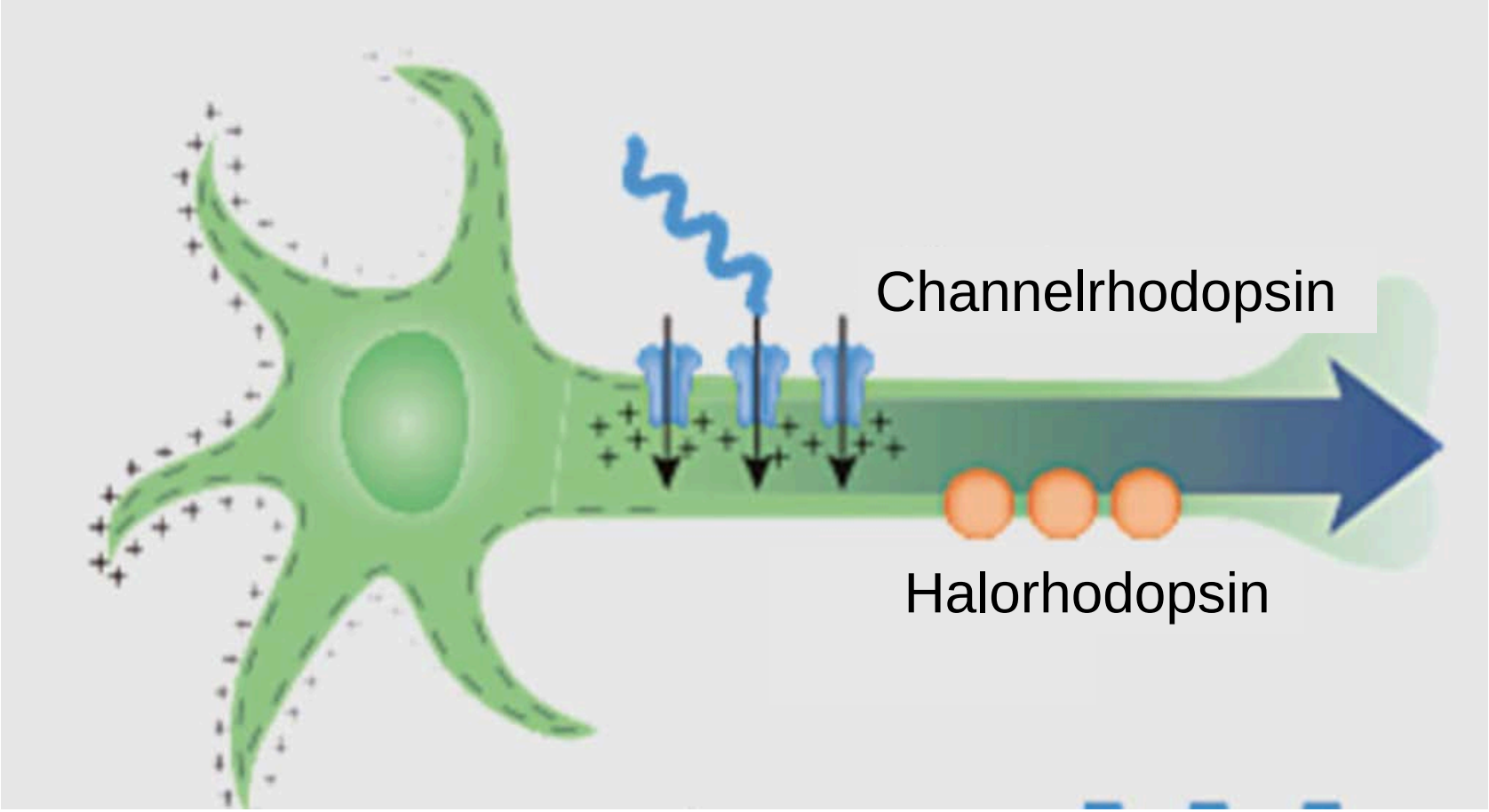


Light sensitive single-cell algae



Light sensitive sodium channel called channelrhodopsin

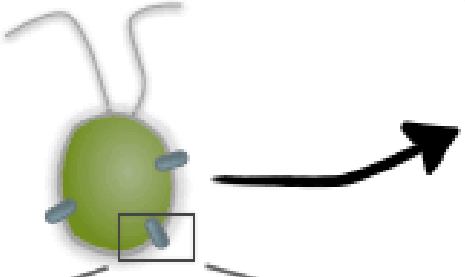
Generation of action potential by light pulse



The blue-light sensitive Channelrhodopsin and the yellow light-activated chloride pump halorhodopsin together enable activation and silencing of neural activity

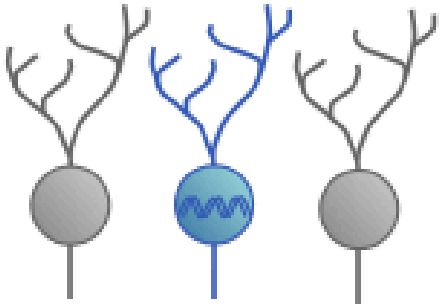
How optogenetics works

A light-sensitive protein from algae

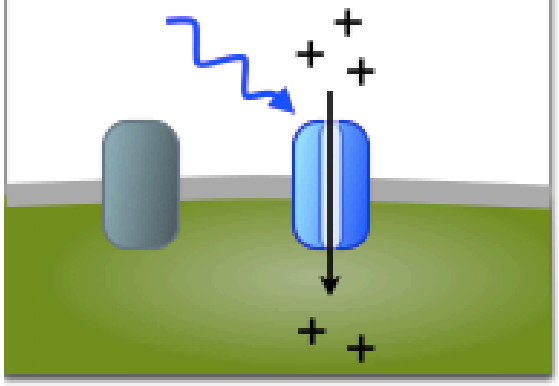


Take the gene for this protein...

... and insert the DNA into specific neurons in the brain

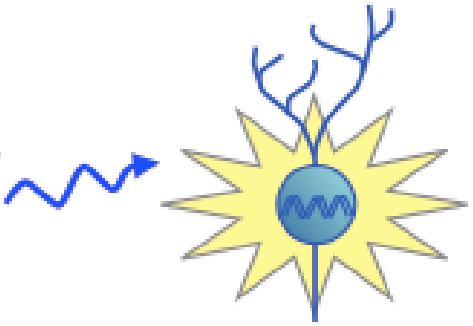


This protein is an ion channel that opens in response to **blue light**



Neurons communicate by "**firing**." This is an electrical signal created by opening & closing ion channels.

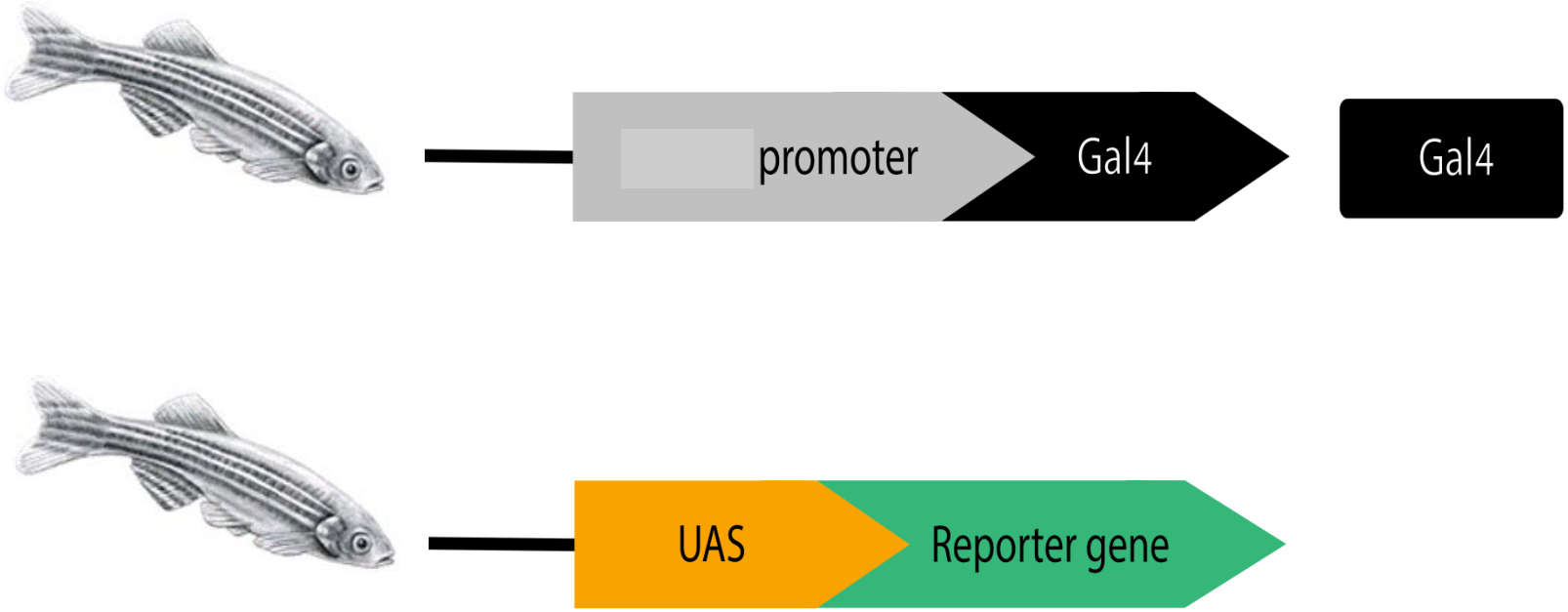
So now you can cause neurons to fire just by flashing **blue light!**



With the right combination of neurons, you can activate an entire brain circuit to control specific behaviors (like movement)

https://www.youtube.com/watch?v=I64X7vHSHOE
https://www.youtube.com/watch?v=rfEKc_0iaJo
https://www.youtube.com/watch?v=IW4j8_k8pmE

What is the advantage of using the GAL4-UAS system?



Promoters

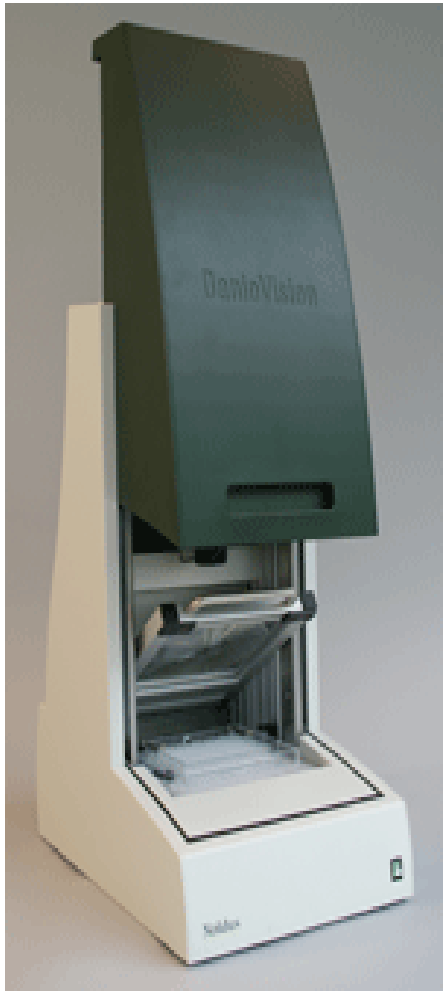
- Gene 1
- Gene 2
- Gene 3
- Gene 4
- Gene 5
- Gene 6
- Gene 7
- Gene 8
- Gene 9
- Gene 10

Reporters

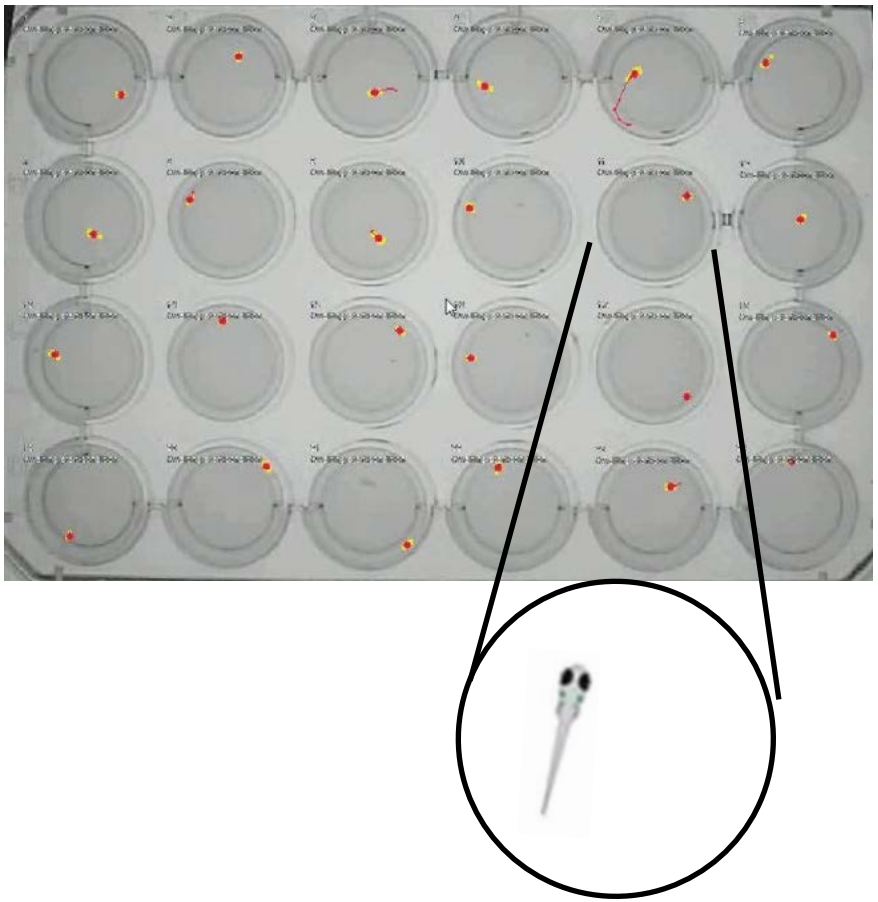
- EGFP
- Tetanus toxin
- nfsB (nitroreductase)
- Synaptophysin-EGFP
- Cannelrhodopsin
- GCaMP (Ca sensor)
- Halorhodopsin

= 70 combinations with 17 Tg lines

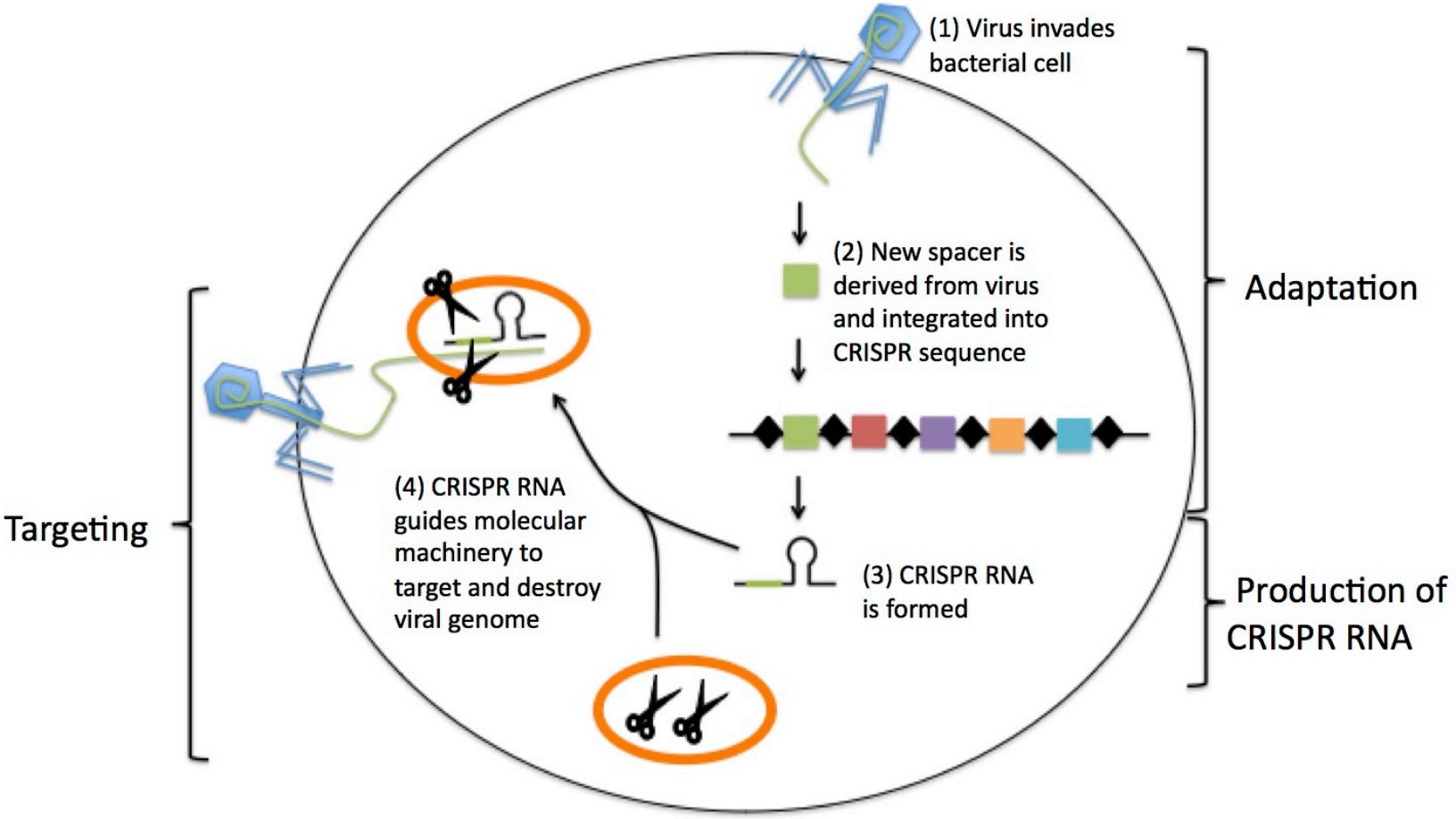
Monitoring locomotor activity of zebrafish larvae

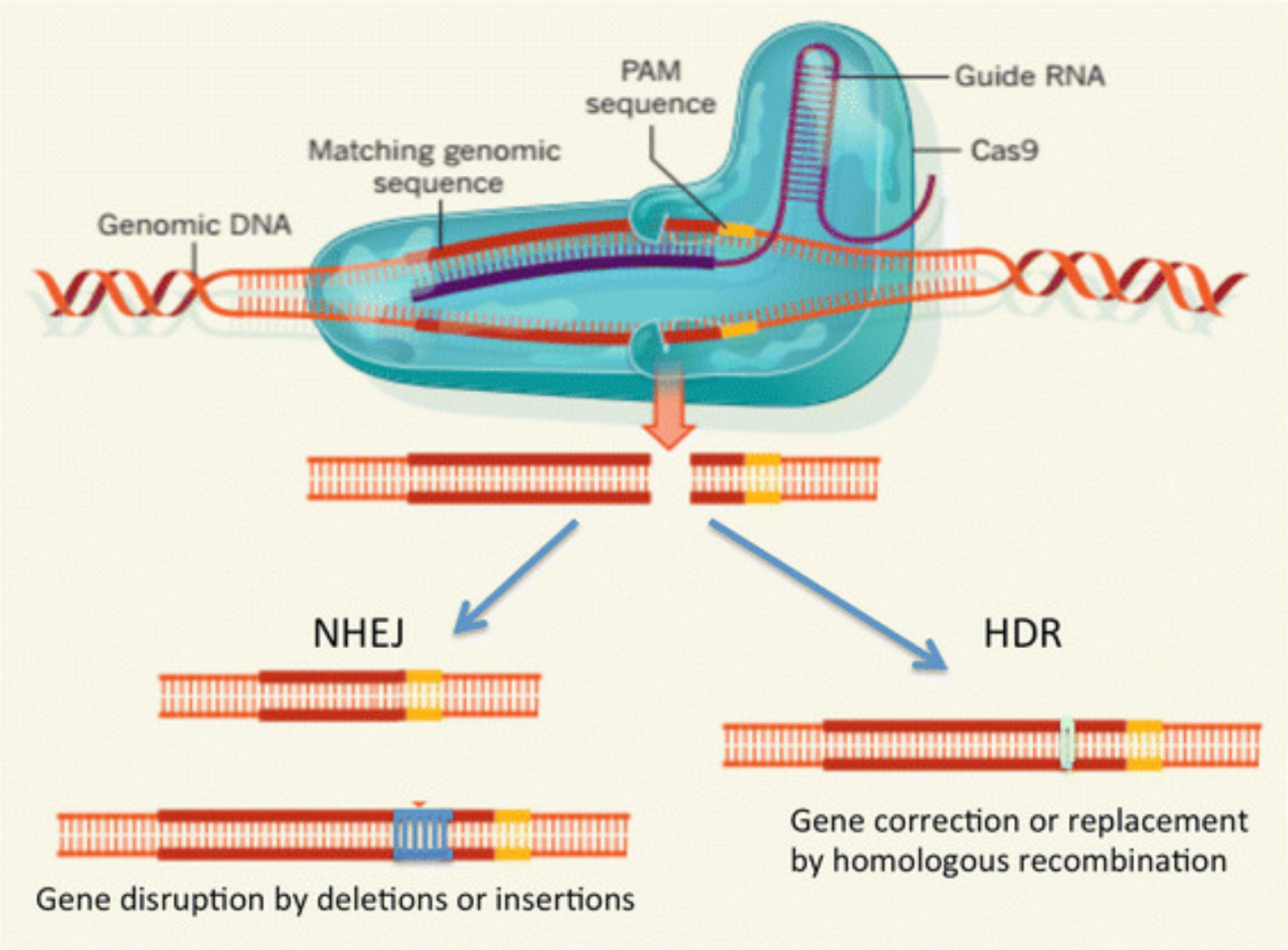


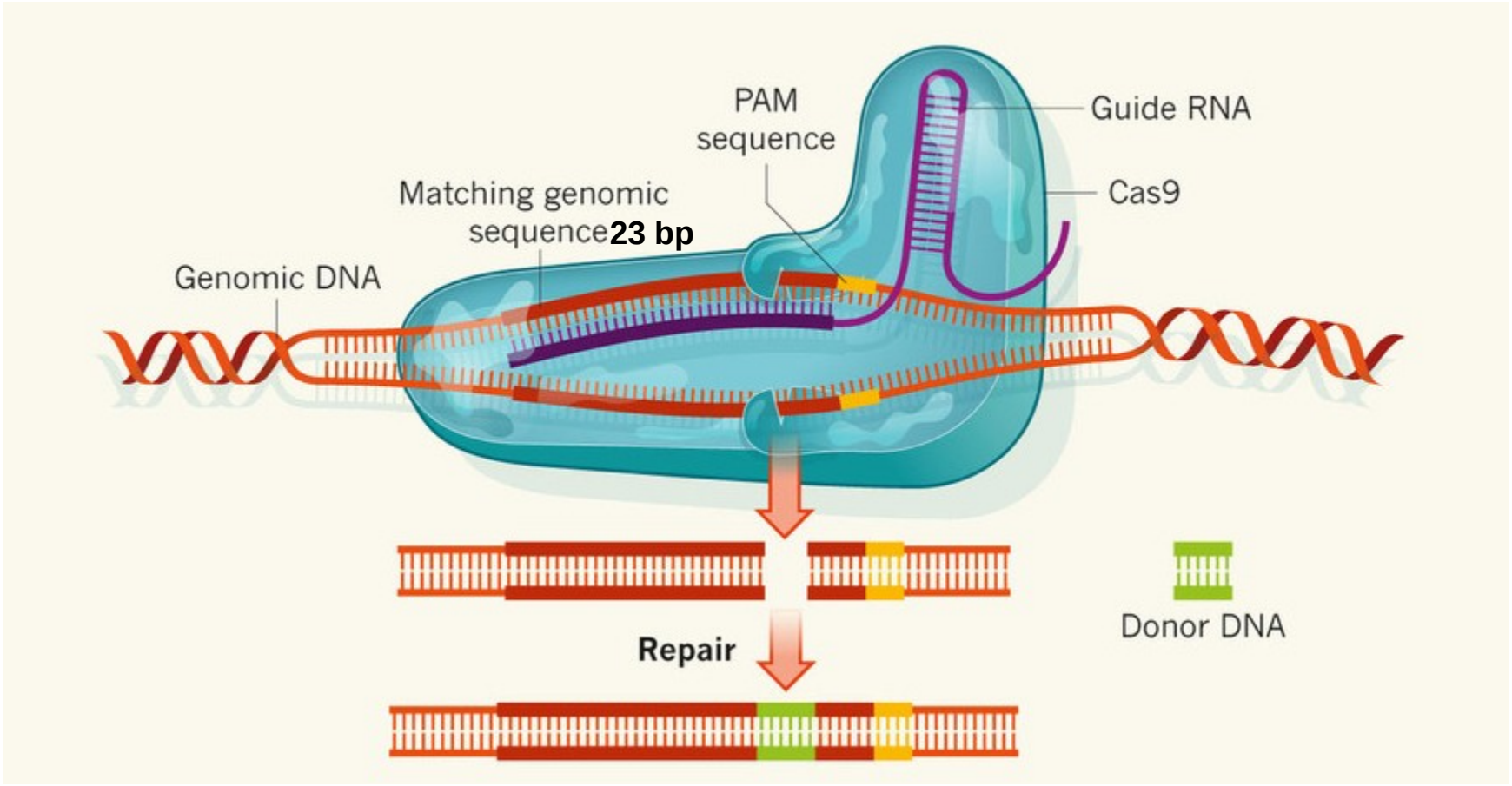
Danio Vision, Noldus

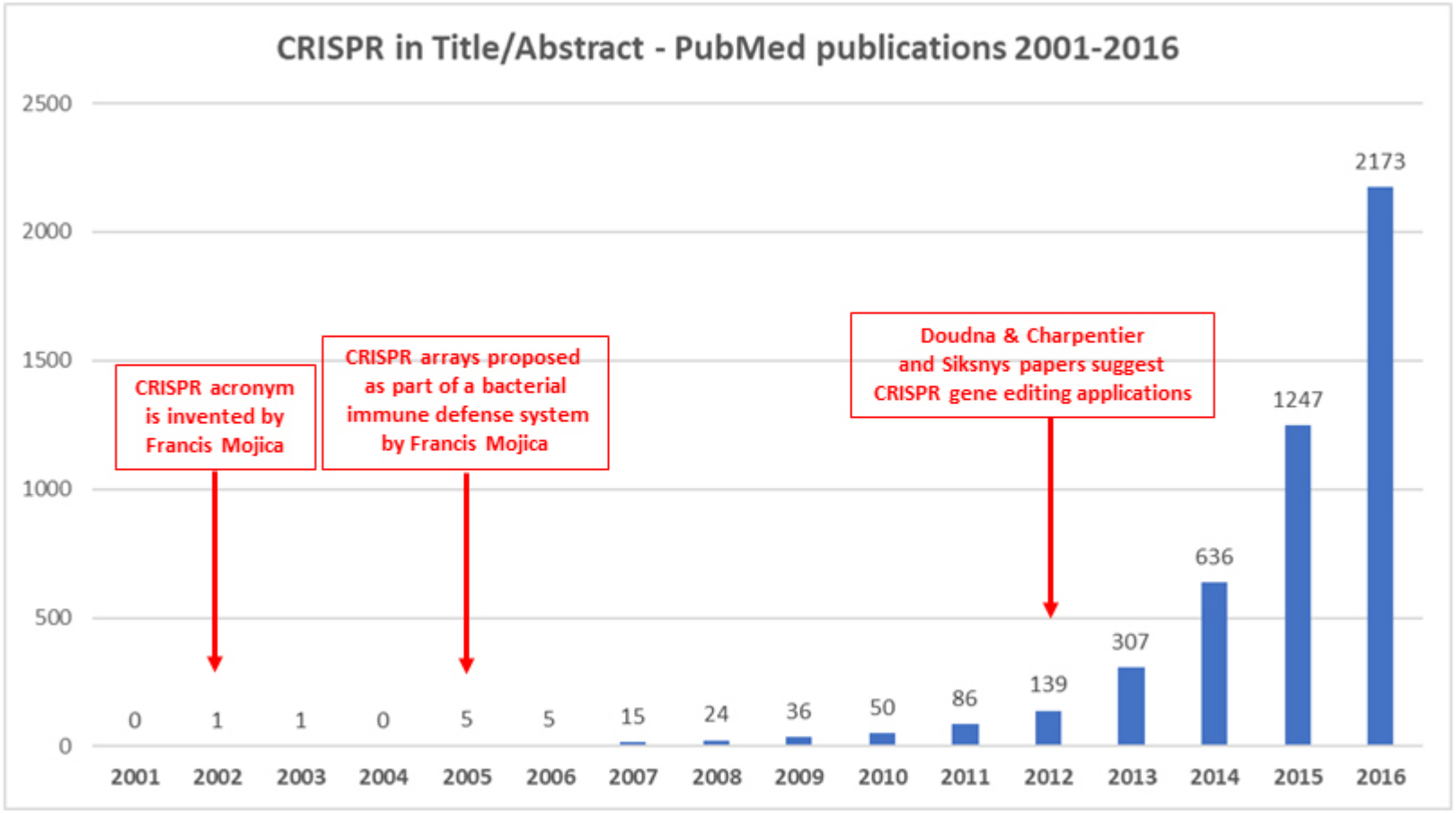


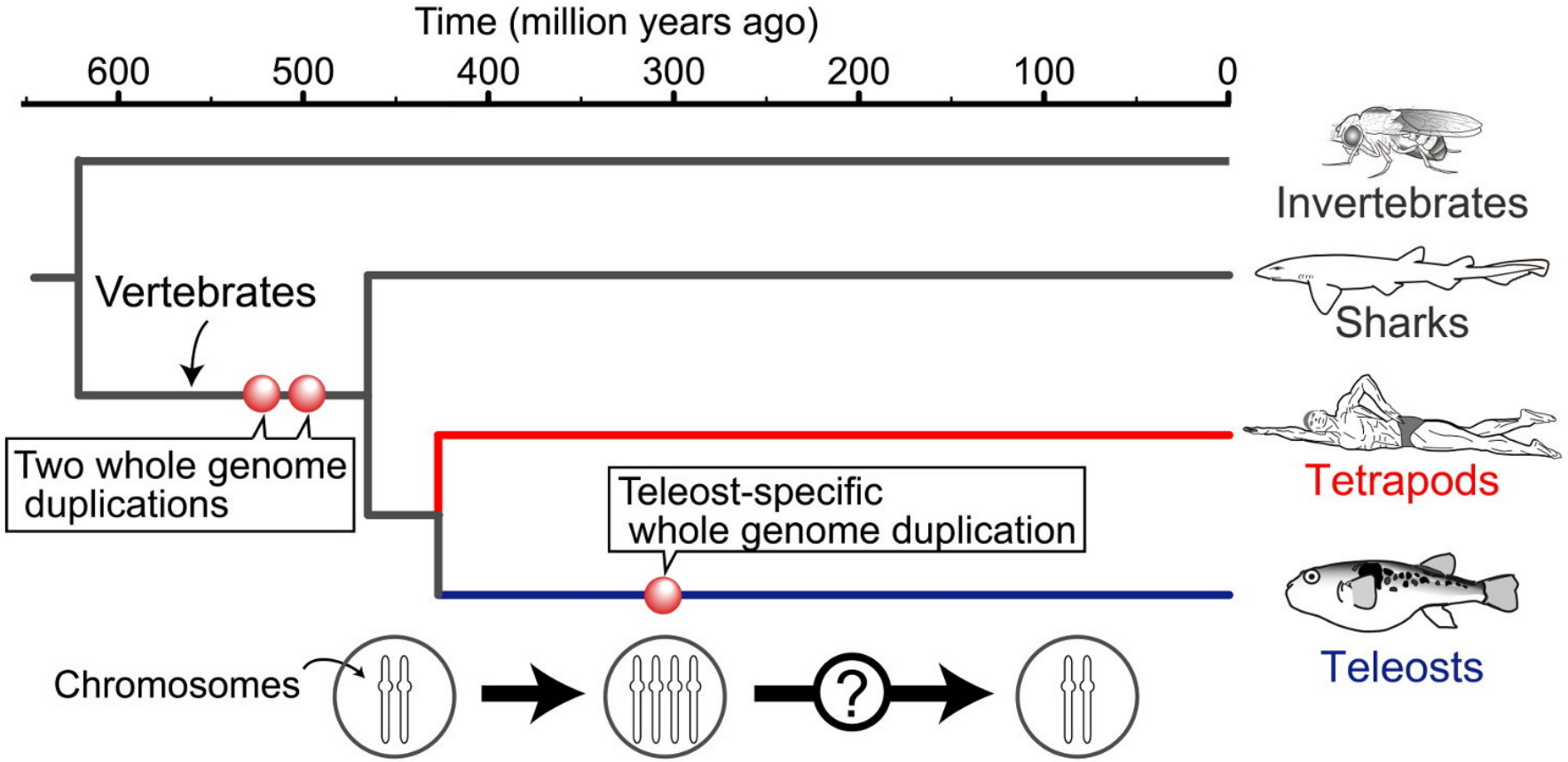
clustered regularly interspaced short palindromic repeats (CRISPR)




PAM
sequence
Guide RNA
Cas9
Matching genomic
sequence
Genomic DNA
NHEJ
HDR
Gene disruption by deletions or insertions
Gene correction or replacement
by homologous recombination


PAM
sequence
Guide RNA
Cas9
Matching genomic
sequence
23 bp
Genomic DNA
Repair
Donor DNA

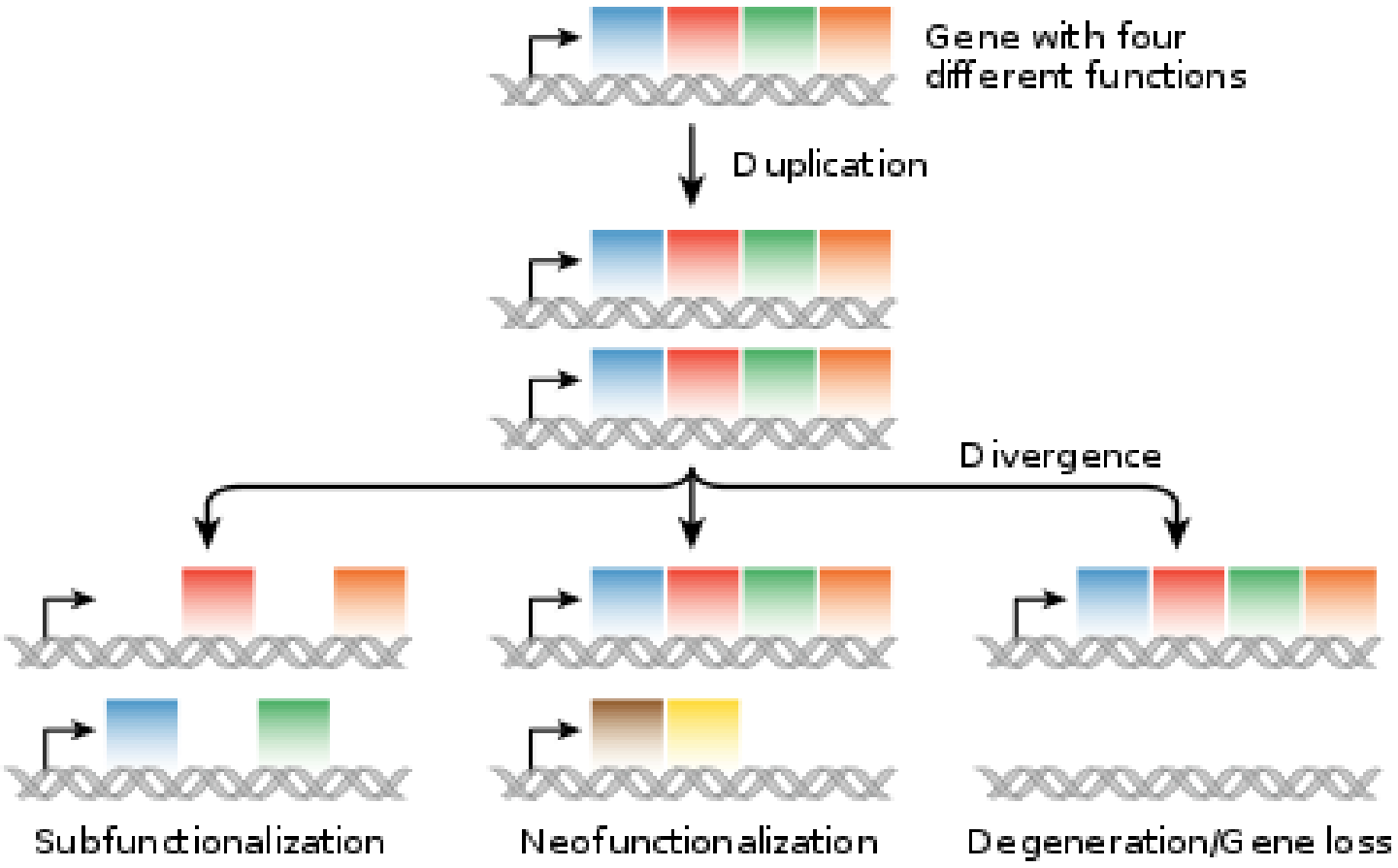

CRISPR in Title/Abstract - PubMed publications 2001-2016
2500
2000
1500
1000
500
0
CRISPR acronym is invented by Francis Mojica
CRISPR arrays proposed as part of a bacterial immune defense system by Francis Mojica
Doudna & Charpentier and Siksnys papers suggest CRISPR gene editing applications
0
1
1
0
5
5
15
24
36
50
86
139
307
636
1247
2173
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016



Whole genome duplication occurred as a result of failure of homologous chromosomes to separate properly during meiosis which results in additional copies of the entire genome.

Whole genome duplication is a rare evolutionary event that has played a dramatic role in diversification.

Whole genome duplication: What could be the consequence and why is this a consideration?



- What are the advantages and disadvantages of zebrafish as a model for human genetic diseases?

Thank you for your patients!!