A zebrafish model for the FA/BRCA pathway and connecting fish medical models to human health

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UNIVERSITY OF OREGON

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http://www.exoticfishaquarium.com/zebra-fish-danio-rerio/

A zebrafish model for the FA/BRCA pathway and connecting fish medical models to human health

- What makes zebrafish a good biomedical model?
- A small molecule screen to rescue Fanconi anemia.
- Connecting fish genomes to human biology.

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Fish provide models for biomedicine

Cleft palate

Eberhart 2008 *Nature Genetics*

Li 2010 *Am. J. Med. Genet.*

Osteopenia

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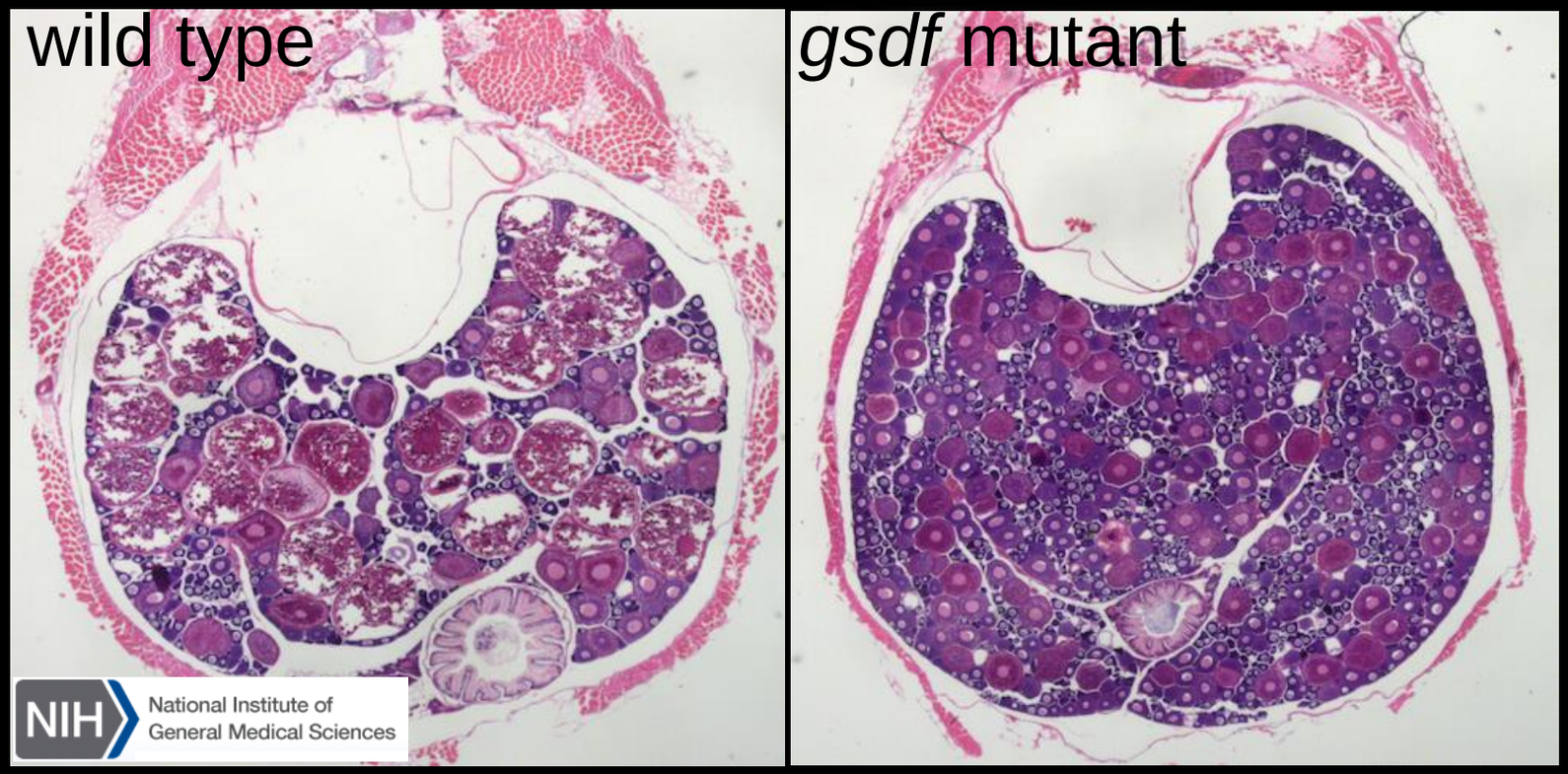
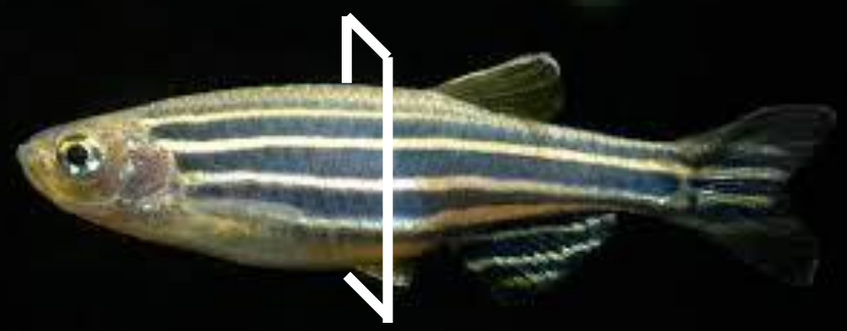
Fish provide models for biomedicine



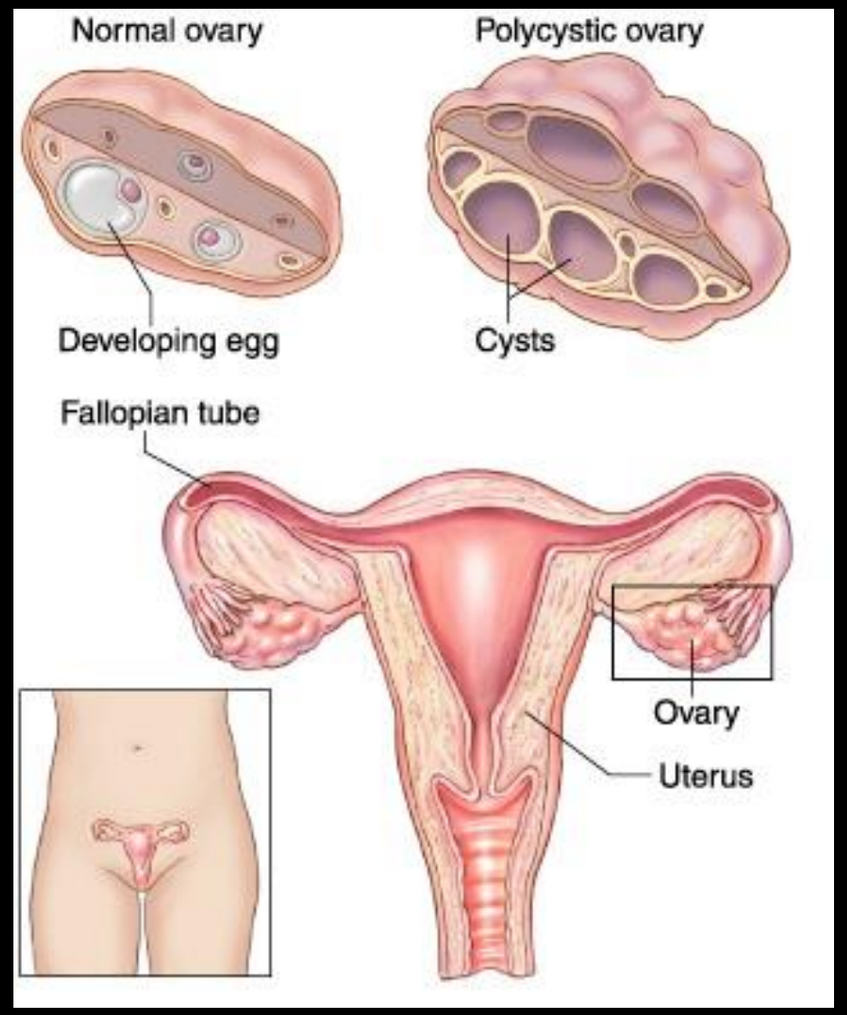
follicles mature

follicles accumulate

Reproductive health



polycystic ovary syndrome



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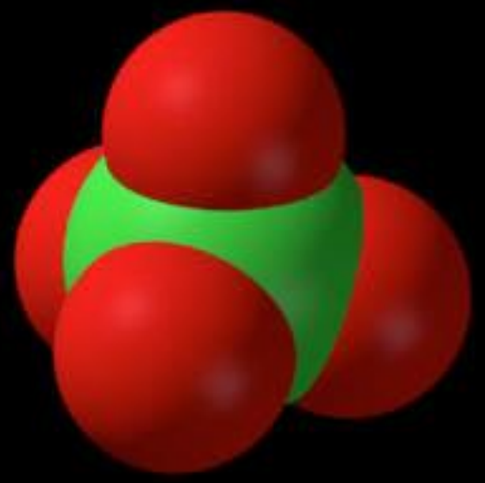
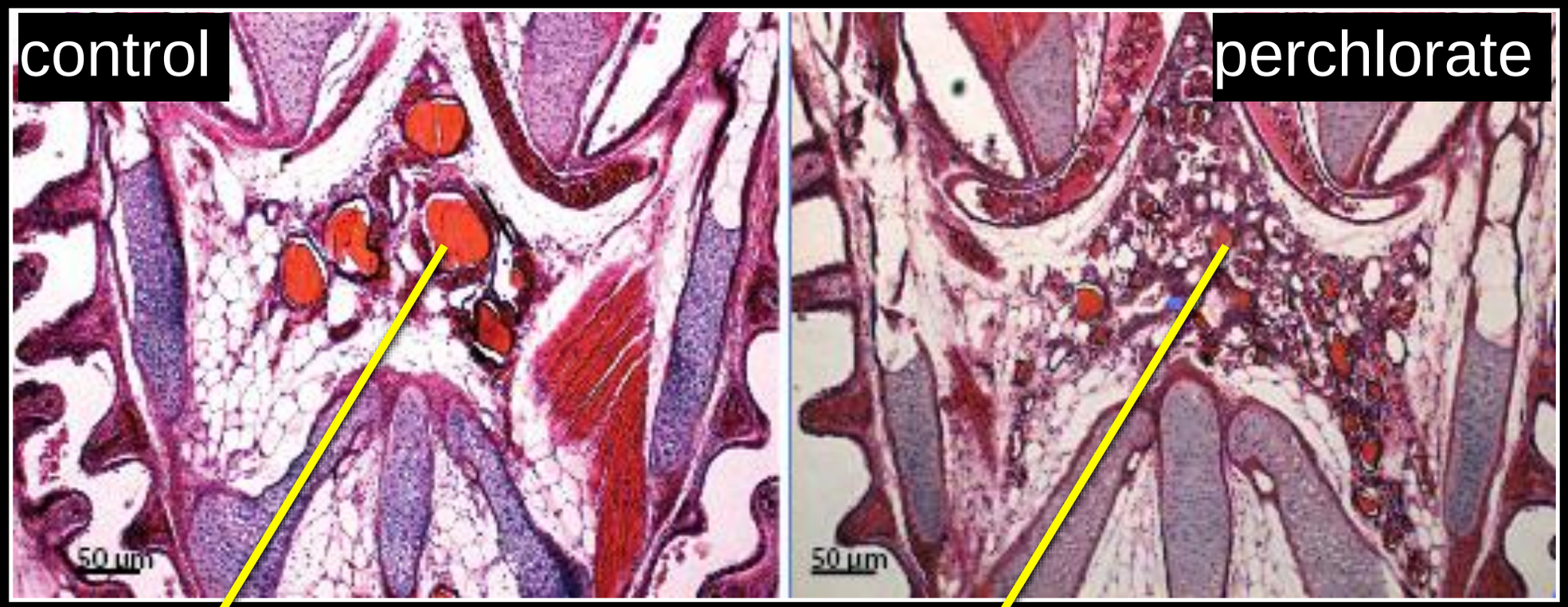
http://www.ovarydisease.com/p/polycystic-ovary-syndrome.html

http://www.glowm.com/section_view/heading/Transvaginal%20ultrasonography%20and%20female%20infertility/item/325

Fish provide models for environmental health

stickleback





few, large thyroid follicles many, small thyroid follicles

- What makes zebrafish a good biomedical model?

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NIH National Institute of Environmental Health Sciences
Your Environment. Your Health.

http://www.serdp.org/Featured-Initiatives/Cleanup-Initiatives/Perchlorate
http://www.usbr.gov/pmts/tech_services/tracy_research/photos/fish/TracyFishImages072.html

A zebrafish model for the FA/BRCA pathway and connecting fish medical models to human health

- What makes zebrafish a good biomedical model?
- A small molecule screen to rescue Fanconi anemia.
- Connecting fish genomes to human biology.

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- What makes zebrafish a good biomedical model?

Embryos develop outside the mother.

Karlstrom & Kane 1996 *Development*

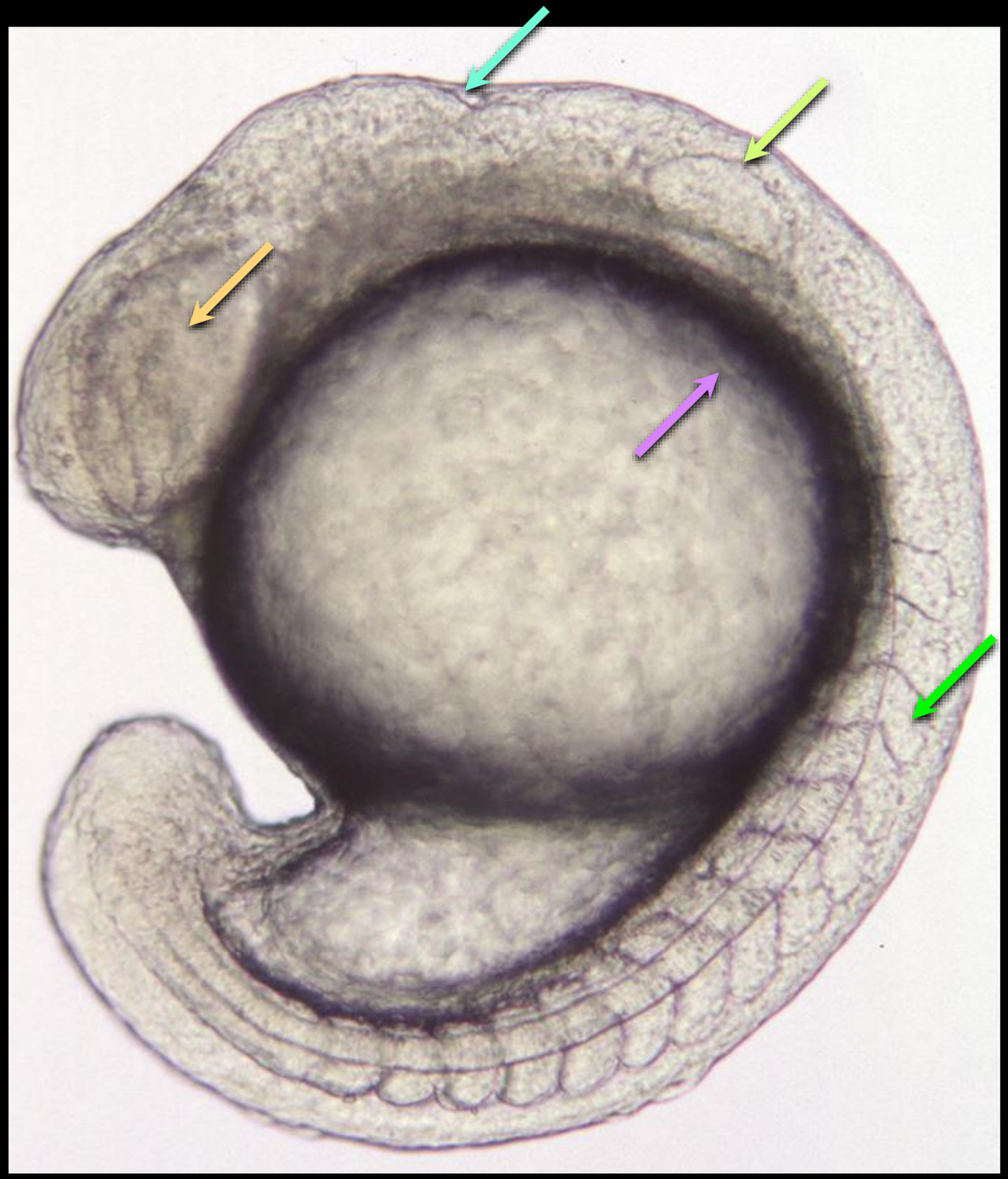
- What makes zebrafish a good biomedical model?

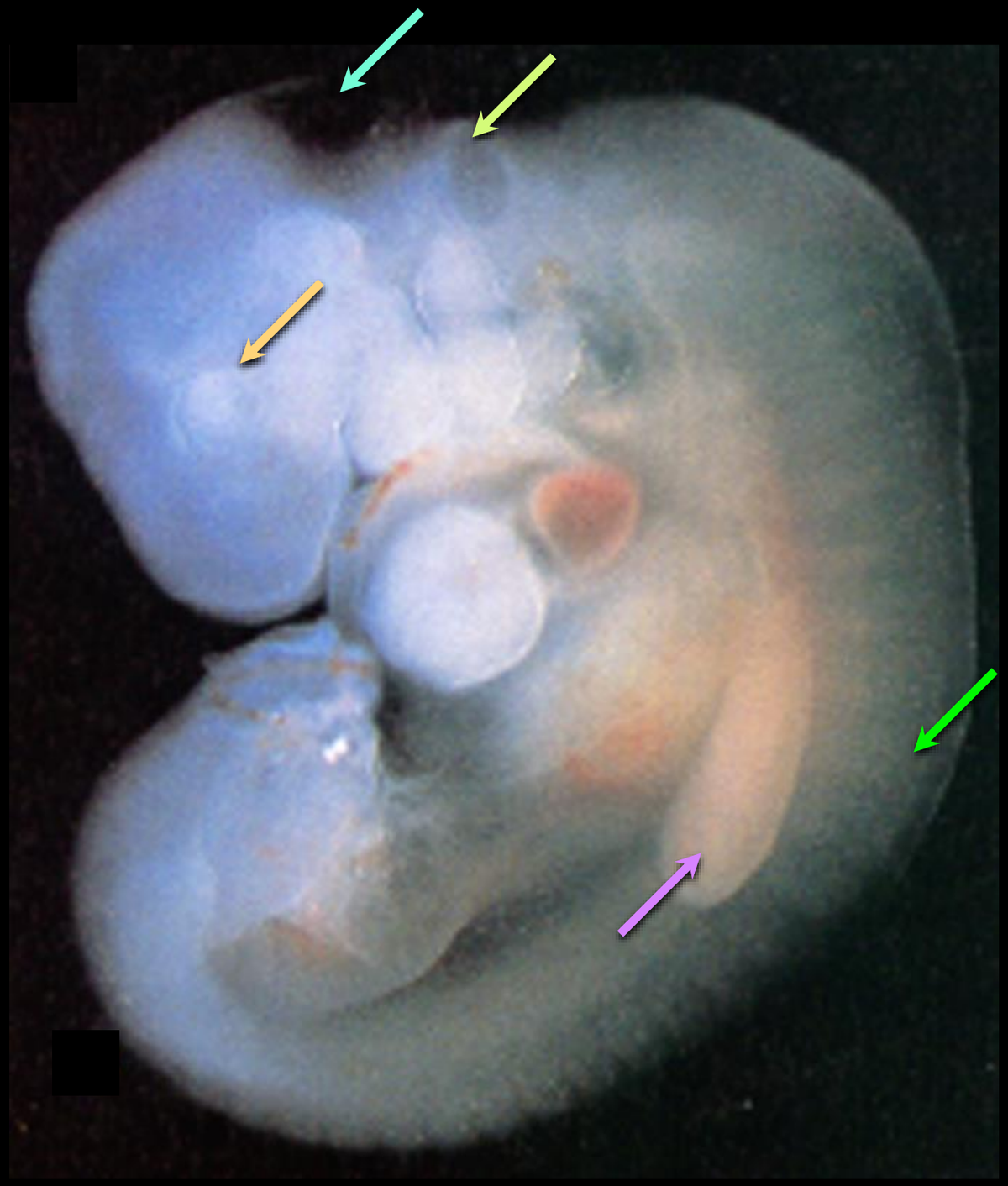
Embryos develop outside the mother.

Karlstrom & Kane 1996 *Development*

Zebrafish embryos develop like early human embryos.

28 day human embryo





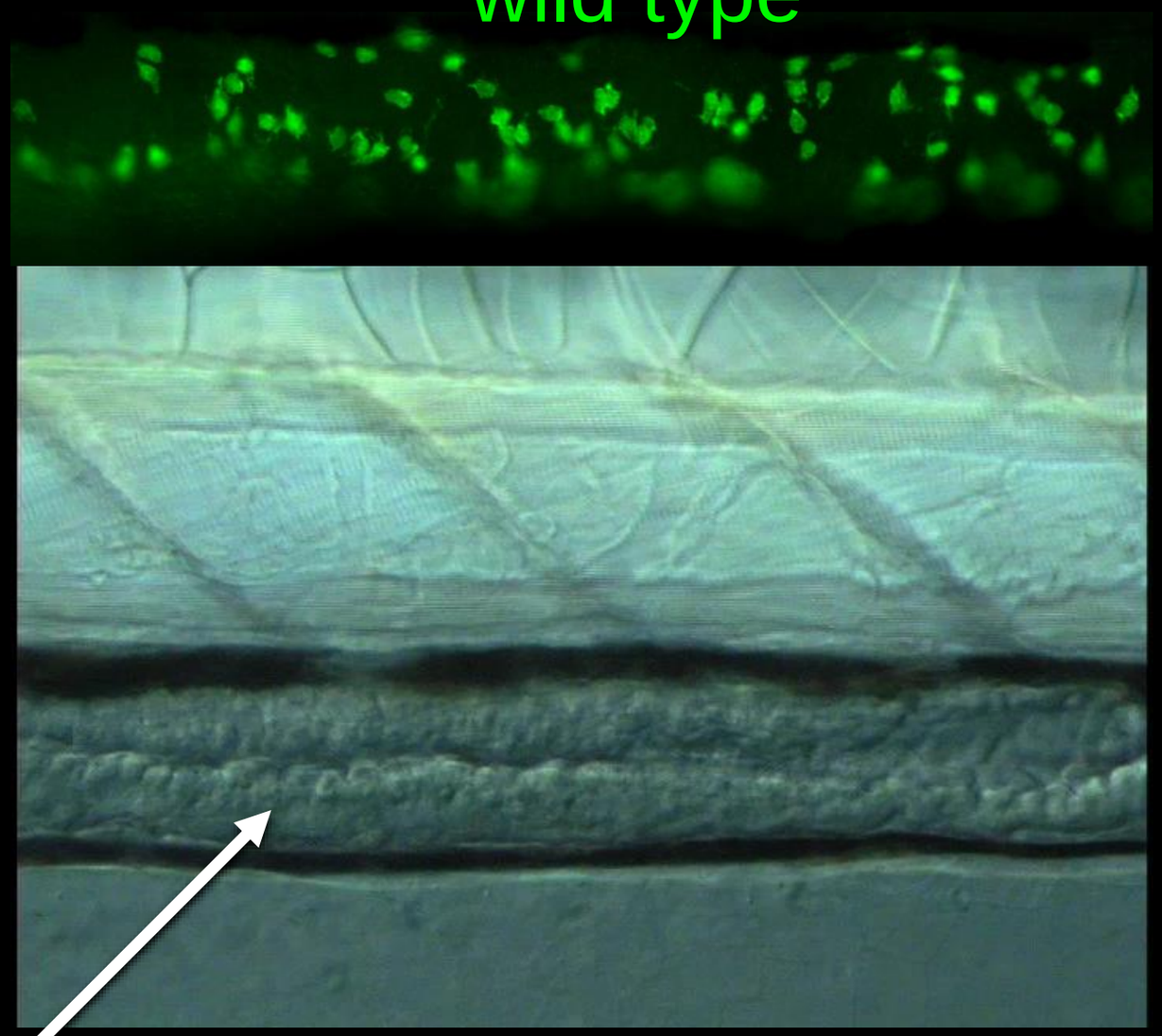
Zebrafish allows forward mutagenesis

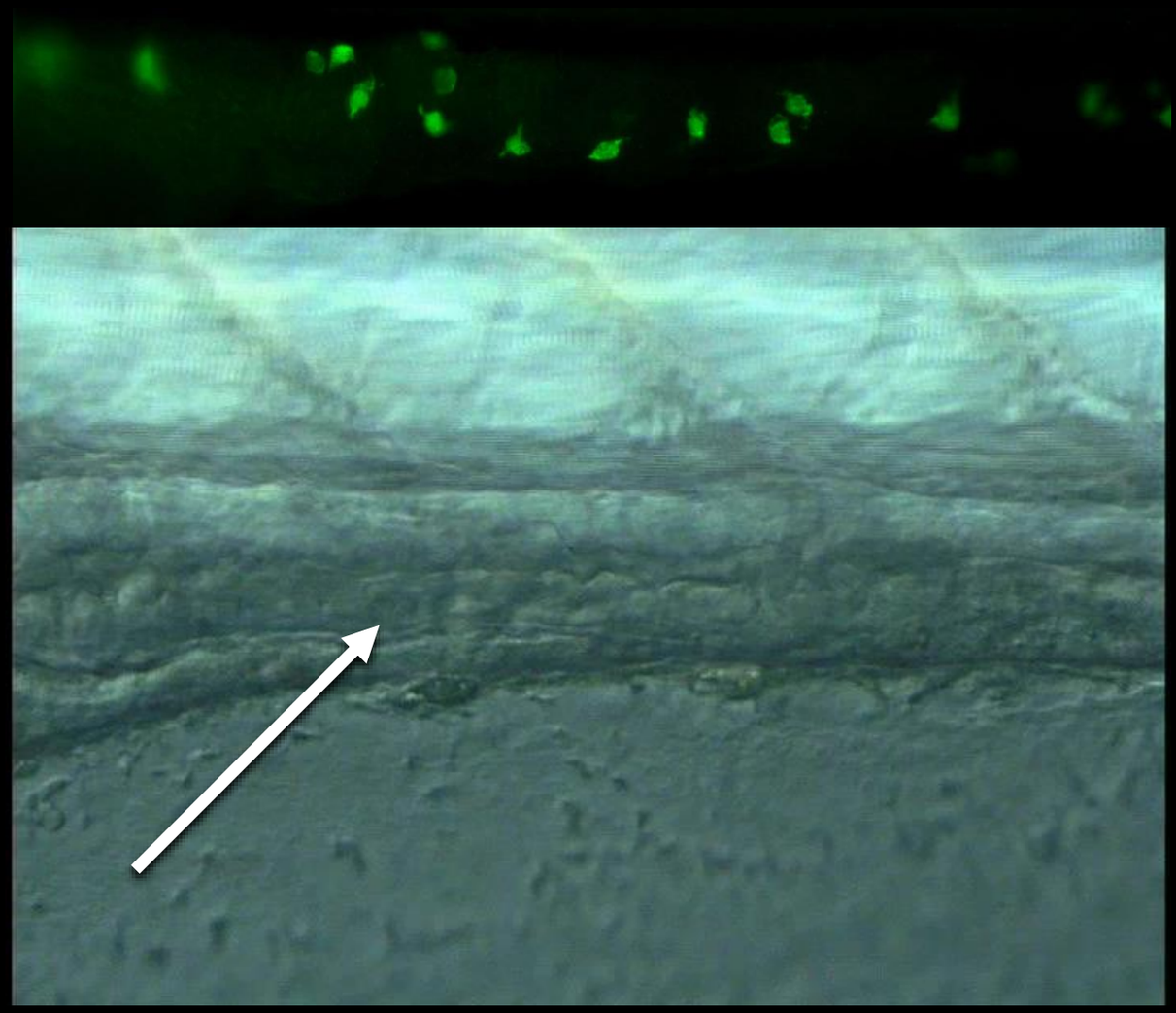
Zebrafish allows forward mutagenesis

enteric neurons

wild type

growler mutant





Model for Hirschsprung disease

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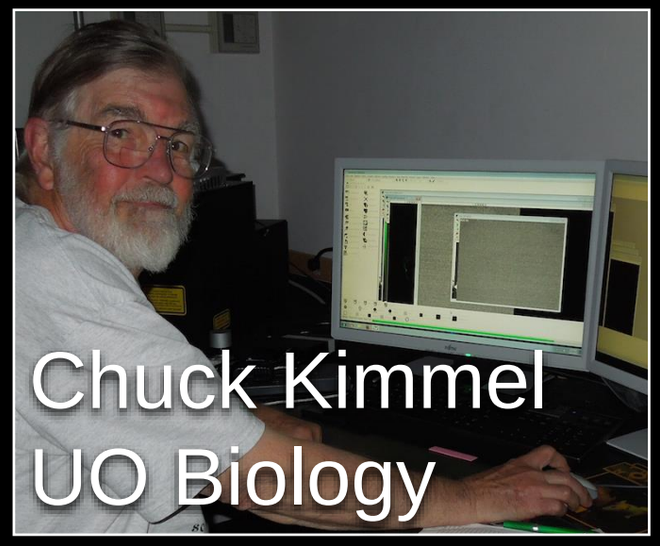
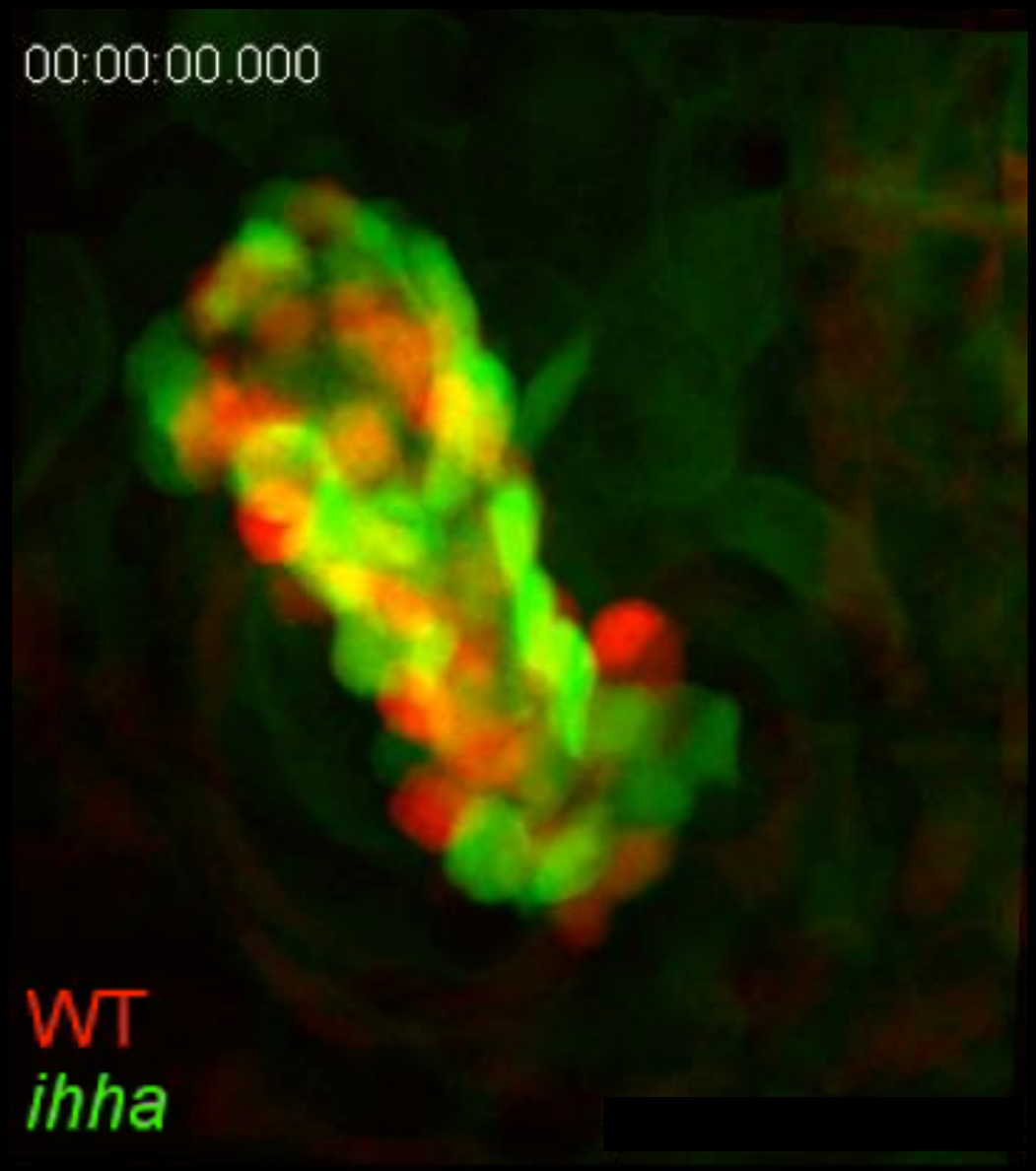
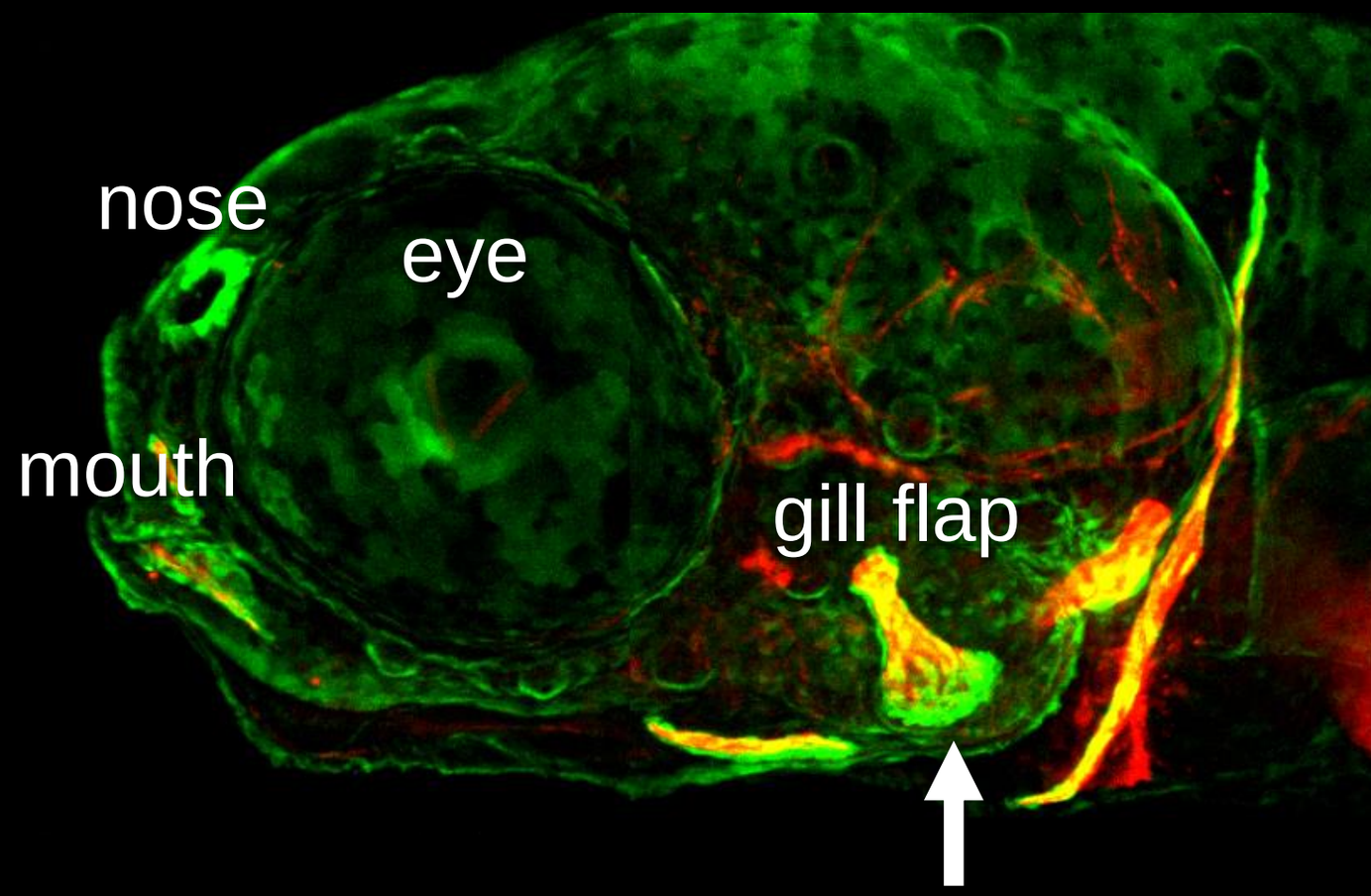
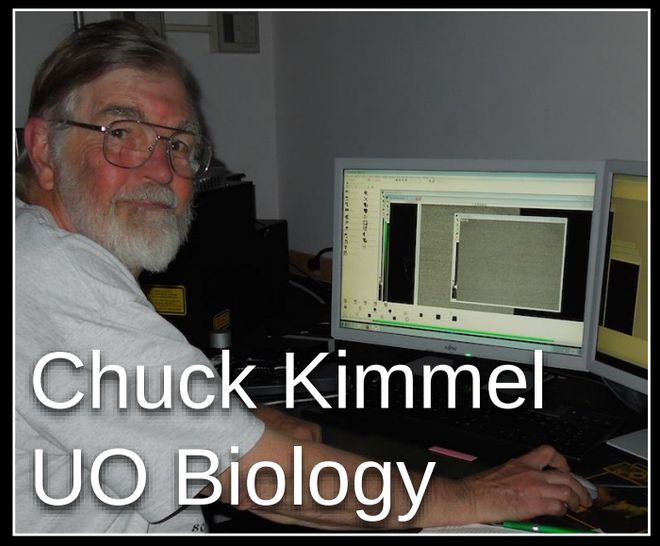


Zebrafish has stereotypic development

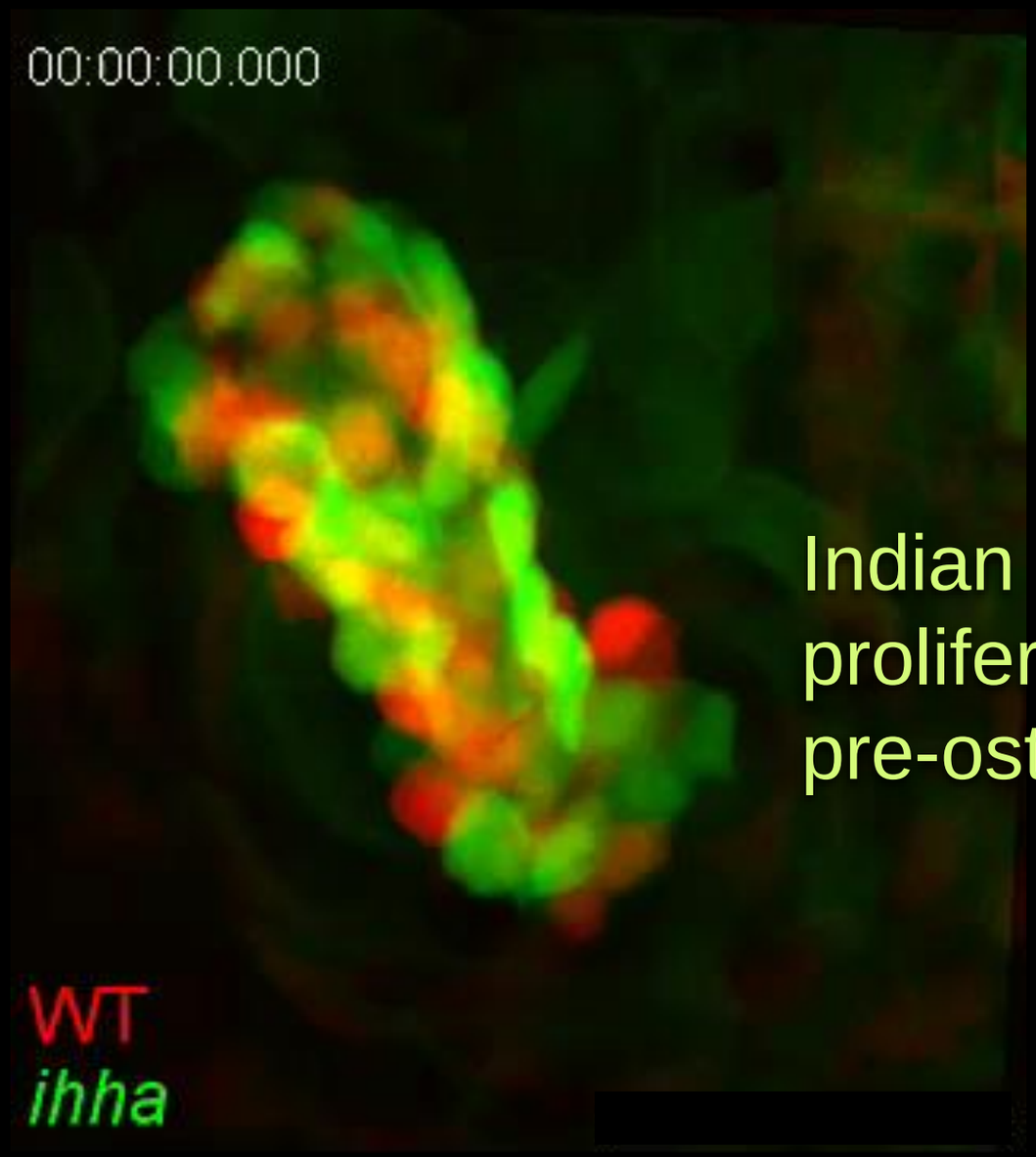


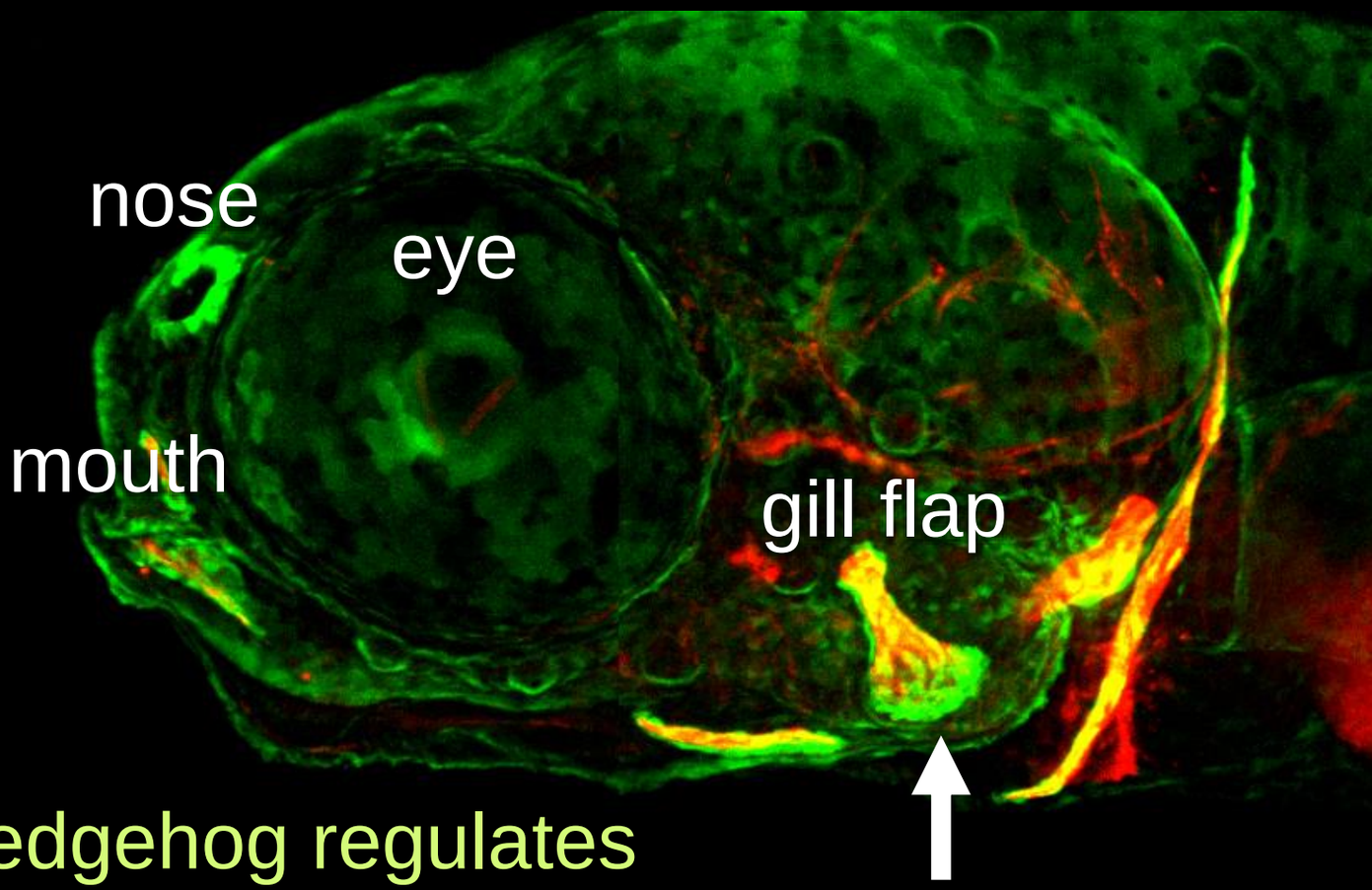


NIH National Institute of Dental and Craniofacial Research



Stereotypic development





Indian hedgehog regulates
proliferation of distal margin
pre-osteoblasts

NIH National Institute of Dental and Craniofacial Research

- What makes zebrafish a good biomedical model?

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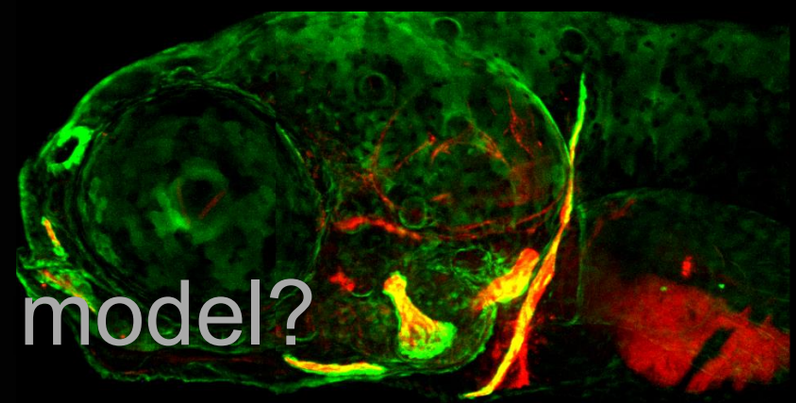
Zebrafish International Resource Center

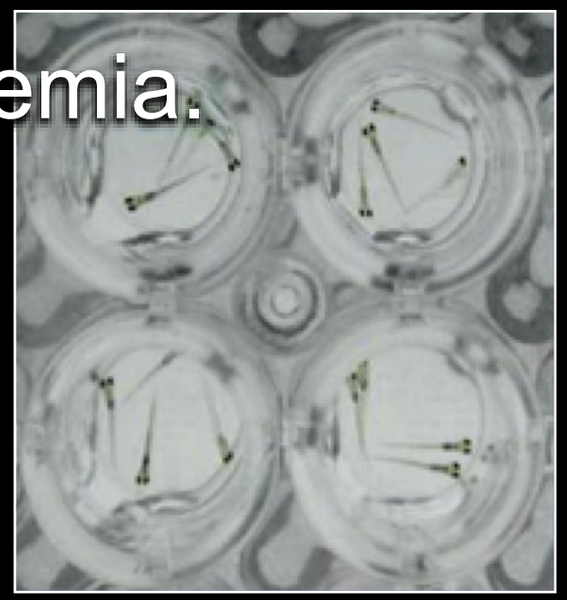
ZFIN

Zebrafish Information Network

A zebrafish model for the FA/BRCA pathway and connecting fish medical models to human health



- What makes zebrafish a good biomedical model?
- A small molecule screen to rescue Fanconi anemia.



- Connecting fish genomes to human biology.

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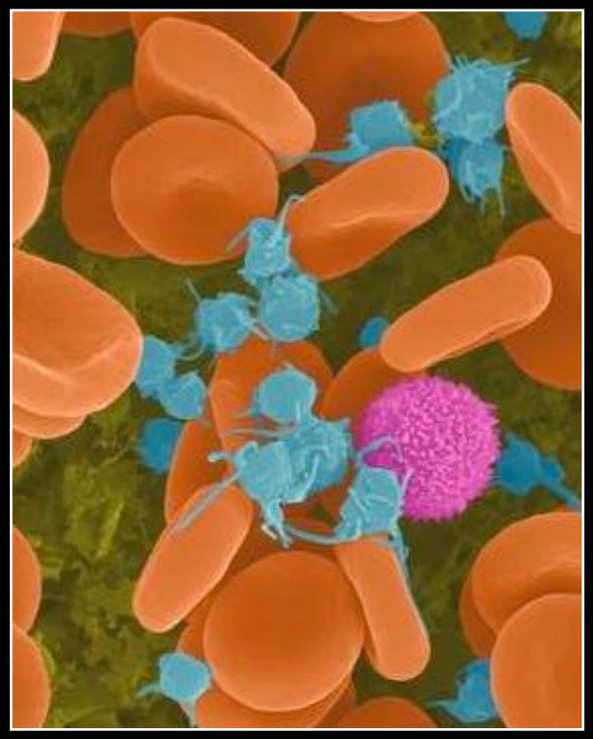
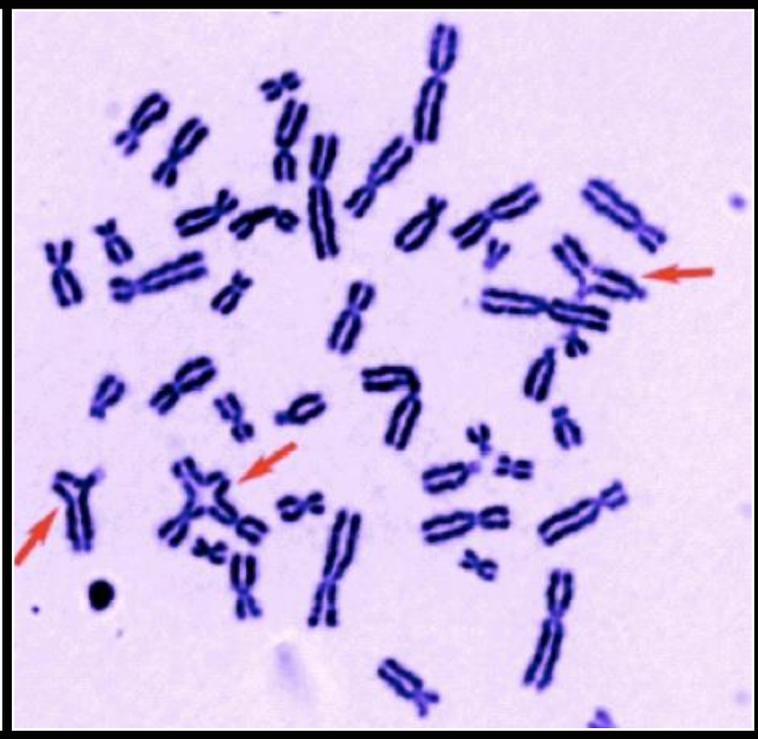
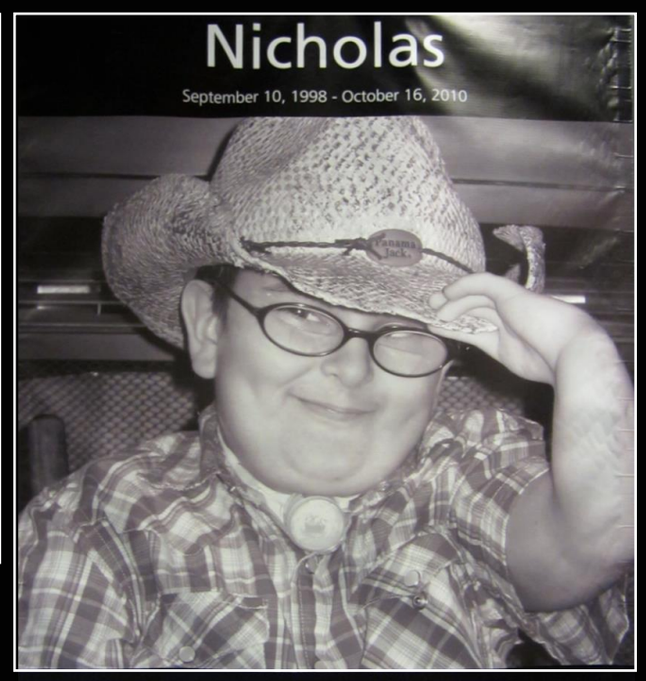
Fanconi Anemia

- Clinical features
  - Most common inherited bone marrow failure disease
    -red blood cells
    -white blood cells
    -platelets
- Thumb and radius





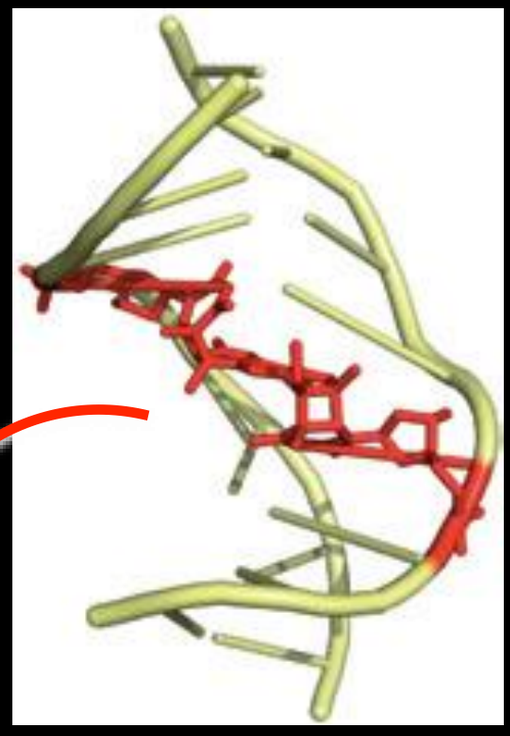
- Induced inter-strand DNA crosslinks
- Rare (1 per 350,000)
- acute myelogenous leukemia up 8,000X

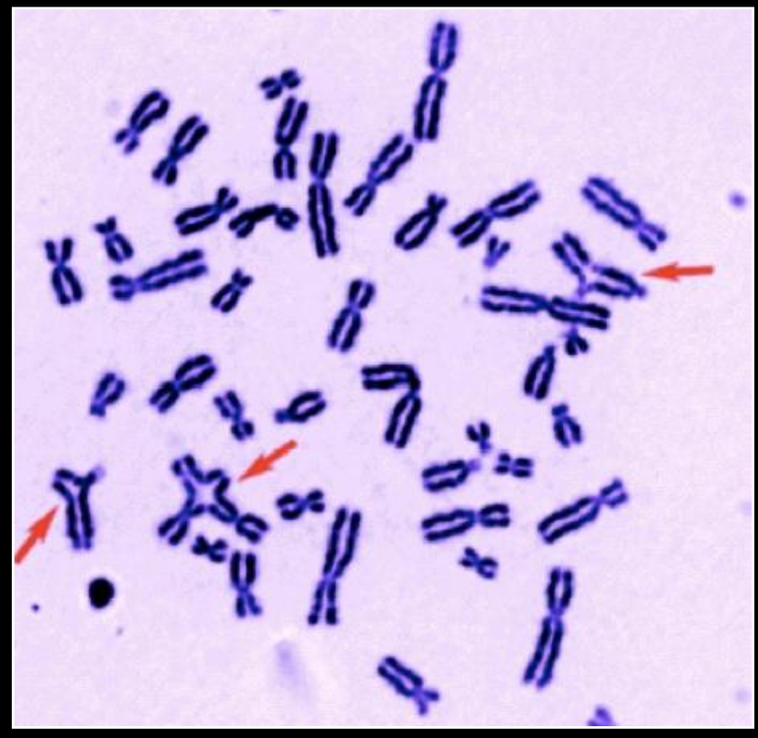


- Hypogonadism
- Small head & eyes

FA proteins help repair DNA damage.

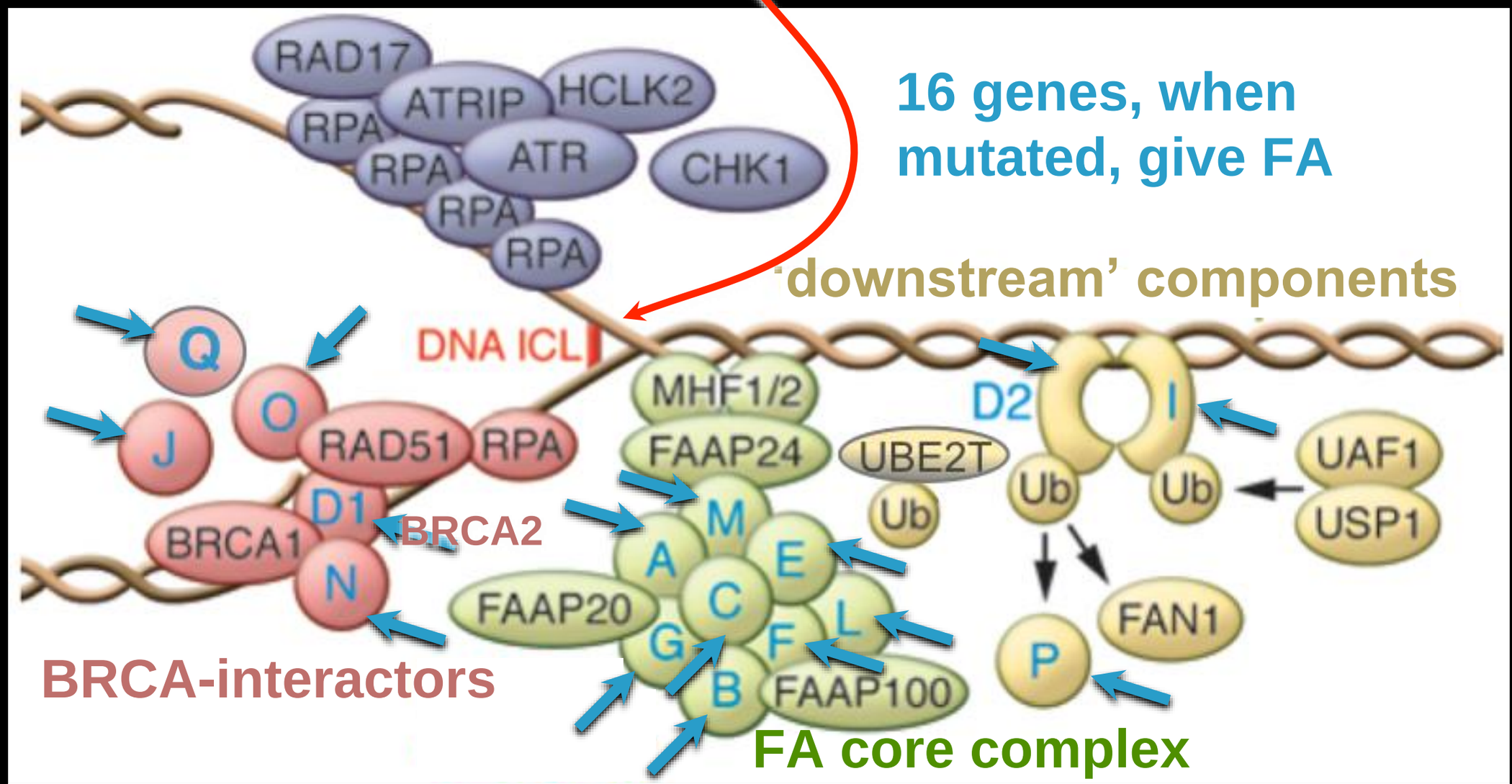
Does the molecular genetics suggest therapies?





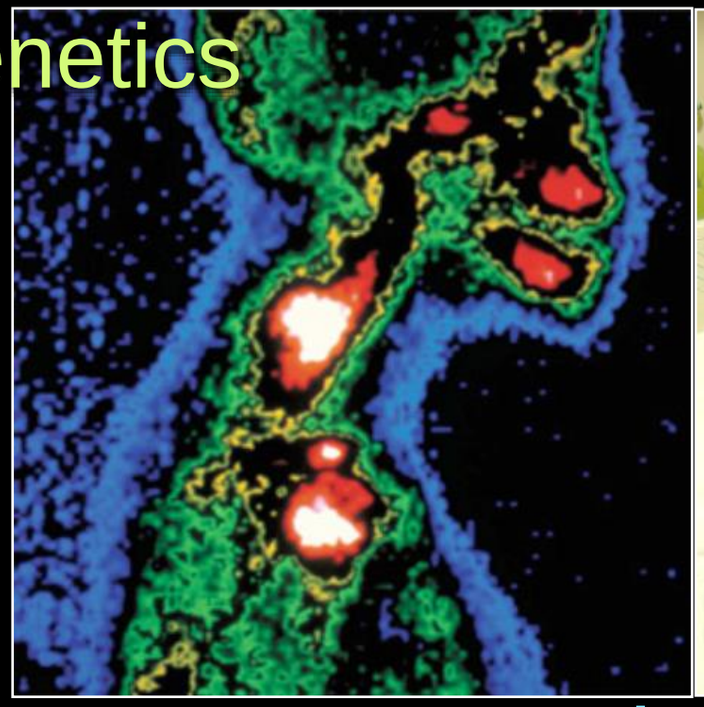
Induced interstrand DNA crosslinks

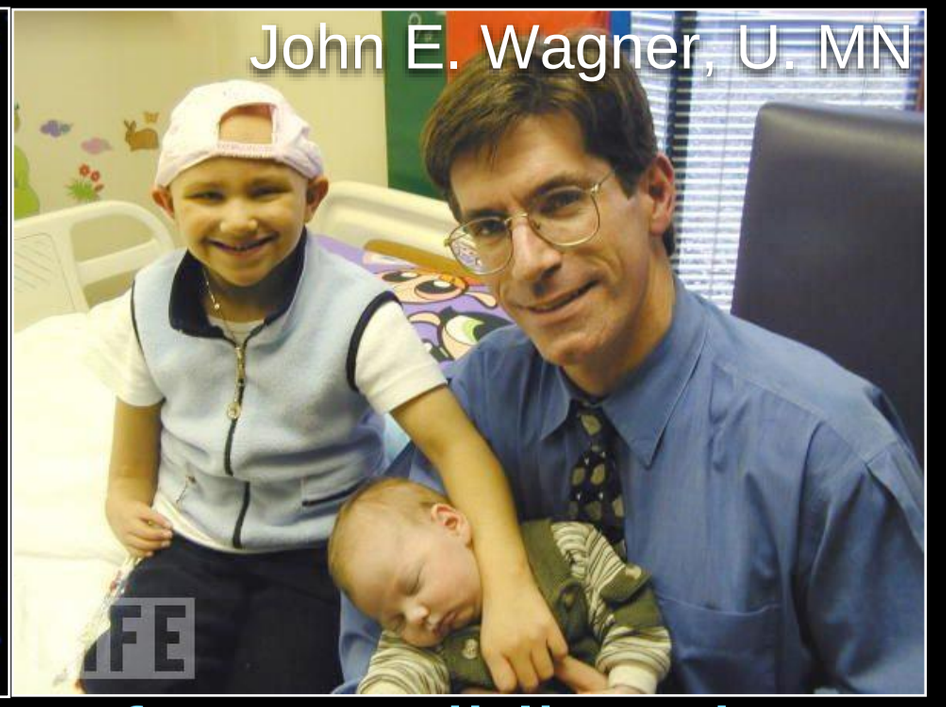
16 genes, when mutated, give FA



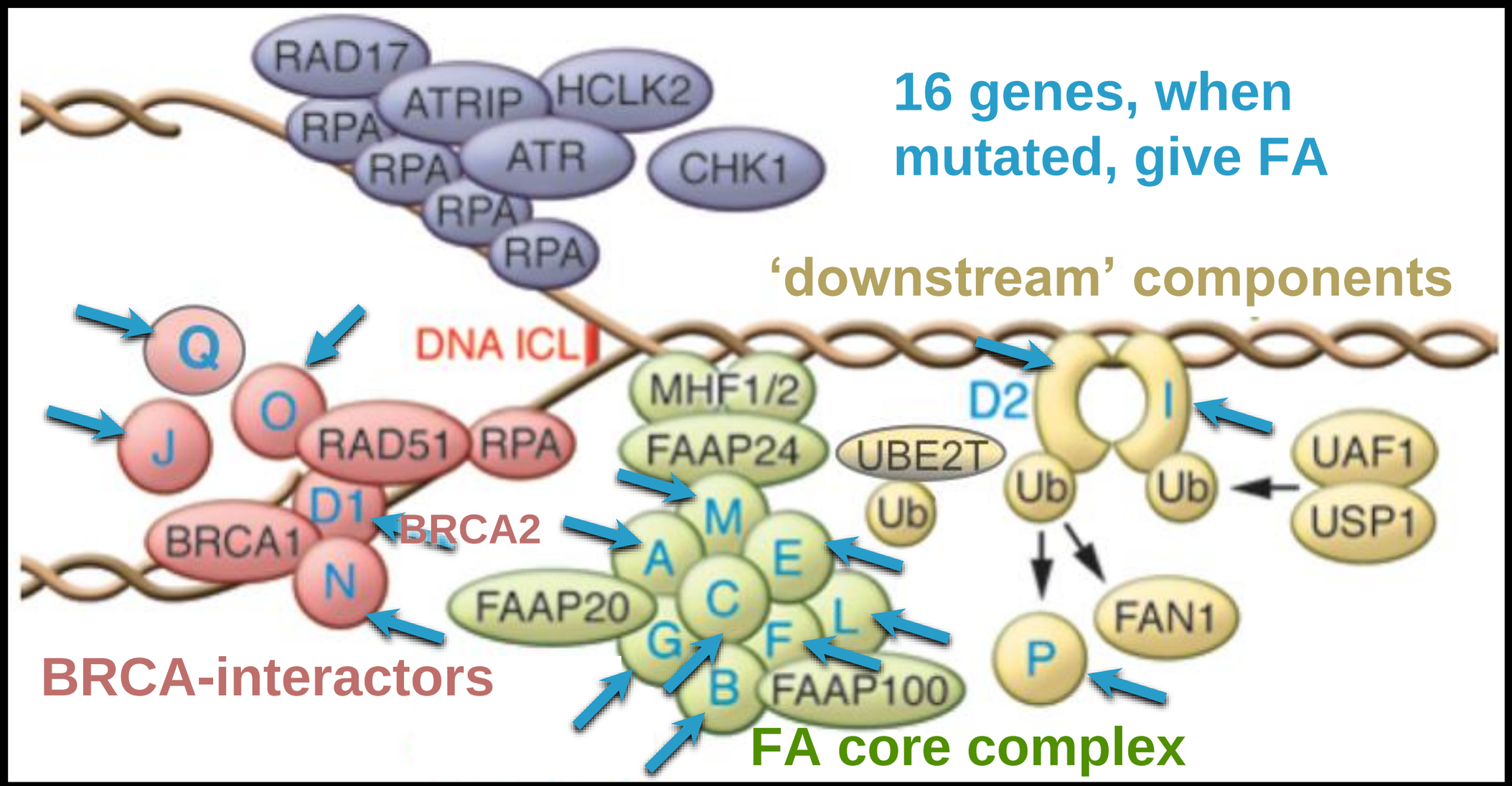
Does the molecular genetics suggest therapies?

- squamous cell carcinoma of head and neck up 500X





Therapy: bone marrow transplant from a sibling donor



http://www.enttoday.org/details/article/1006495/Treatment_Options_Study_reports_neck_dissection_effective_in_patients_with_resid.html

Does the molecular genetics suggest therapies?

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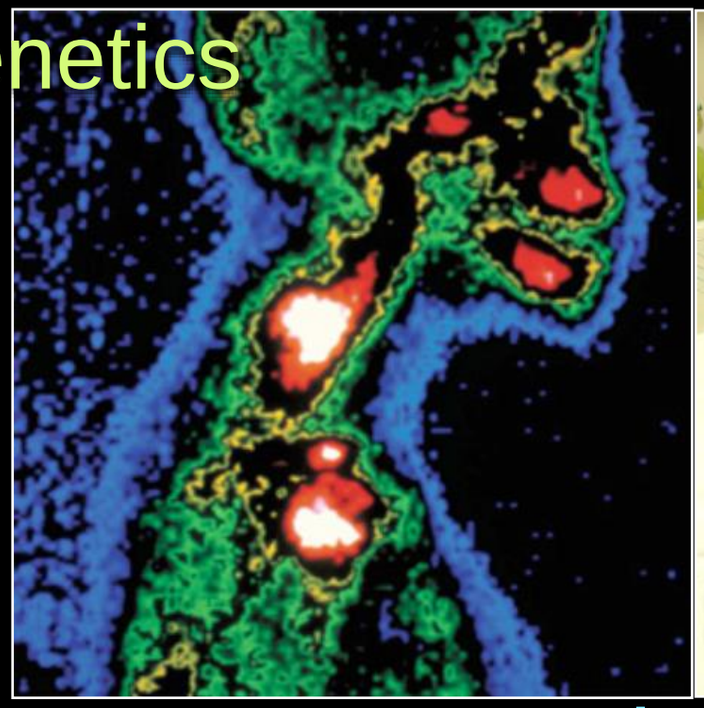

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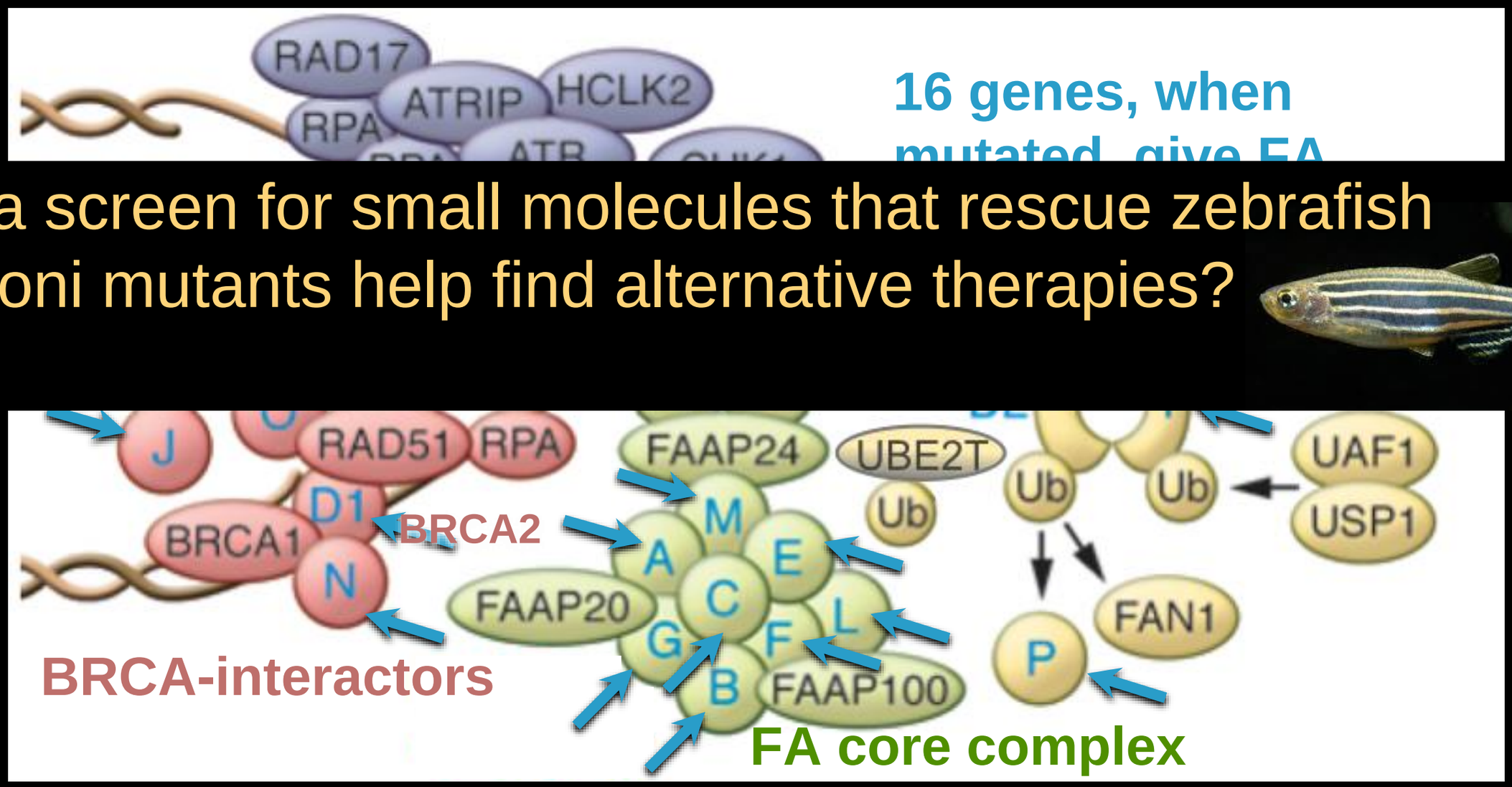
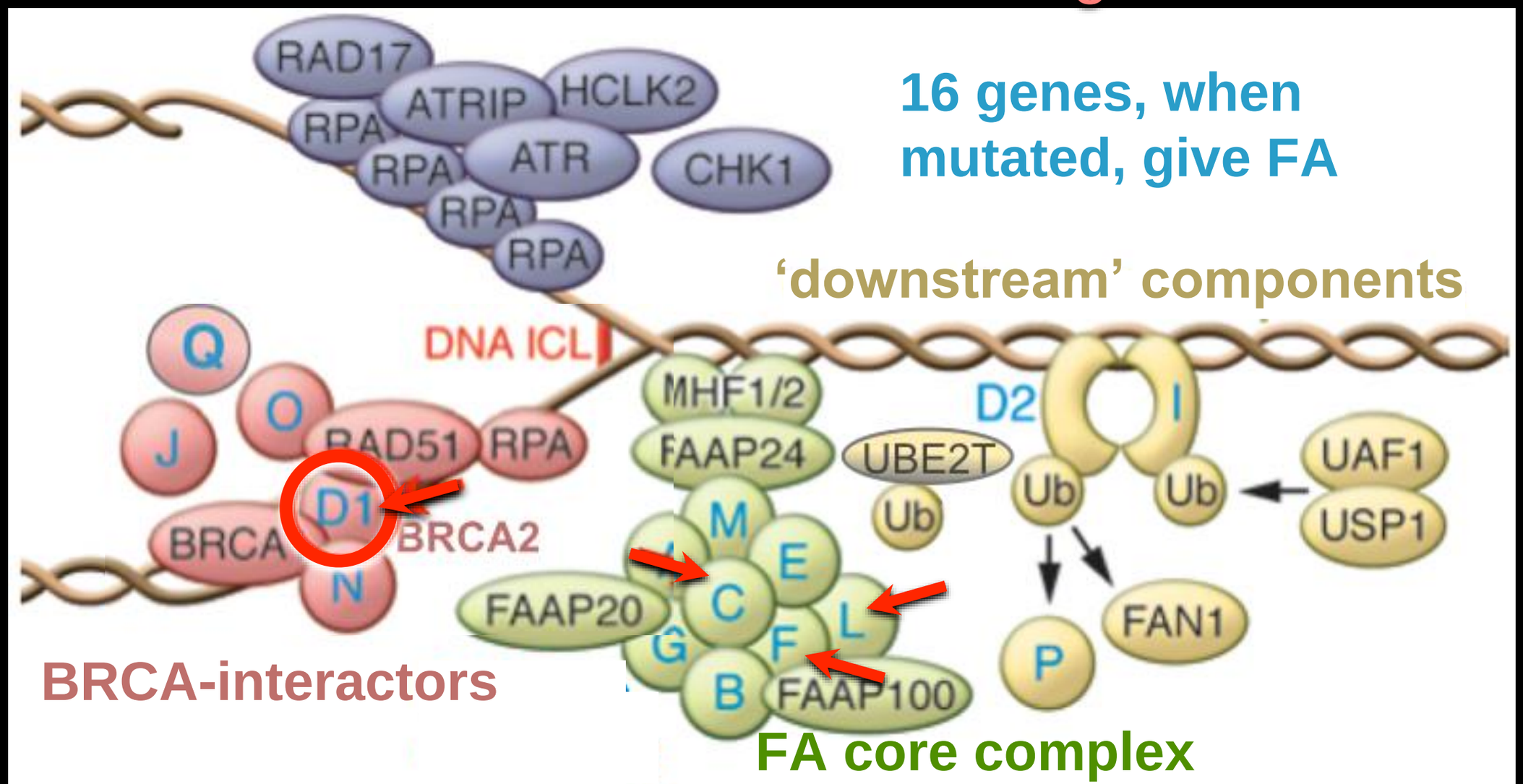


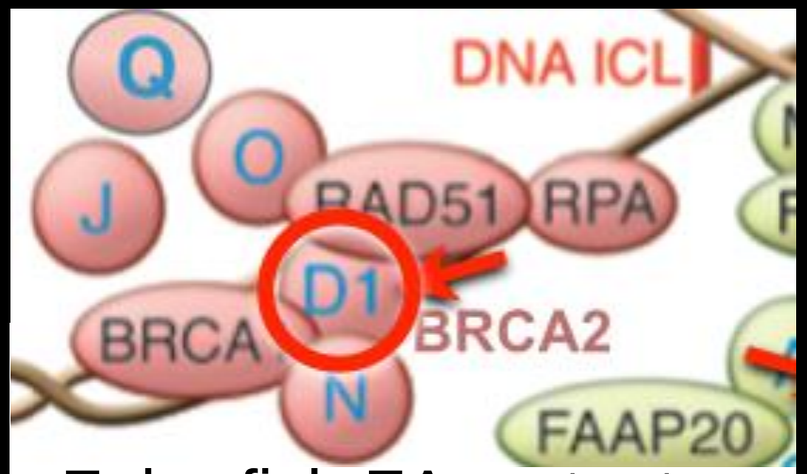
16 genes, when mutated, give FA

Can a screen for small molecules that rescue zebrafish Fanconi mutants help find alternative therapies?

Can a screen for small molecules that rescue zebrafish Fanconi mutants help find alternative therapies?

We made mutations in four genes





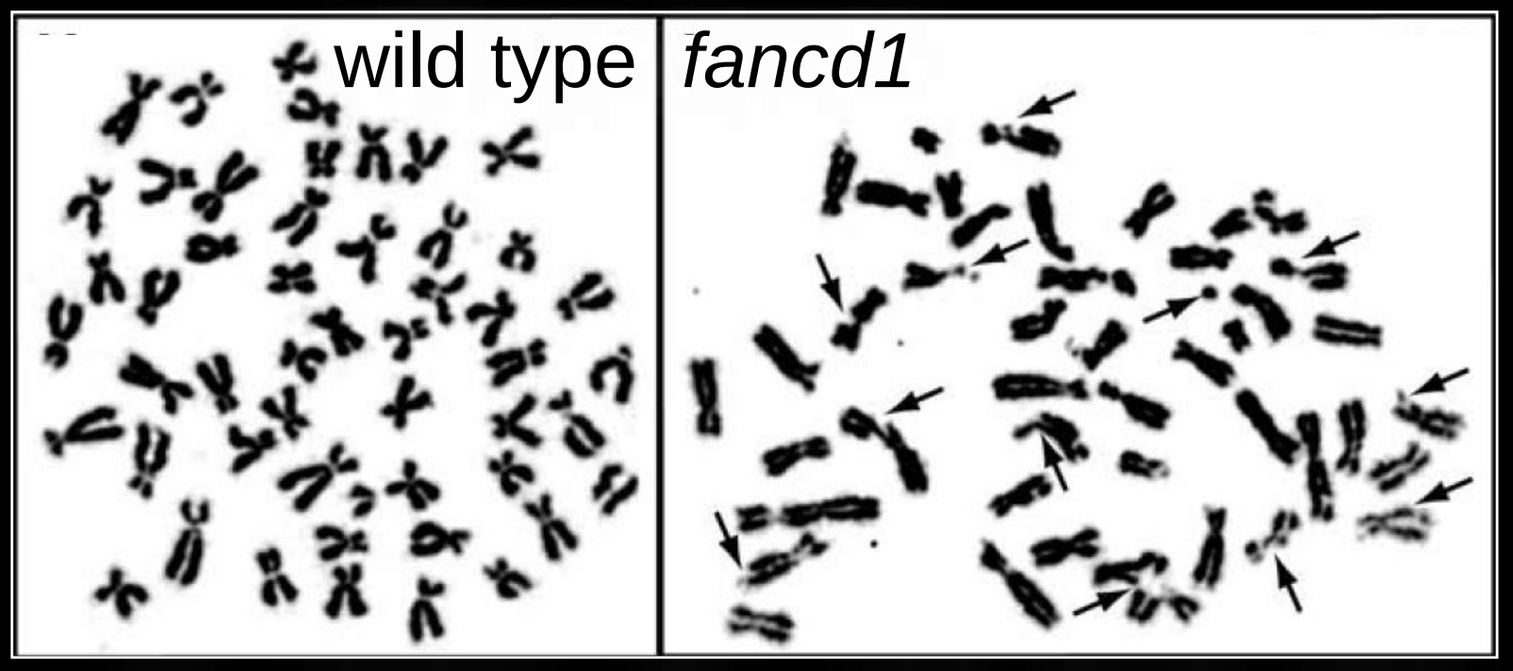
Zebrafish FA mutant

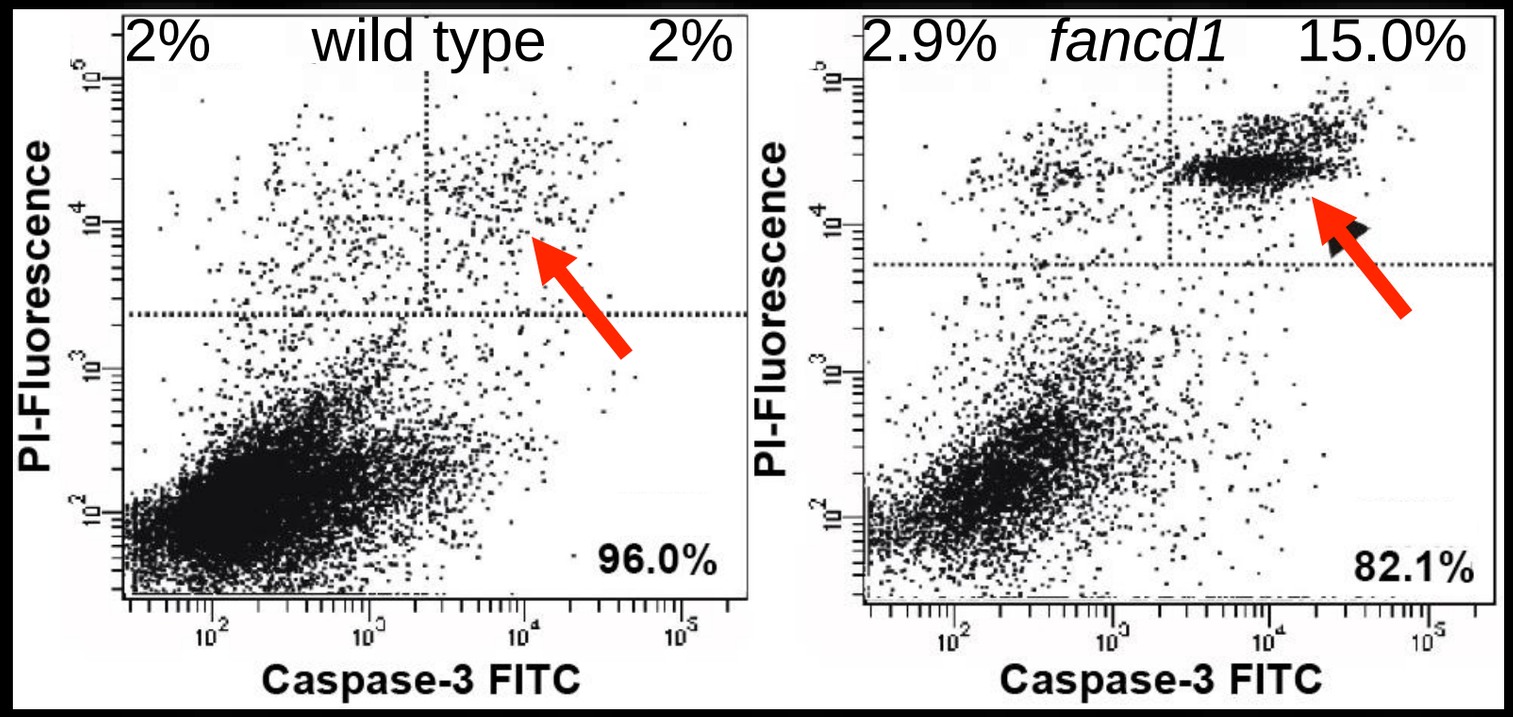
- genome instability
- embryonic cell apoptosis

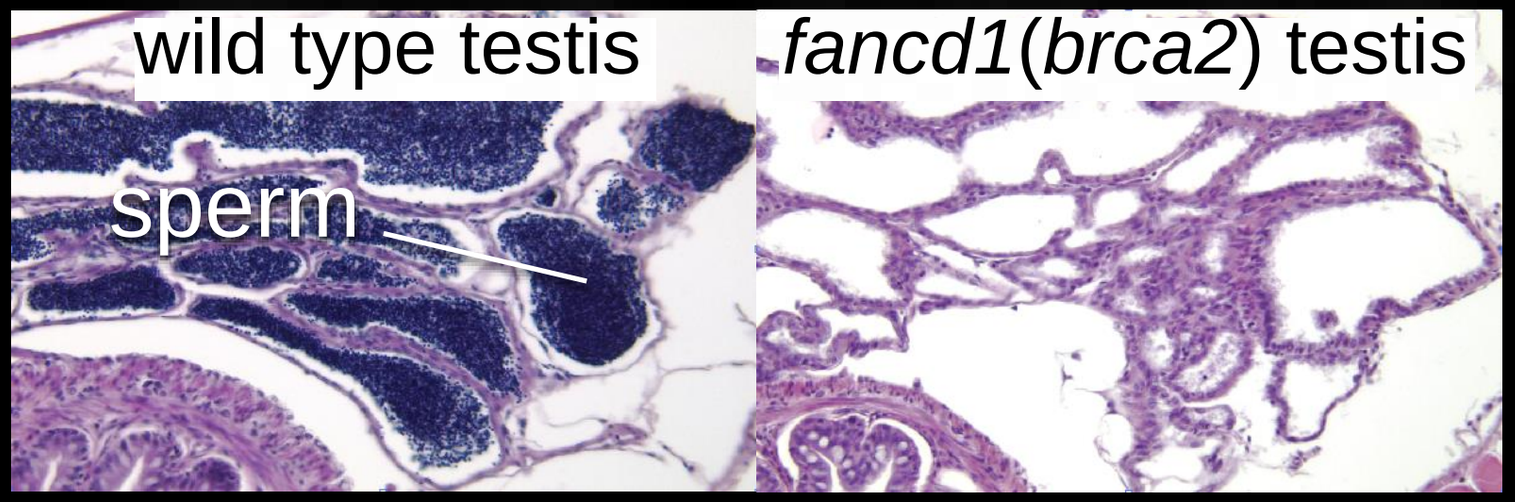
Zebrafish FA mutants have phenotypes like FA patients.

Can a small molecule screen help find alternative therapies?

- germ cell stem cell defect; sterility



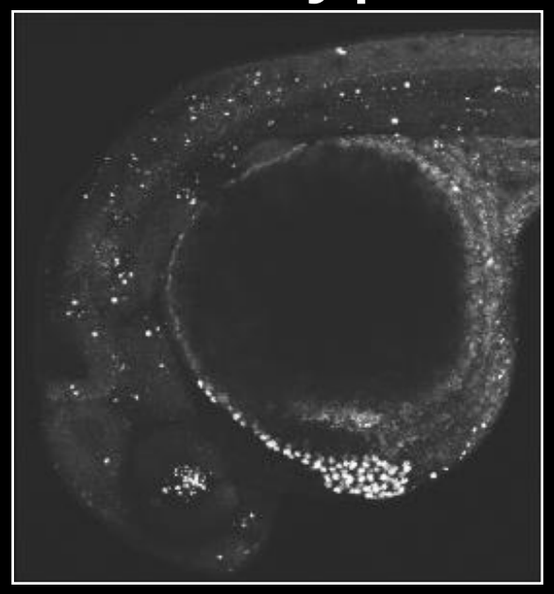


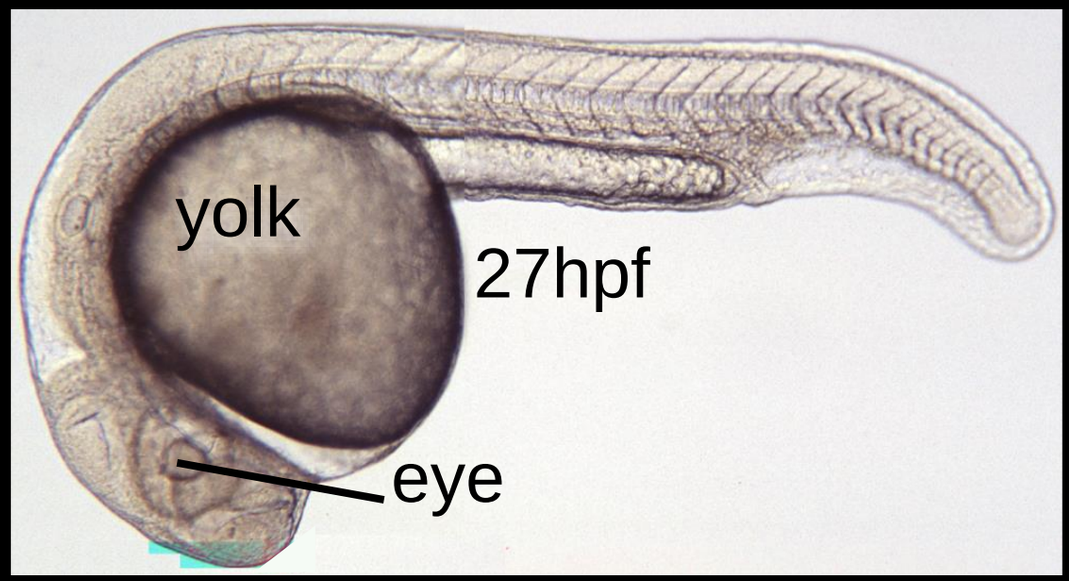


Can a screen for small molecules that rescue zebrafish Fanconi mutants help find alternative therapies?

wild type

control



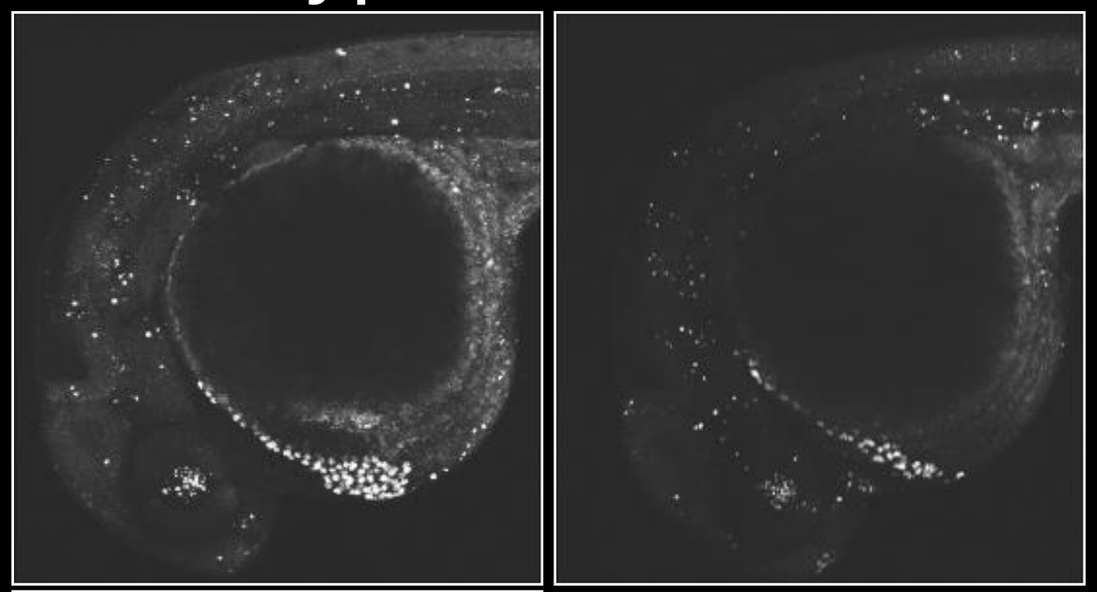


acridine orange,
stains broken DNA

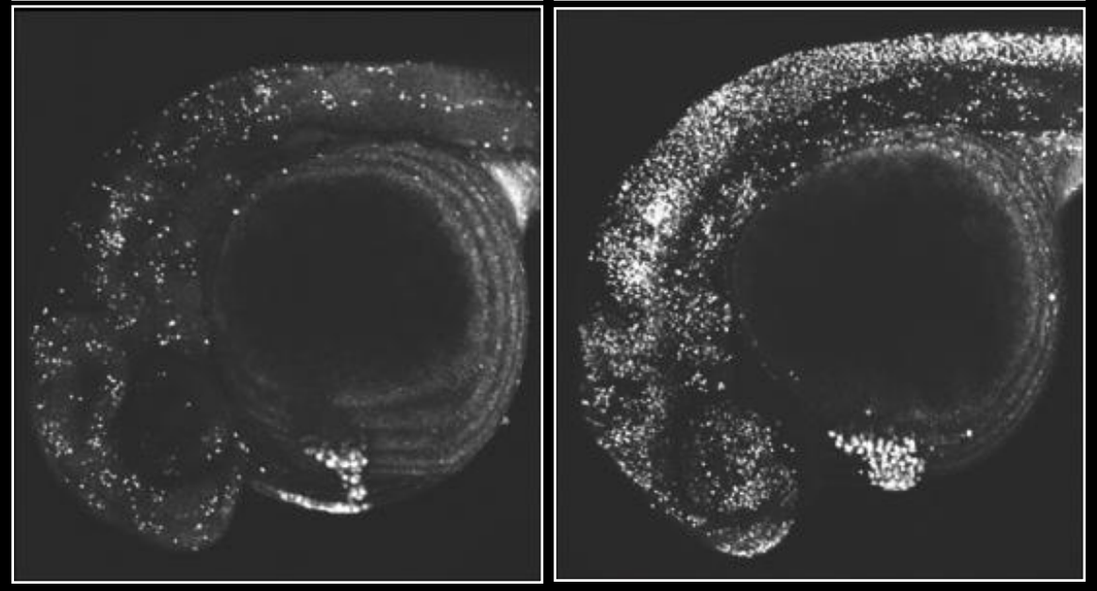
Can a screen for small molecules that rescue zebrafish Fanconi mutants help find alternative therapies?

wild type | *fancd1*$^{-/-}$

control



DEB

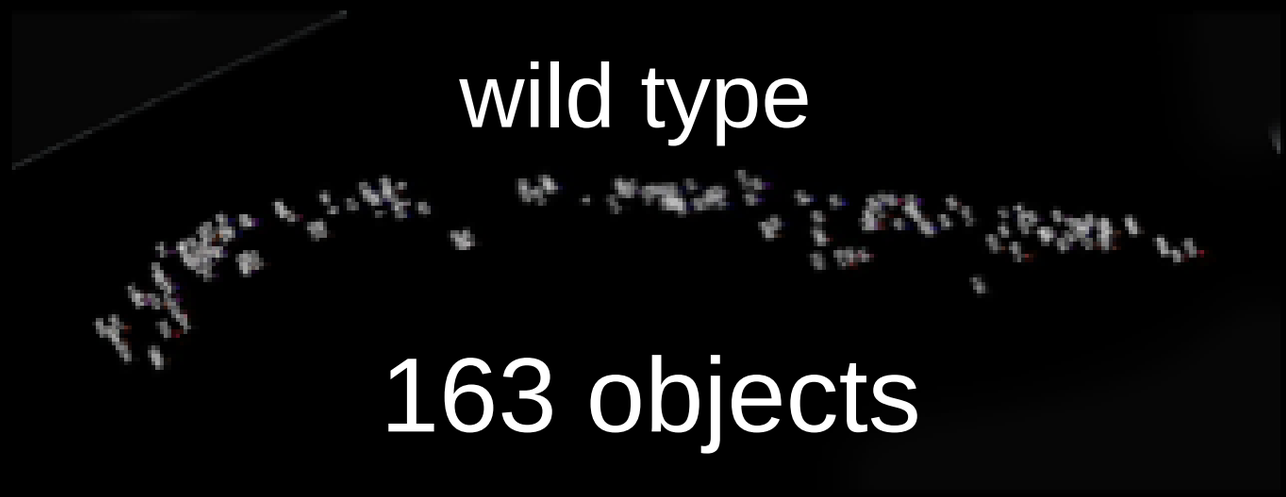


DEB increases acridine orange staining of wild type embryos

fancd1 mutants are more sensitive to DEB

fancd1 mutants stain about the same as wild types

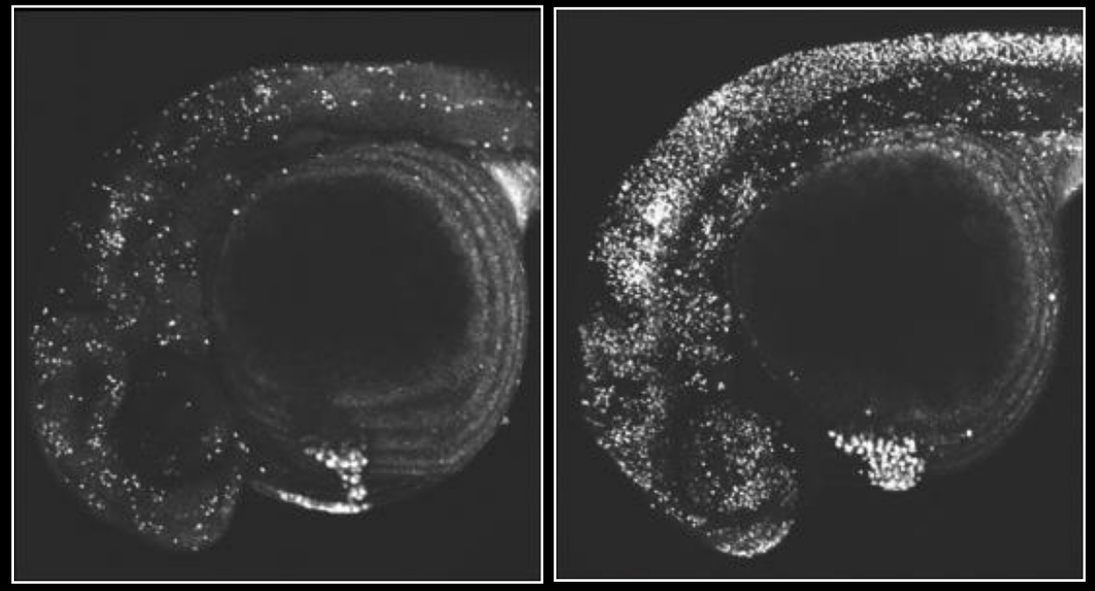
Can a screen for small molecules that rescue zebrafish Fanconi mutants help find alternative therapies?

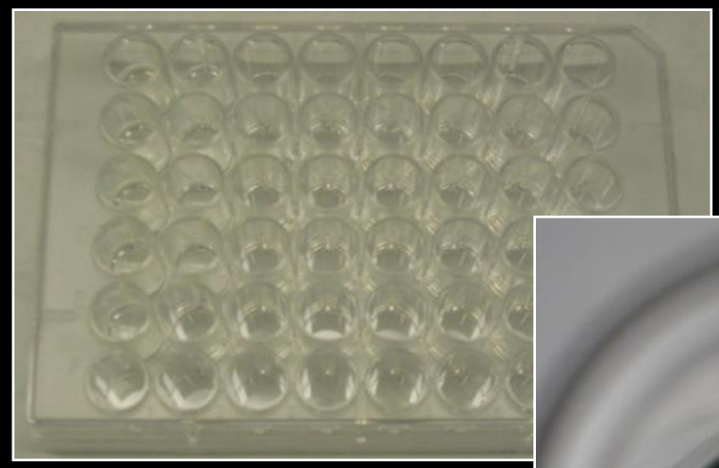


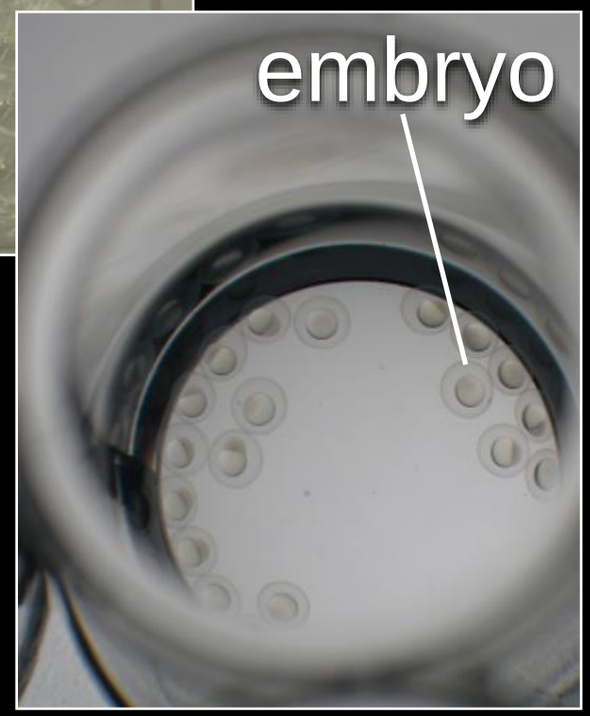
ImageJ
Image Processing and Analysis in Java

wild type *fancd1*$^{-/-}$

DEB







Can we find a compound that makes *fanc* mutants look like wild types?

Can a screen for small molecules that rescue zebrafish Fanconi mutants help find alternative therapies?

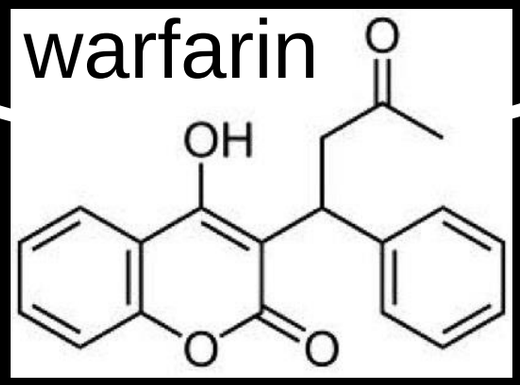
wild type

fancd1$^{-/-}$

163 objects

920 objects

warfarin



731 compounds used in clinical trials

How does warfarin rescue FA zebrafish?

How does warfarin rescue FA zebrafish?

warfarin

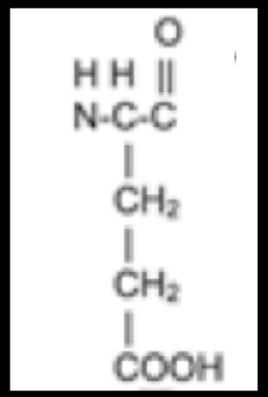
morpholino antisense oligonucleotide

vitamin-K epoxide reductase complex-1

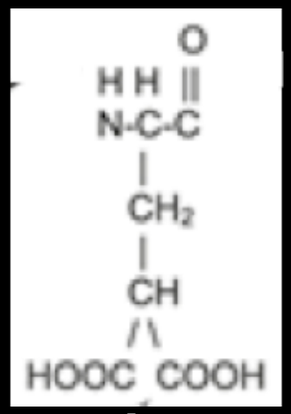
warfarin + exogenous vit-K

reduced vit-K

oxidized vit-K



carboxylase + CO_2



pre-thrombin

pro-thrombin

Which of the several vit-K-dependent proteins is the FA-relevant target?

Strong AO staining in *fancd1* mutants

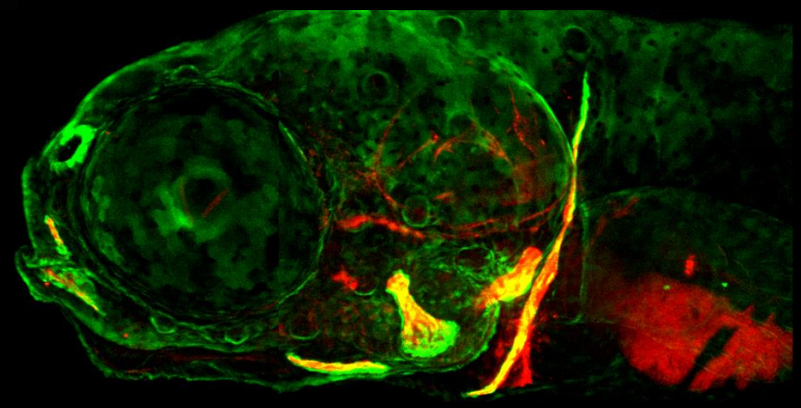
Rescues AO staining in *fancd1* mutants

Warfarin rescue fails with exogenous vit-k

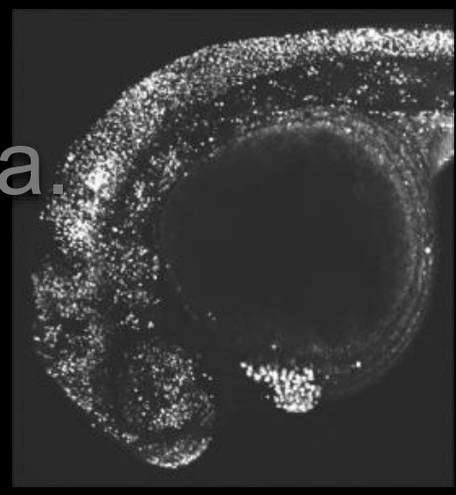
Knockdown of VORC1 rescues *fancd1* mutants

Conclude: warfarin rescues *fancd1* mutants by a vitamin-K dependent mechanism.

A zebrafish model for the FA/BRCA pathway and connecting fish medical models to human health



- What makes zebrafish a good biomedical model?
- A small molecule screen to rescue Fanconi anemia.



- Connecting fish genomes to human biology.

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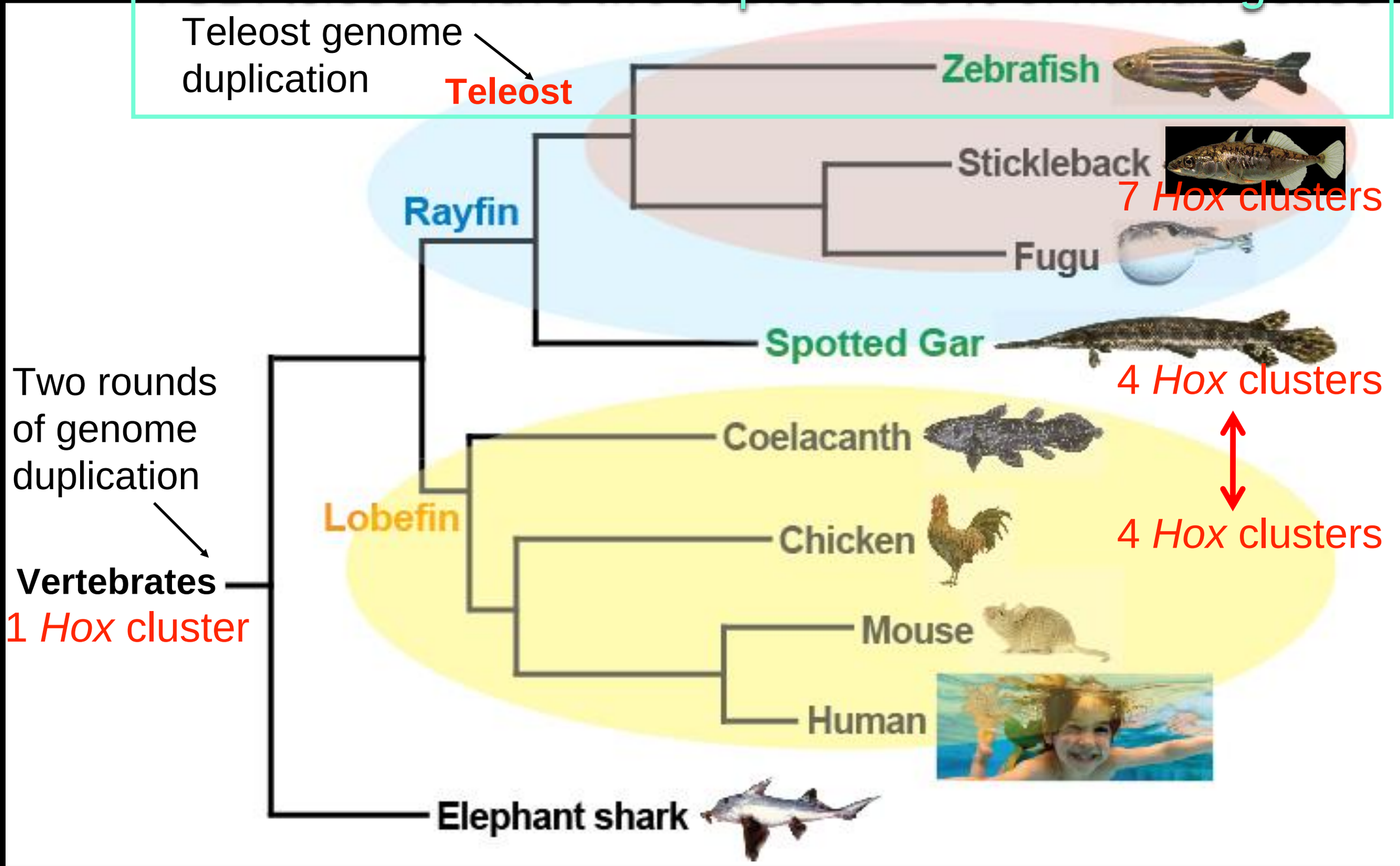
- Connecting the zebrafish genome to human biology.

TGD: teleosts have two copies of 25% of human genes



Gar biology is more similar to teleost biology,
but the gar genome is more similar to the human genome.

Amores 2011 *Genetics*

Gar links human and zebrafish conserved non-coding elements (CNEs)

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Mouse as comparator

Vista plot

CNEs:

- Don’t encode proteins
- Sequence conserved for some other function
- Some or most likely regulatory

Gar links human and zebrafish conserved non-coding elements (CNEs)

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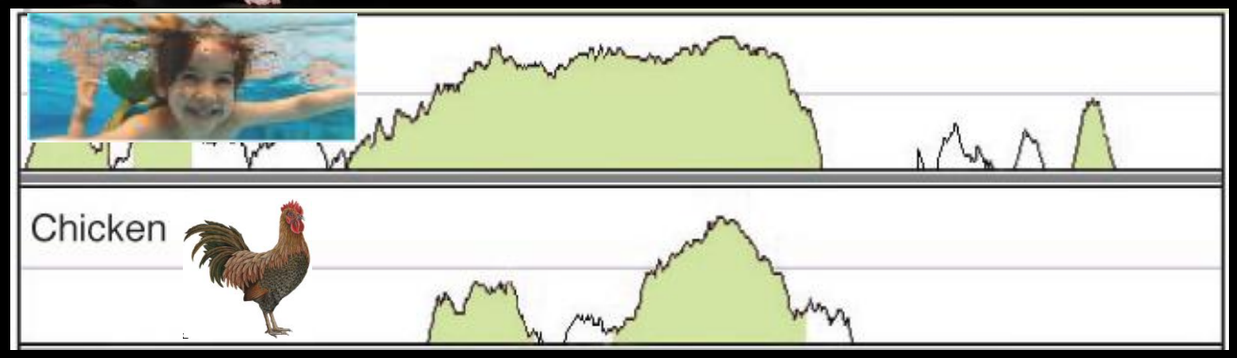
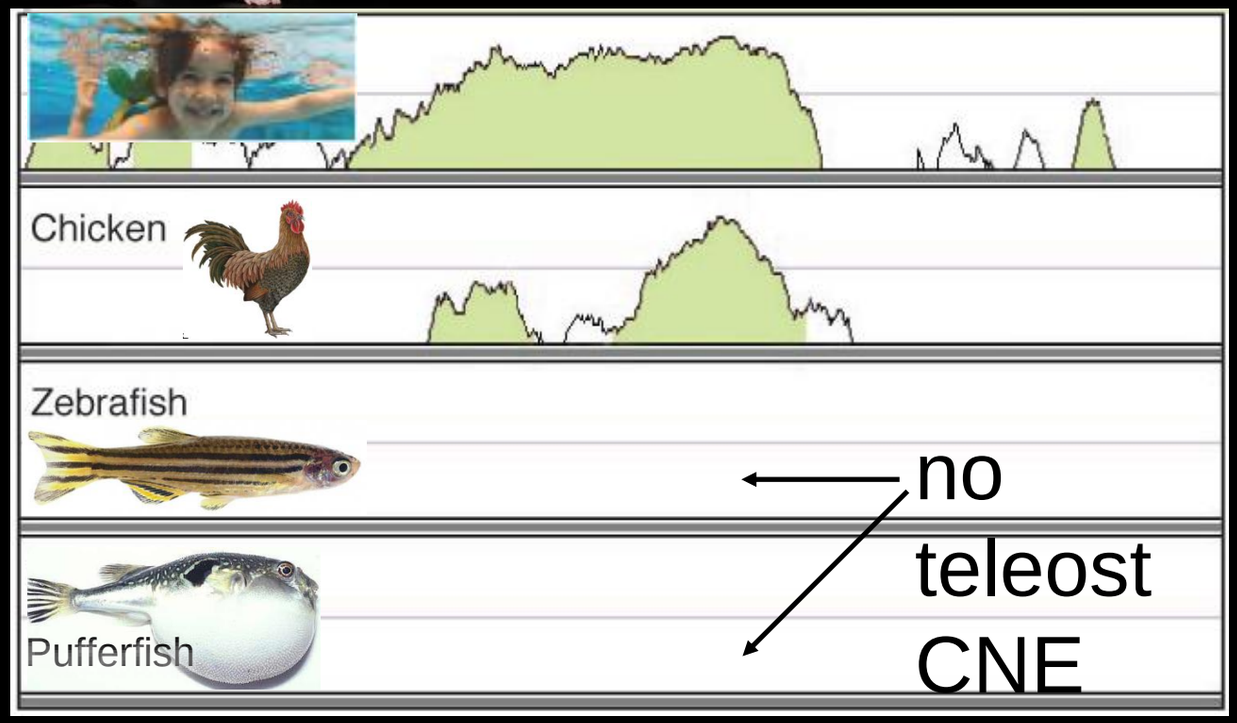
Mouse as comparator



Mouse as comparator

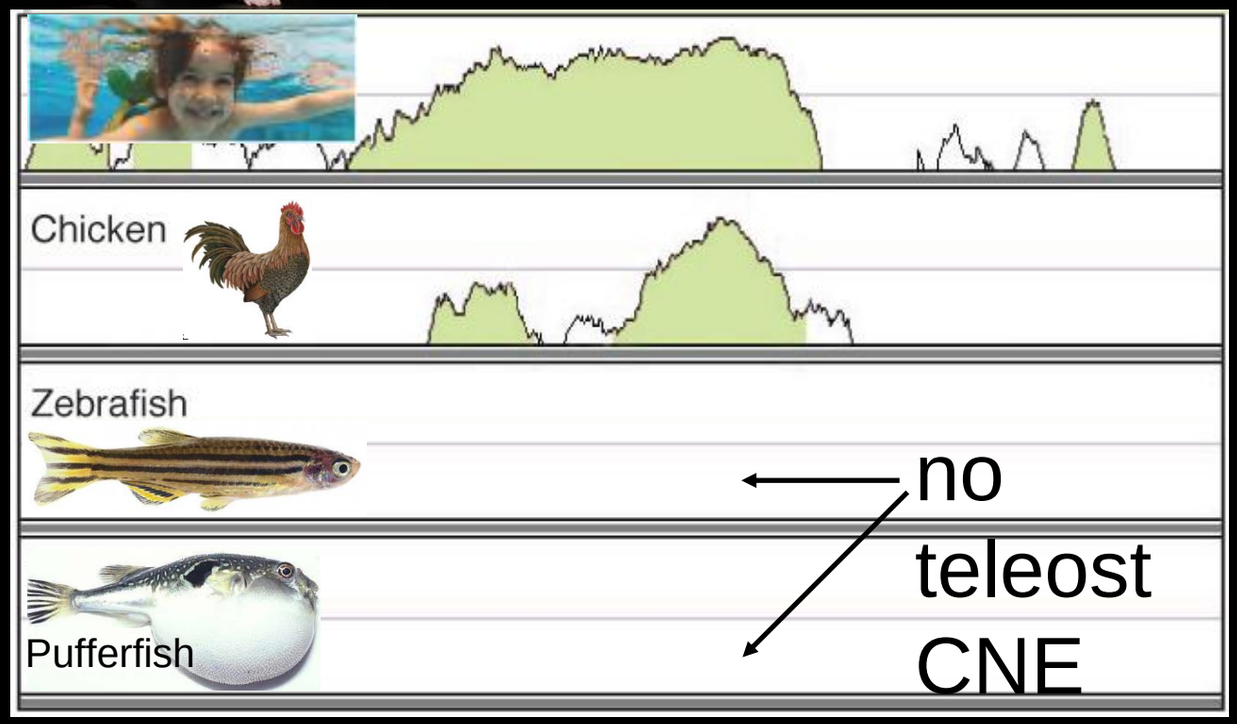


Gar alignments identify CNEs otherwise undetectable.

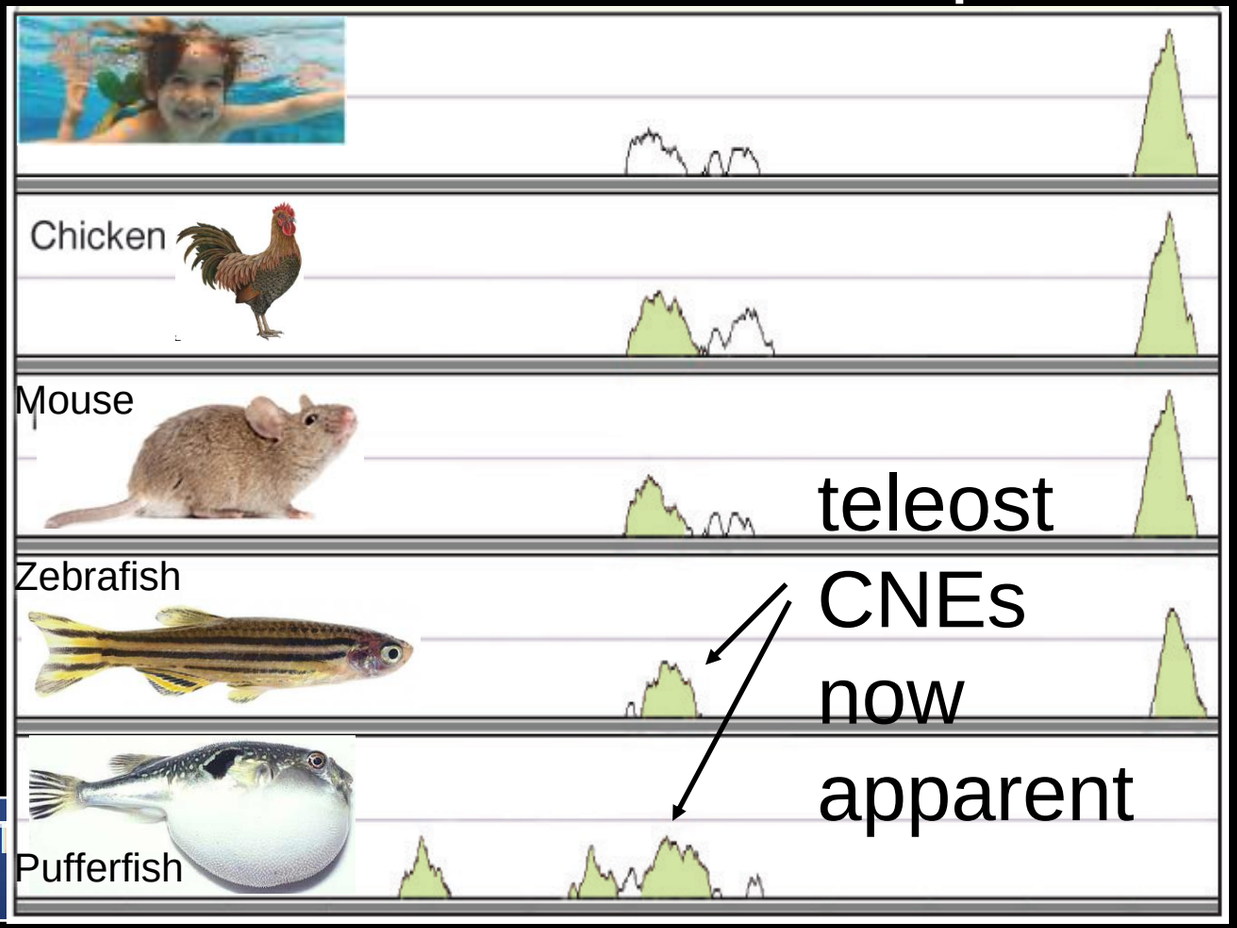
Gar reveals potential regulatory elements teleosts share with human!

We can test them for function

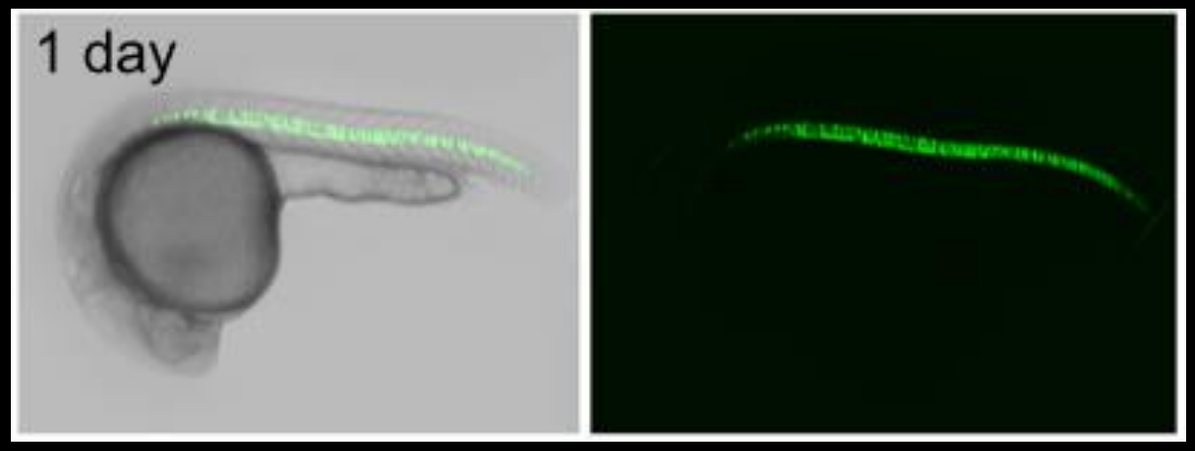
Mouse as comparator

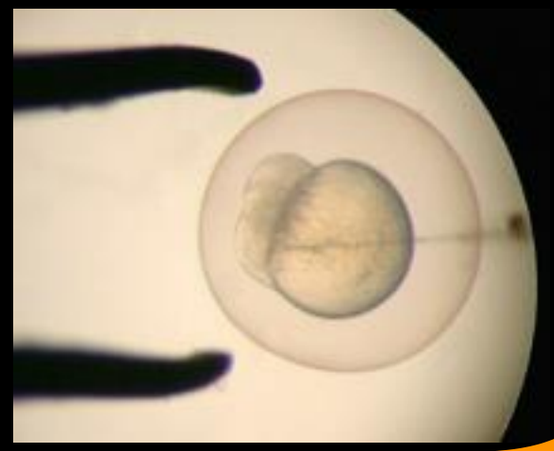


GAR as comparator



We can test them for function





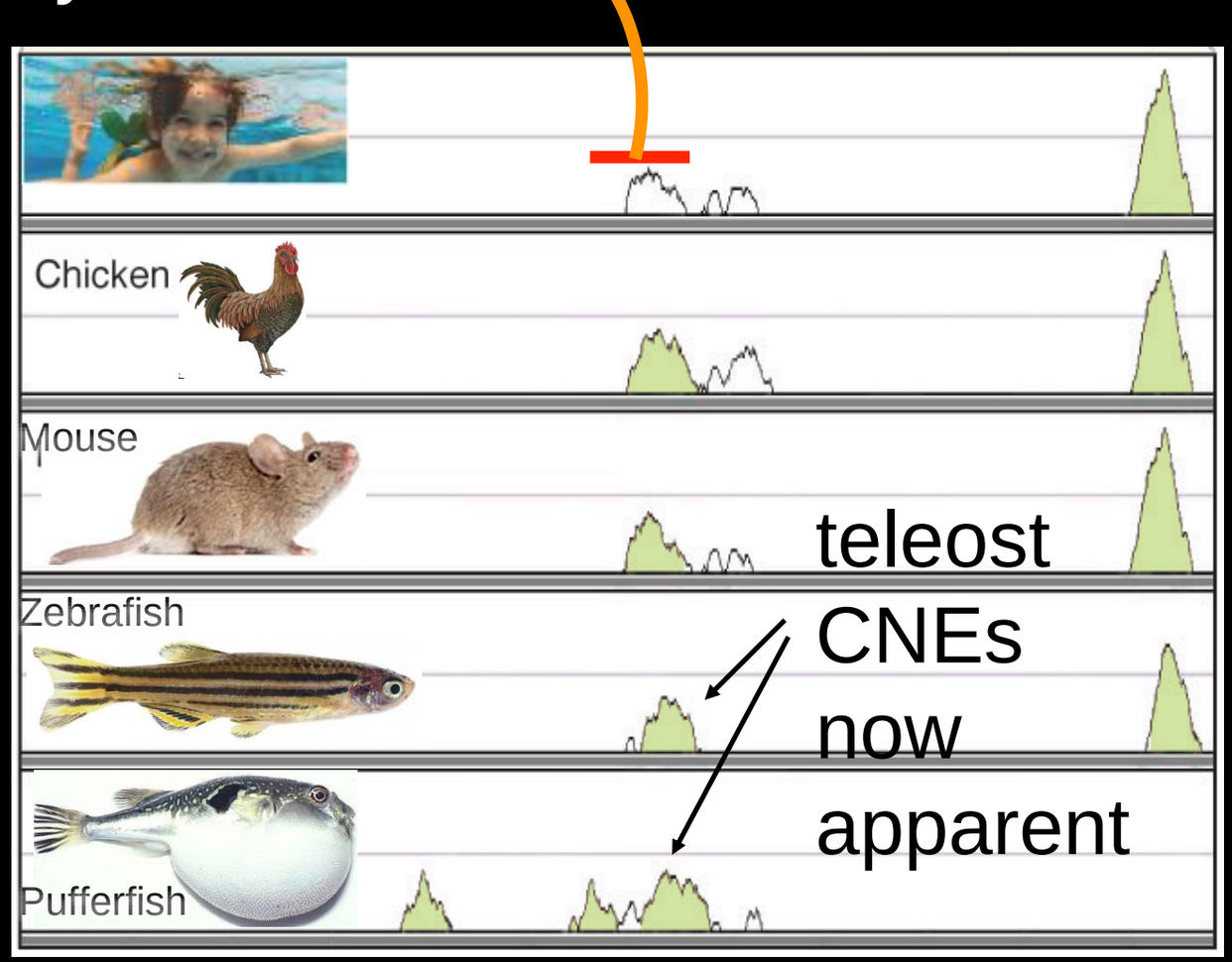
Inject construct

Tol2

GFP

Learn role of CNE by gain of function

Gar reveals hidden shared regulatory elements



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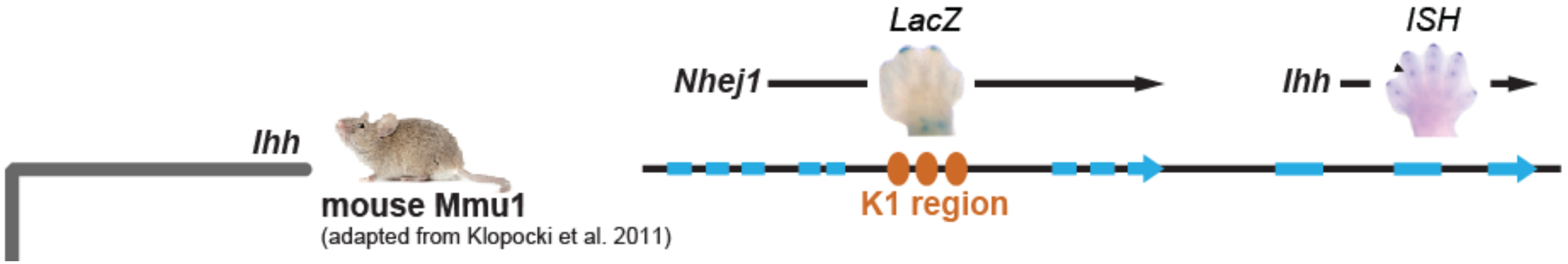
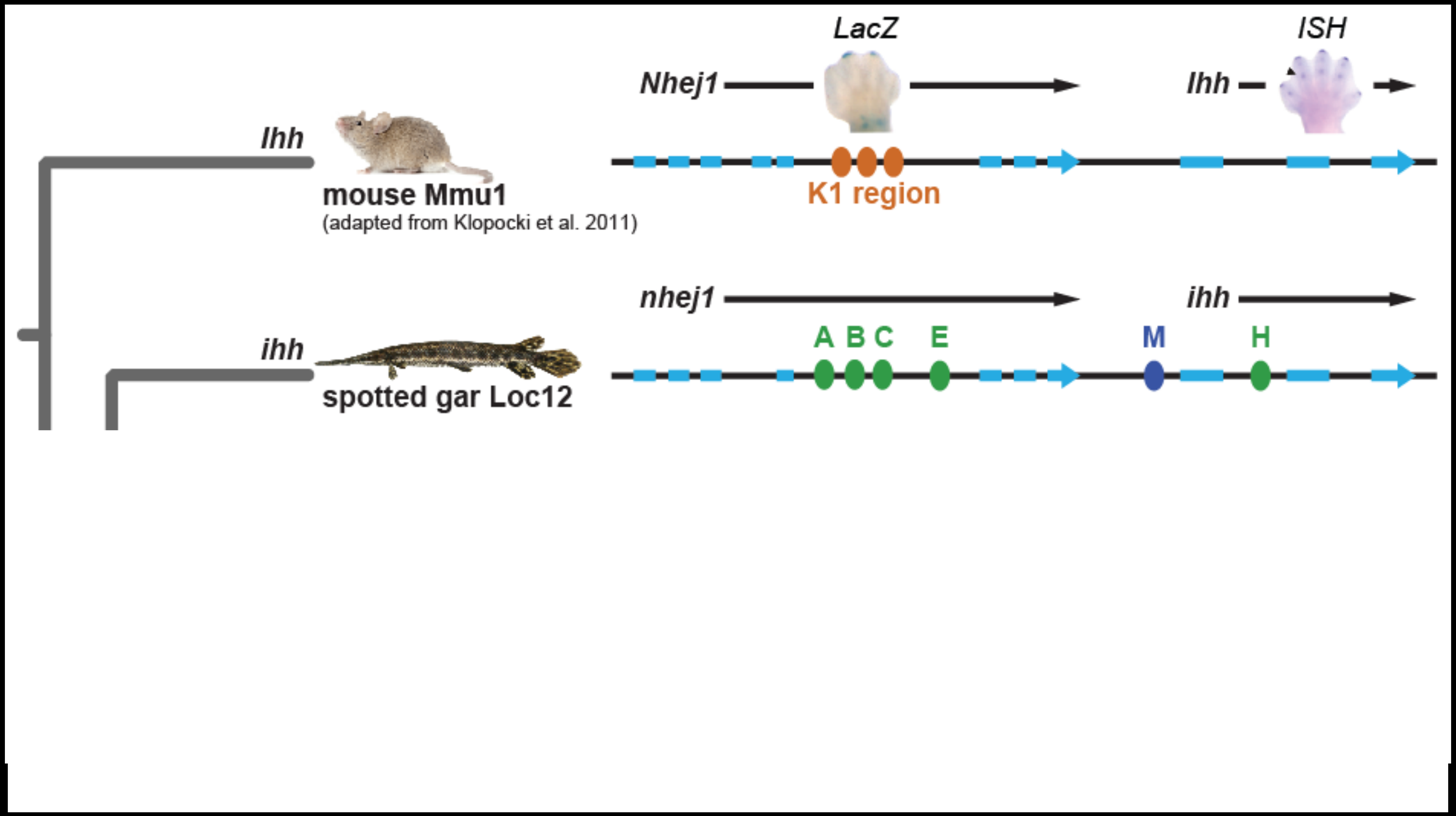
Gar reveals hidden shared regulatory elements



Gar reveals hidden shared regulatory elements



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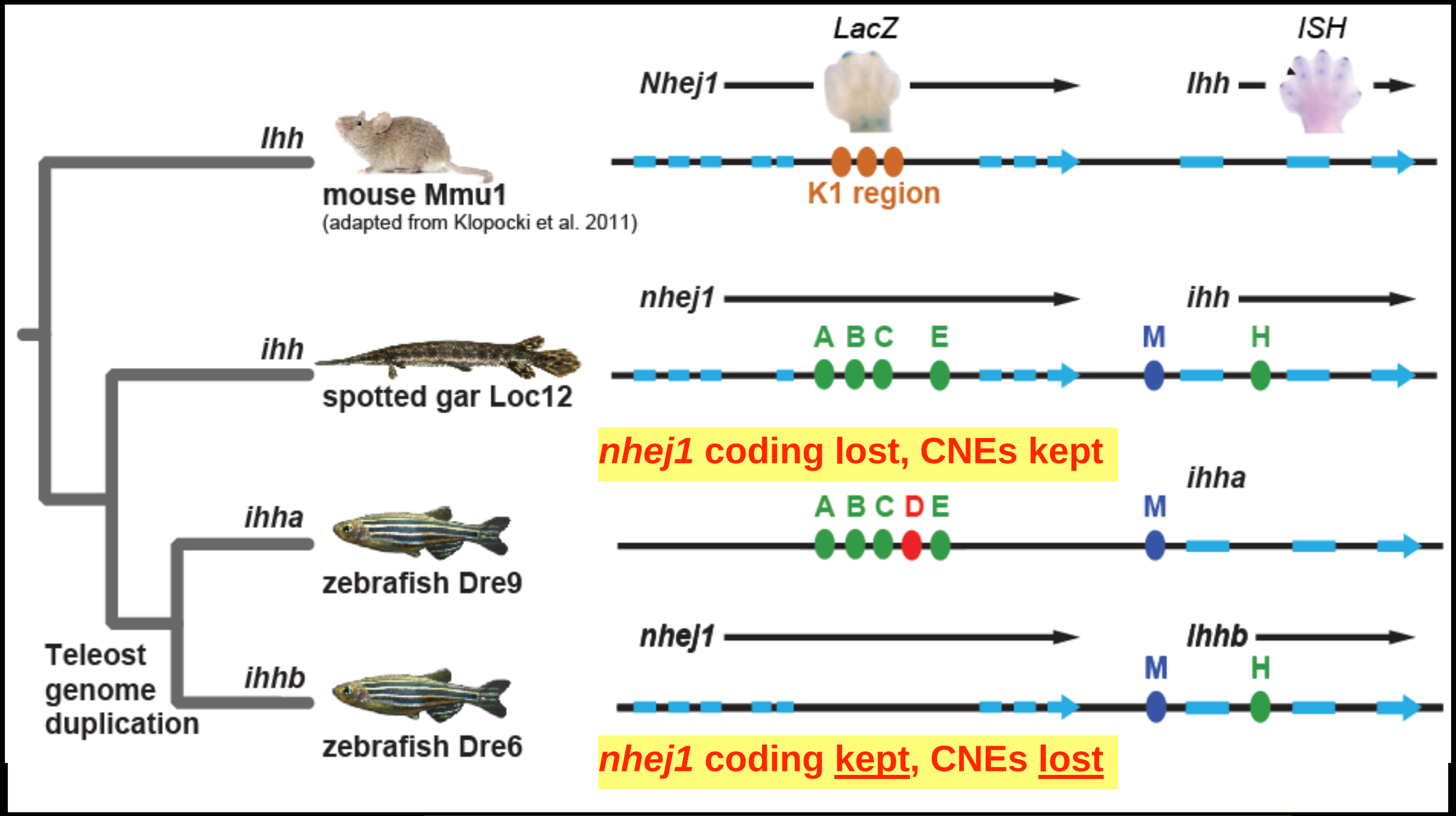
Gar reveals hidden shared regulatory elements



these CNEs aren't regulating *nhej1*

TGD helps assign function to CNEs

Are these CNEs regulatory?

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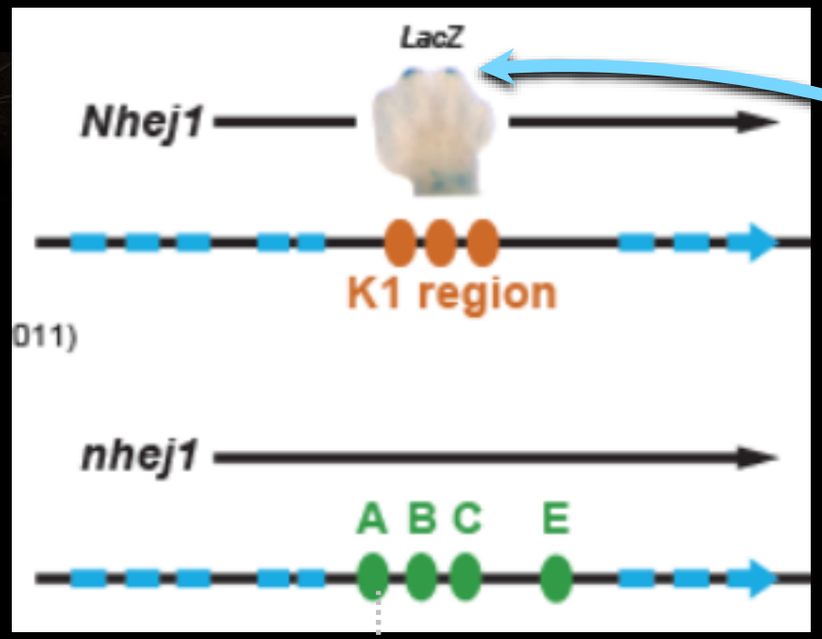
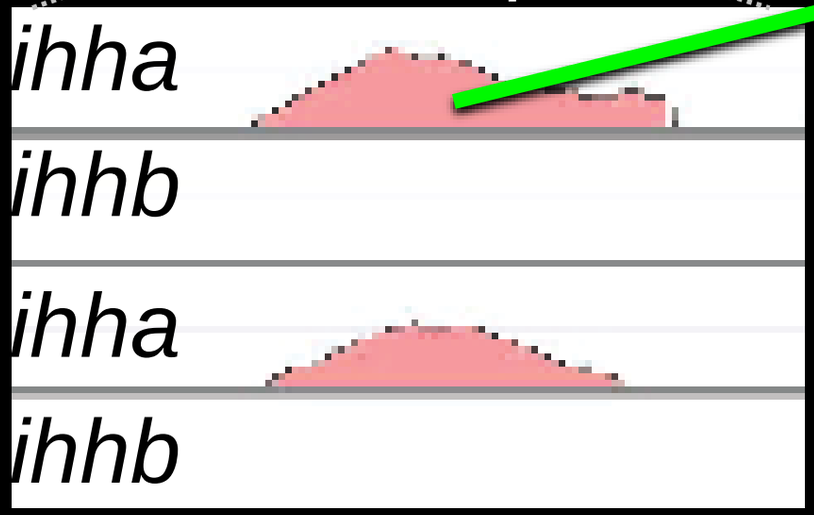
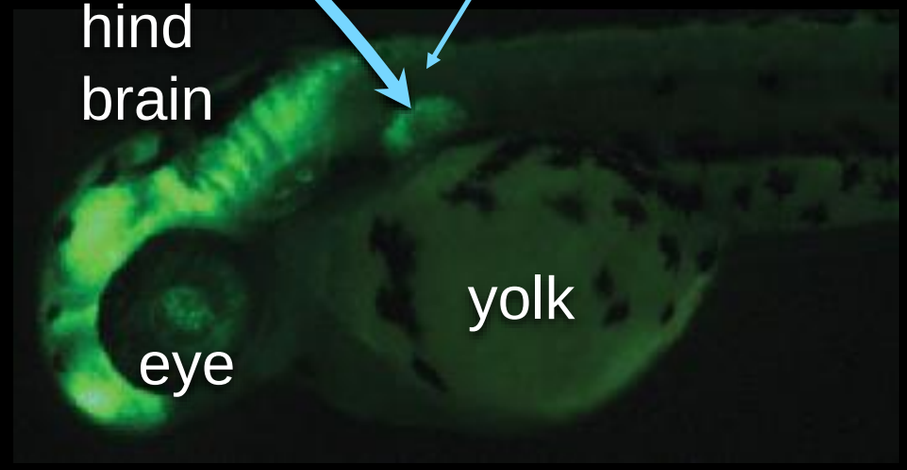
Are these CNEs regulatory?



GAR as comparator



pectoral fin bud



transgenic
test

Gar and zebrafish can help assign function to CNEs.

Thus, gar and zebrafish can help assign function to human GWAS hits

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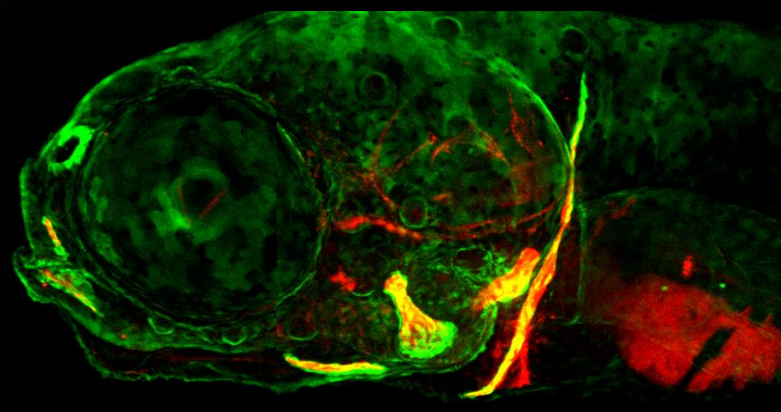
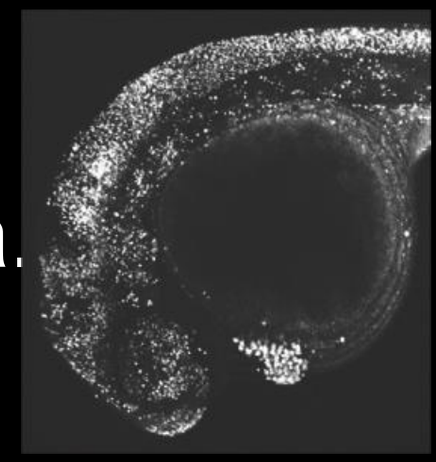
A zebrafish model for the FA/BRCA pathway and connecting fish medical models to human health





- What makes zebrafish a good biomedical model?
- A small molecule screen to rescue Fanconi anemia.
- Connecting fish genomes to human biology.

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Acknowledgements

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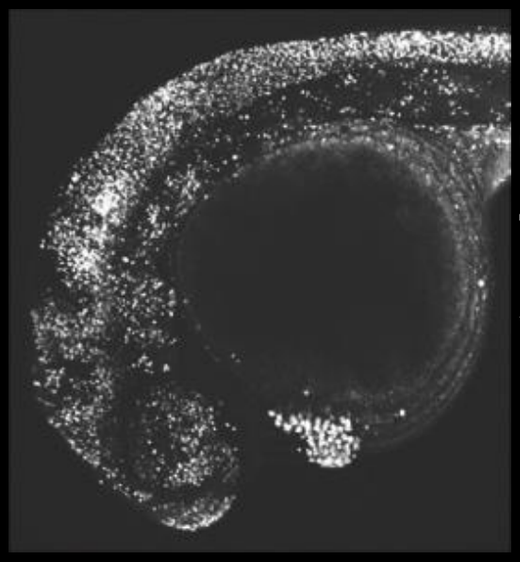

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