Deconstructing disease genes: How zebrafish models of Usher syndrome can help develop therapies for vision loss

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Vision for a Cure

USHER 1F COLLABORATIVE
מבט לעתיד

Our goal: To give you the stars

Our method: learn how to stop retinal degeneration by studying zebrafish models of Usher syndrome.

Why do we need animal models?

A disease model is a necessary prerequisite to developing and testing treatments

Animal models that are most like humans are desirable, but not always an option

Human cell cultures can provide some info, but don't behave like complex, functioning bodies

All animal models have pros and cons

Similar to humans

Dissimilar to humans

Expensive

Slow generation time

Small number of offspring

Genetically “messy”

Ethically controversial

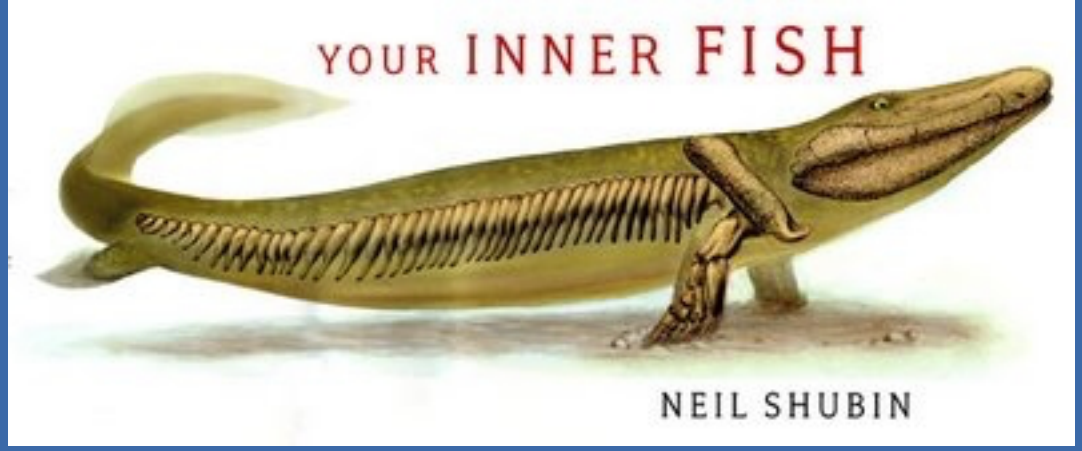
Cheap

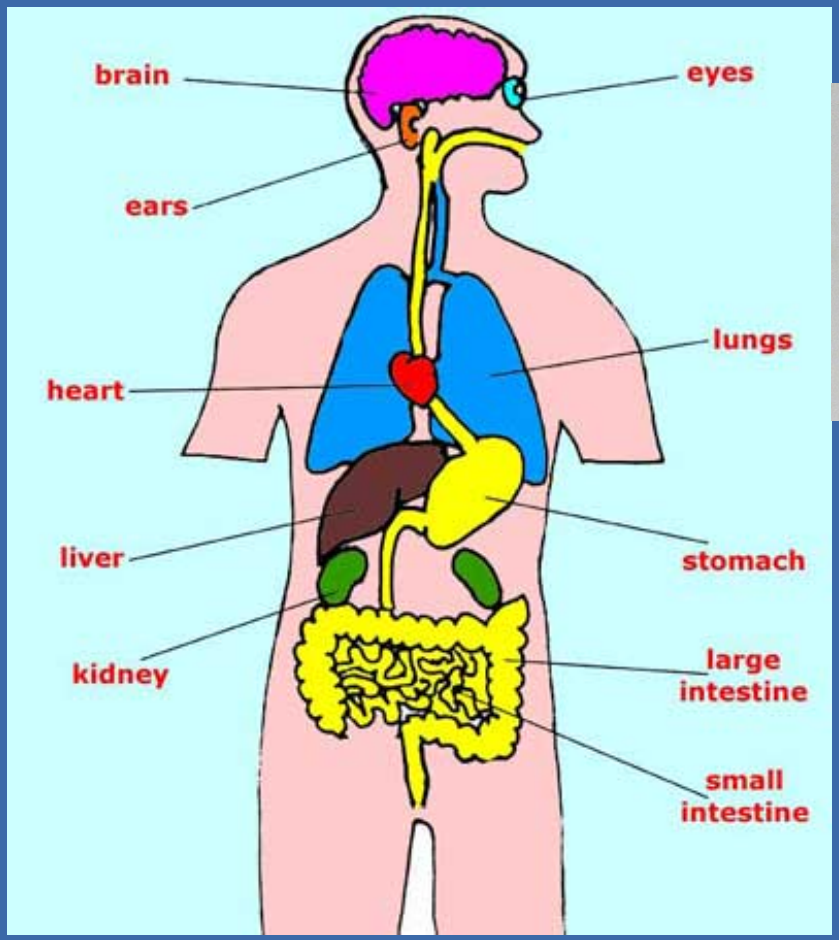
Fast generation time

Lots of offspring

Genetically tractable

Ethically uncontroversial


YOUR INNER FISH
NEIL SHUBIN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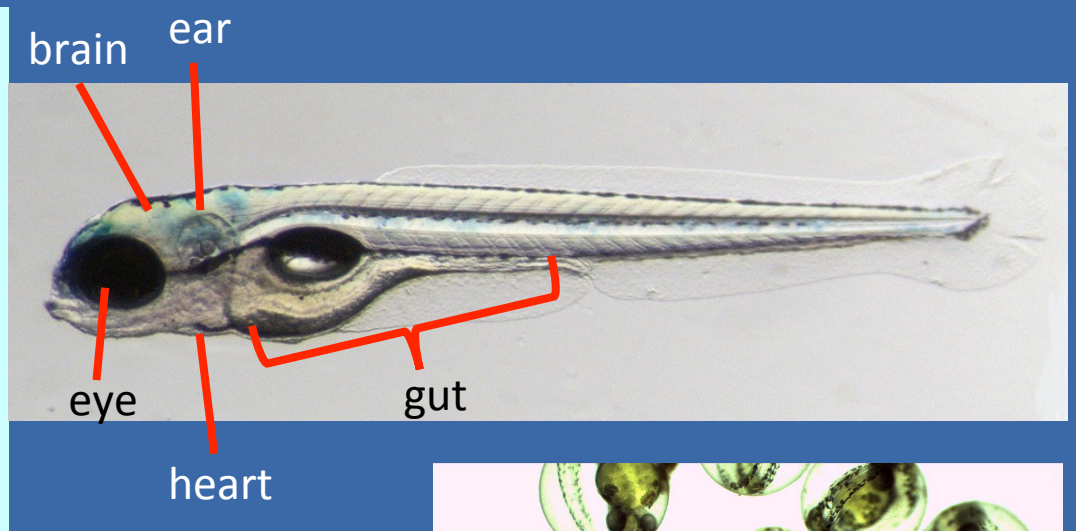
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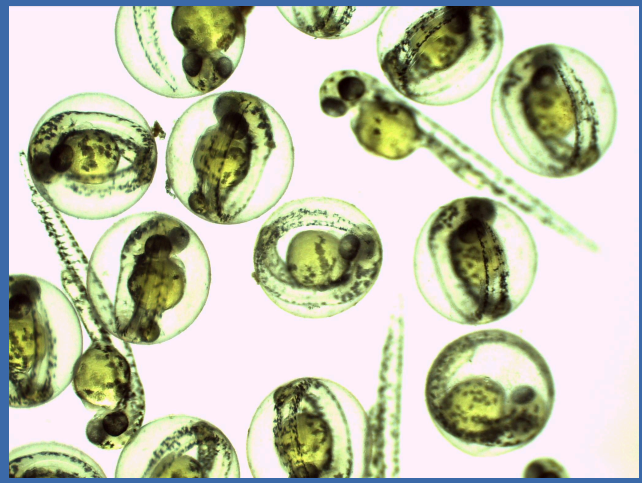


Zebrafish and other animal models help us understand molecular and cellular events that are the
basis for genetic disorders like Usher syndrome

Factors in the cell read the genetic code like an instruction manual for building proteins.

“Assemble items 1 through 2000. Then, stop”

When the DNA code changes due to mutation, the message in the manual can be incomplete or inaccurate

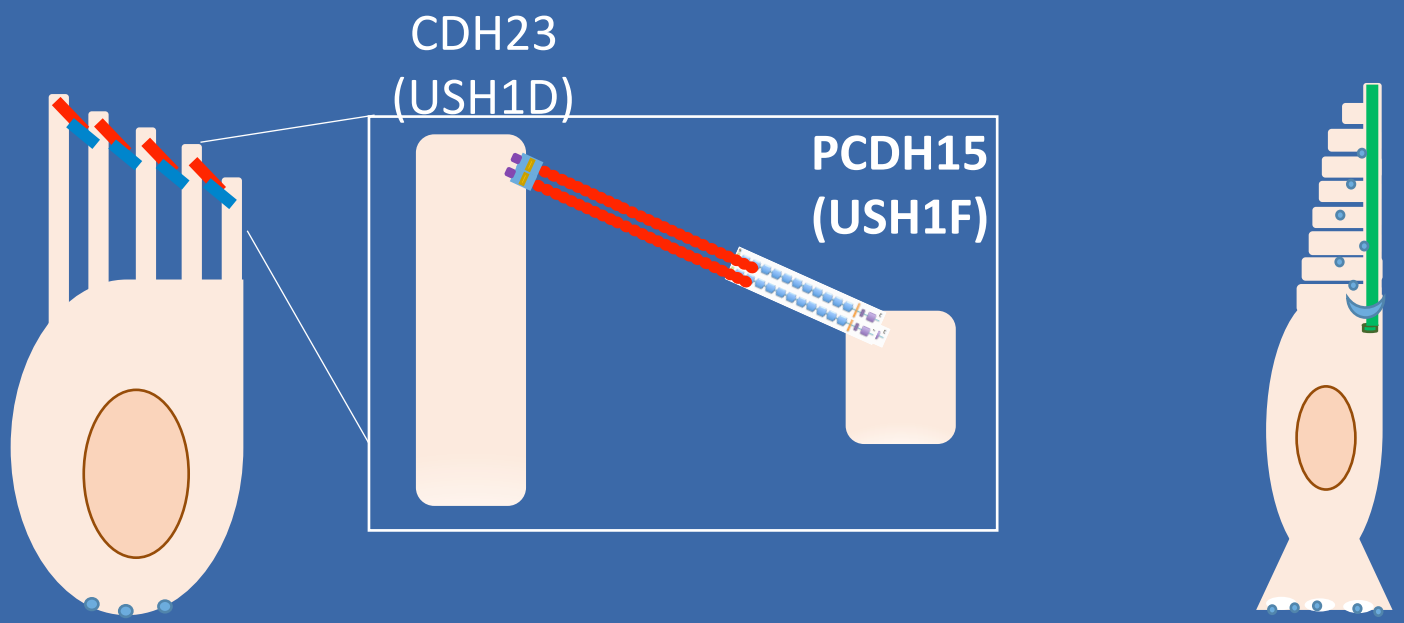
“Assemble items 1 through 244. Then, stop”

Genetic disorders are the result of such misprints

The Westerfield lab has active projects on most of the zebrafish USH genes. Today I will talk about two:

Usher type	Human gene	Zfish gene
USH1B	*MYO7A*	*myo7aa*
		myo7ab
USH1C	*USH1C*	*ush1c*
USH1D	*CDH23*	*cdh23*
USH1F	*PCDH15*	*pcdh15a*
		pcdh15b
USH1G	*USH1G*	*ush1ga*
		ush1gb
USH1J	*CIB2*	*cib2*
USH2A	*USH2A*	*ush2a*
USH2C	*GPR98*	*gpr98*
USH2D	*CIP98*	*cip98a*
		cip98b
USH3A	*CLRN1*	*clrn1*

Usher proteins provide important structural support within sensory cells



Mutations in Usher genes cause disruptions to the development and function of these sensory cells.

A common mutation in the USH1F gene ("R245X") creates an error in the instruction manual that results in a short, non-functional protein:

Normal USH1F

R245X USH1F

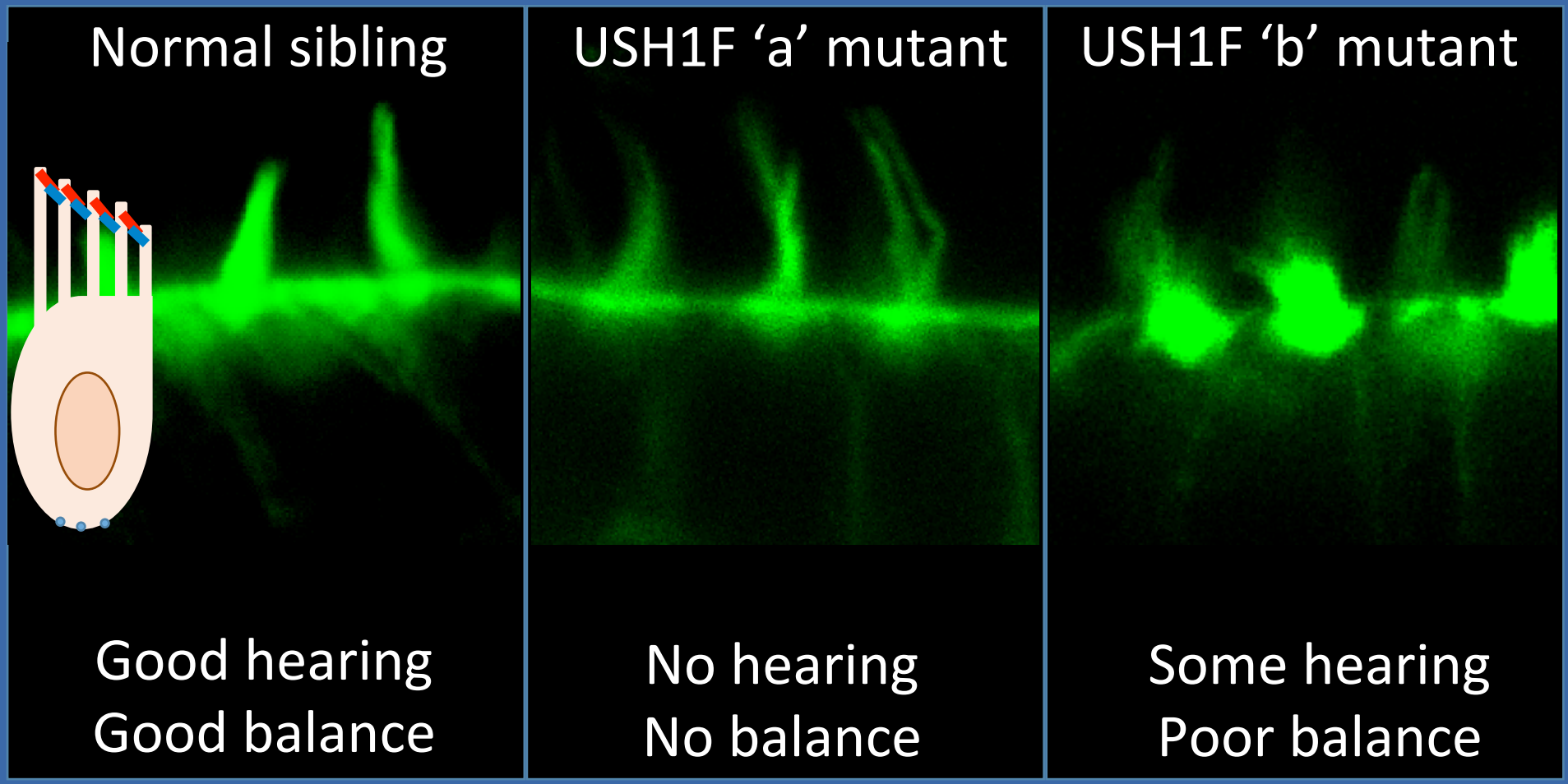
What can we do to compensate for this misprint?

We made zebrafish models that make the same ‘wrong’ protein produced in R245X patients.

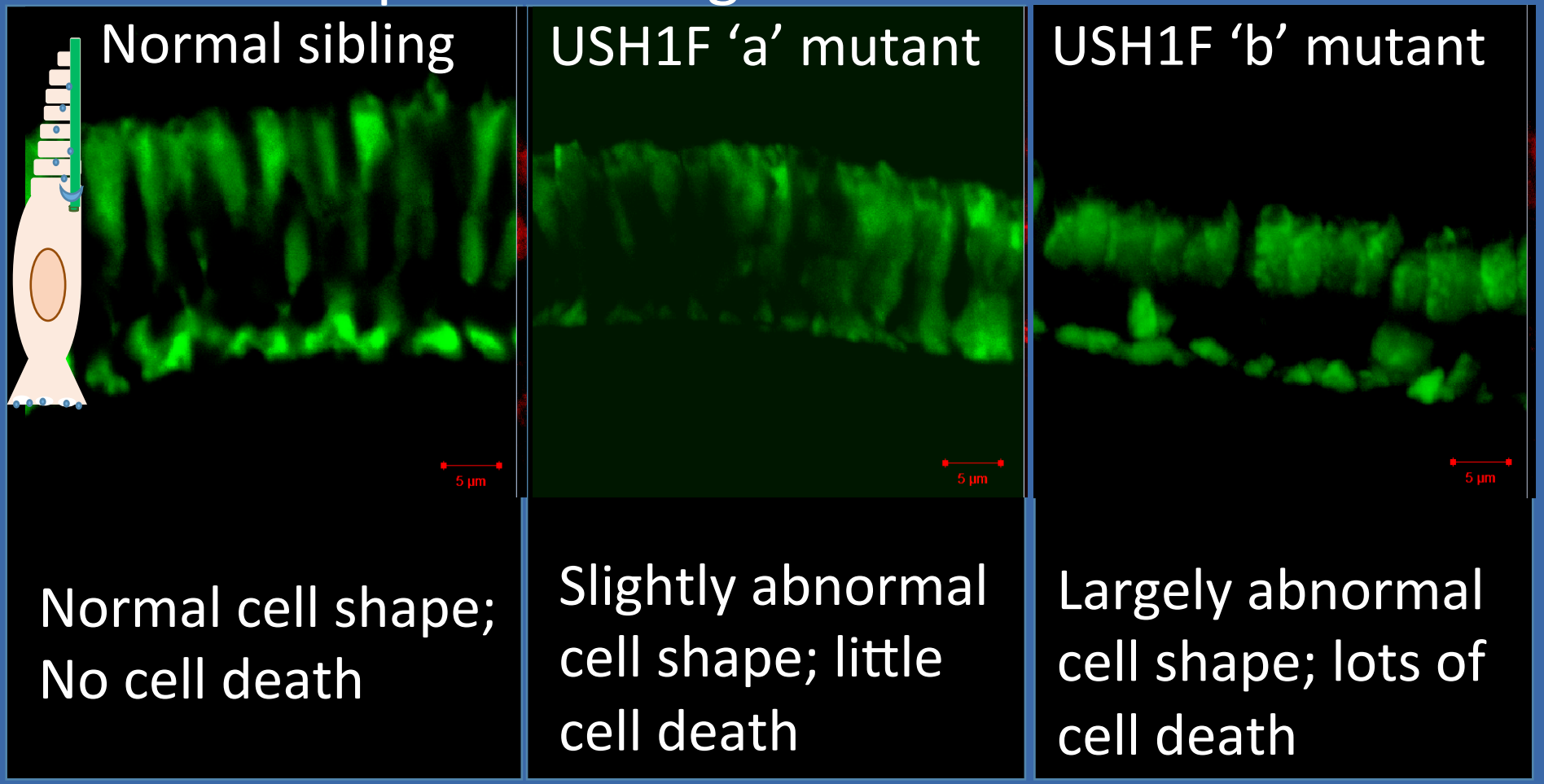
Since zebrafish have two copies of the USH1F gene, we made two models in parallel.

Our study revealed that both zebrafish USH1F genes, “a” and “b”, are important for vision and hearing in the zebrafish. That means we now have two models to test potential USH1F therapies on.

The absence of normal USH1F proteins in these zebrafish models disturbs cellular structures of the inner ear. This produces similar symptoms to those experienced by USH1F patients.



Photoreceptors in USH1F mutant zebrafish are poorly formed and prone to degeneration.



Normal cell shape; No cell death

Slightly abnormal cell shape; little cell death

Largely abnormal cell shape; lots of cell death

These problems in the zebrafish retina develop at a young age, as seen in people with USH1F

We are now using these models to test different gene therapy options for USH1F R245X, trying to work around the ‘misprint’

Exon skipping: If we remove a section of the manual, can it still build a functional protein?

Normal USH1F protein:
"Assemble items 1-2000. Then, stop."

Exon skipped USH1F protein:
"Assemble items 1-2000, but omit items 241-290. Then, stop."

Nonsense Read-through: Can we convince the cell to read past the mistake and follow the complete assembly instructions?

"Assemble items 1 through 2000. Then, stop"

"Assemble items 1 through 244. Then, stop"

+ Read-through drug:

"Assemble items 1-2000. When you get to item 245, KEEP GOING. When you reach item 2000, stop."

Zebrafish models of USH1F and what they can tell us:

Genetic mutations that cause early termination of the protein in the same region as R245X.
What is the exact role of USH1F in eyes and ears?
What exact processes go wrong in the cells of R245X patients?

Genetic alterations that skip the region of R245X, but leave the surrounding information intact.
Is the skipped portion of the protein expendable?
Will a protein lacking this region perform better than the non-functional R245X protein?

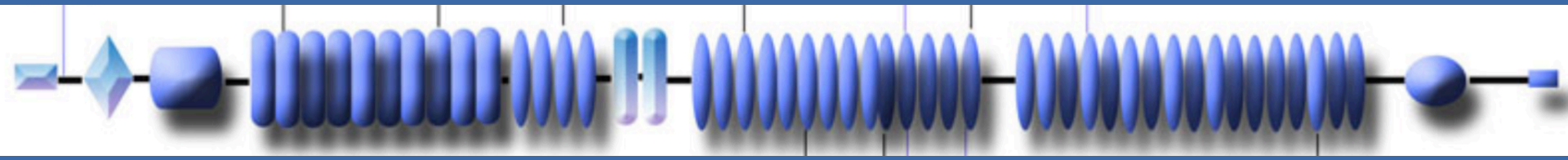
Zebrafish models of USH1F and what they tell us:

Genetic alterations that transplant the portion of Human DNA sequence containing the R245X region into the Zebrafish USH1F genes.

Can the read-through drug successfully bypass the Human R245X mutation sequence and complete assembly of the full length USH1F protein?

Asking these questions in zebrafish models saves money & time, preserving resources to go forward with the options most likely to succeed in clinical trials.

USH2A: the most common form of Usher syndrome

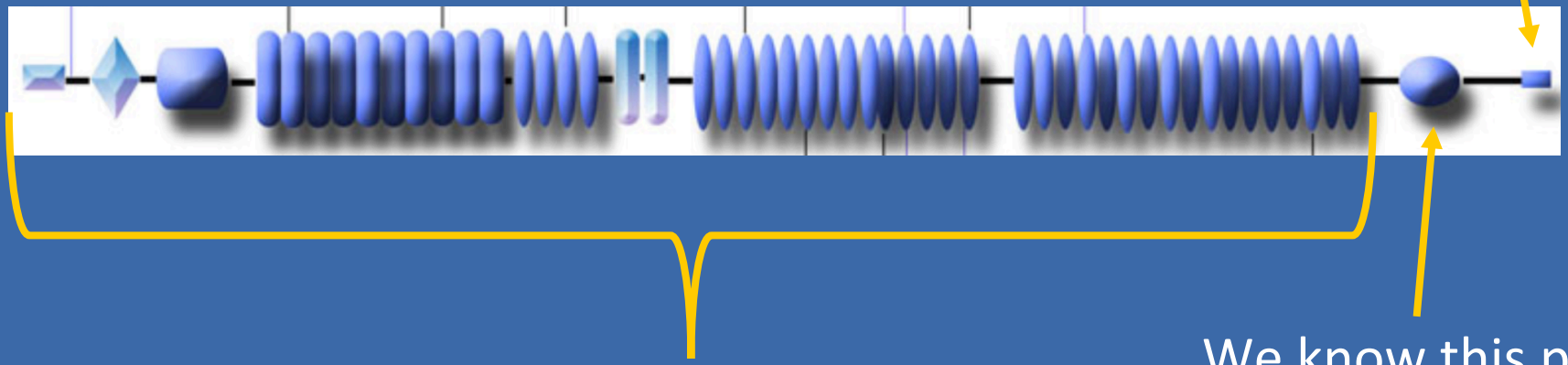


The USH2A gene is LARGE

How can we overcome the difficulty of such a large target for gene therapy? How can we analyze all the different mutations that can arise in such a vast area?

The functional importance of most of the USH2A protein is unknown

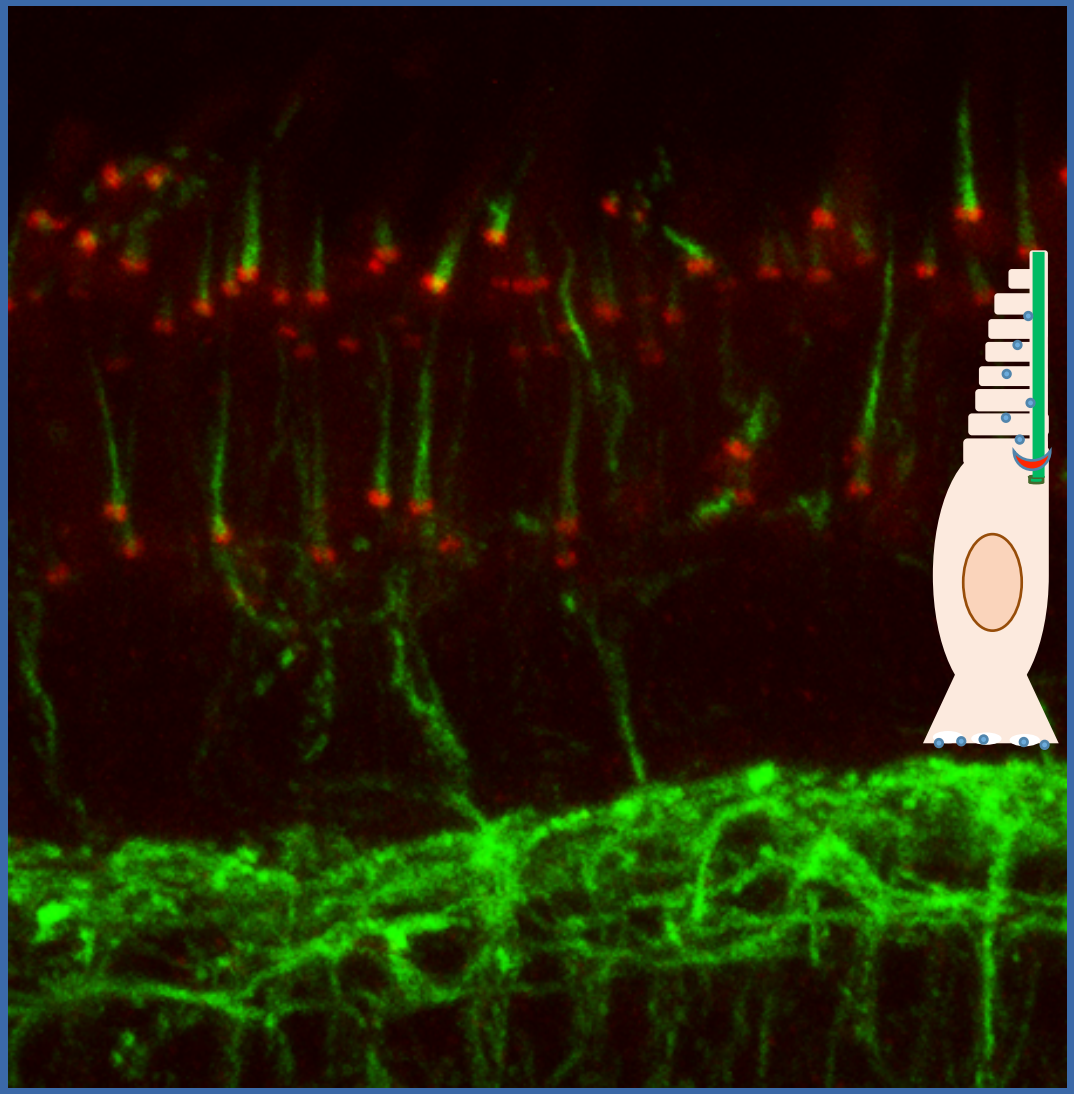
We know this part is needed to interact with other USH proteins

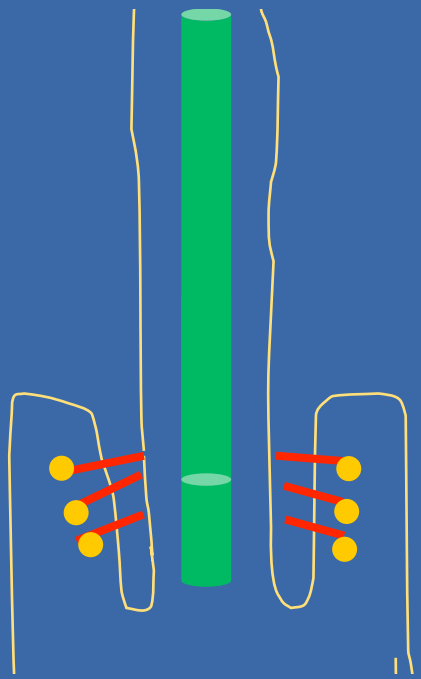


We don't know what any of these parts do aside from providing a defined size and shape

We know this part is needed to interact with the cell membrane

Many Usher proteins reside in a particular part of the photoreceptor where they physically interact with each other and work together as a unit.



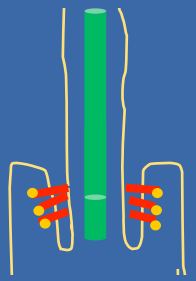


What does loss of USH2A look like in zebrafish models?

Inner ear cells are not as disturbed as what we observe in type 1 zebrafish.

When young fish are raised in normal daylight, their photoreceptor cells begin to degenerate. When they are raised in low light, they don't show degeneration in the first six months.

Other Usher type 2 proteins are not correctly localized in photoreceptors.



What can USH2A zebrafish do for USH2A patients?

Now that we know what USH2A looks like in fish, we have plenty of symptoms to evaluate when testing new therapies.

We can test the read-through drugs on zebrafish USH2A models with the right kind of mutations.

We can test other therapies for the mutations that won't respond to read-through drugs.

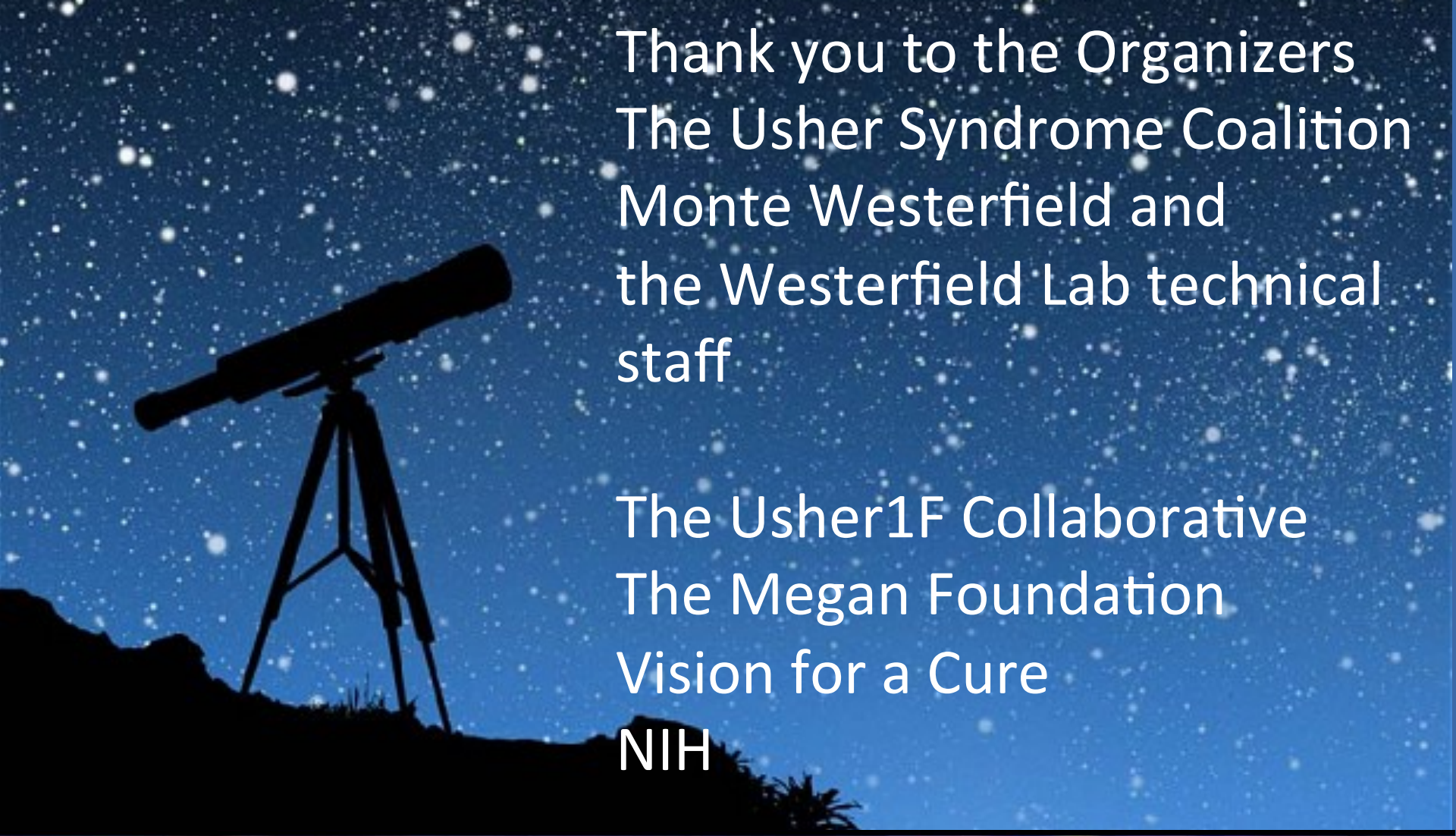
We can use light filters to determine whether some wavelengths are more damaging to retinal cells than others.

What can zebrafish do for the Usher community?

Provide more information about Usher proteins and how they work

Test different variants of Usher genes to determine if they cause Usher syndrome

Provide animal models with Usher symptoms in which to develop and test new therapies



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