SPOT THE DIFFERENCE: ZEBRAFISH

Teacher's notes

OVERVIEW OF THE ACTIVITY

Students are given a worksheet with accompanying flash cards. Each card shows an image of a wild type zebrafish and a similar mutant zebrafish. Some images focus in on a specific part of the body, e.g. a muscle block or fin. Students use their observational skills to identify and record the difference (phenotypic change) between the two images. To aid in their diagnosis of the phenotypic change a glossary is provided to gives hints and clues, as well as definitions for specific scientific terms.

AIM OF THE ACTIVITY

The aim of the activity is to encourage students to identify differences between wild type and mutant zebrafish. The goal of the activity is to raise awareness of the use of model organisms in genome research and how they aid the understanding of the genetic basis of human disease.

Estimated time: 30-45 minutes

Group size: Students can work alone or in pairs

ACTIVITY PREPARATION

The following tasks need to be completed before starting the activity:

Flash cards

Print out all of the flash cards and cut them out. One set of cards is required per student or pair. It is recommended that the cards are laminated to prevent damage, however this is not essential.

RUNNING THE ACTIVITY

To run the activity you will require:

- Introductory PowerPoint
- Flash cards (one set per pair)
- Student worksheets (one per student)
- Teachers' notes

1. Introductory PowerPoint (10 minutes)

The Spot the difference presentation introduces the concept of using zebrafish as a model organism in scientific research. The presentation provides an introduction to model organisms outlining what they are and why they are used in genome research. It also explains the advantages of using zebrafish as a model organism and how they are being used to research human diseases.

The final slide of the presentation introduces the Spot the difference activity and what the students have to do to complete their worksheets.

2. Complete the worksheet (15-20 minutes)

Students should look closely at the images on the flash cards and identify the difference between the wild type and mutant zebrafish shown. They should write down on their worksheets what they think the phenotypic changes are in the six images.

A glossary of terms is provided at the bottom of their worksheets, which will help them to explain what they can see using scientific terms. It also provides hints as to what they could be looking at. For example, there are terms such as melanosomes and erythrocytes that suggest to students that these may be involved in one of the images.

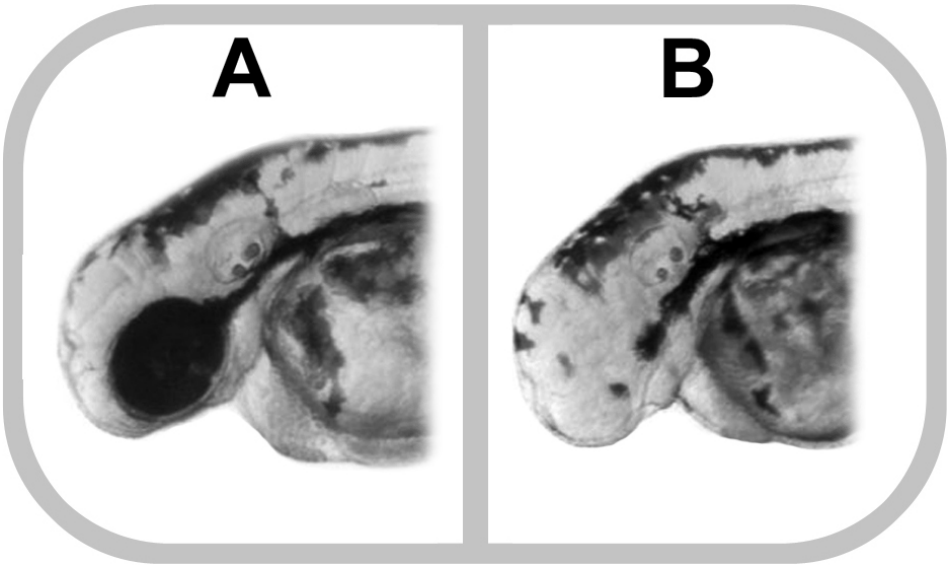
3. Feedback and results (15 minutes)

Once students have completed their worksheets you can reveal the results using the PowerPoint presentation. Make the session interactive by encouraging the students to feed back their observations.

ACTIVITY ANSWERS

Below are the answers to the activity. For each set of images there is a description of the key phenotypic changes and why these mutations are of interest and relevance to human health.

Image 1: Eye development



Rodrigo Young, University College London

What's the difference?

Embryo B has no eye.

Why is this relevant to us?

Zebrafish and humans have a network of proteins called the Wnt pathway that is associated with embryo formation and development. Eyeless embryos, such as the one shown in the picture, can occur through mutations in genes that encode the proteins in the Wnt pathway. Identifying the genes involved in these pathways can aid our understanding of the role of the Wnt pathway in early brain and eye development.

Further information:

Zebrafish research at University College London is looking into the mechanisms that control the development of the vertebrate forebrain. More details about their work are available at: www.ucl.ac.uk/zebrafish-group/research/screening.php

Image 2: Pigmentation

Keith C. Cheng, Penn State College of Medicine.

What's the difference?

Fish B is a lighter, golden colour compared to fish A.

Why is this relevant to us?

Lighter skin colour in humans is linked to a reduced number, size and density of melanosomes. These are structures found in skin cells that contain a dark pigment called melanin. People with darker skin have lots of large and tightly-packed melanosomes in their skin cells. You can see the difference between the melanosomes in the darker striped fish and golden fish in the picture above. This difference in colour has been linked to a gene called *SLC24A5* which is present in both zebrafish and humans.

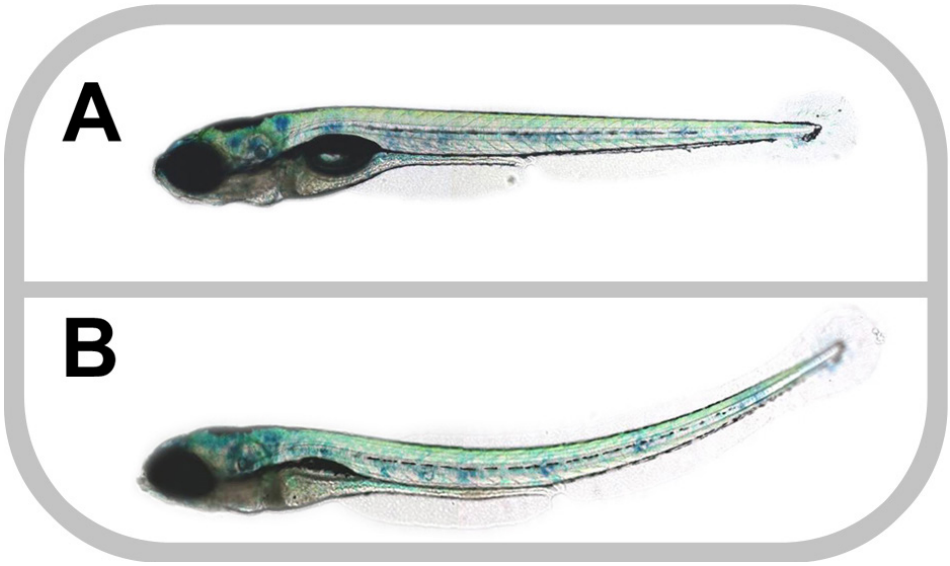
The *SLC24A5* gene encodes a protein that has a major influence on natural skin colour variation. It is thought to have played a key role in the evolution of light skin in humans of European ancestry. In 2005, a one base pair change in the gene sequence was found to be the major difference between the pale skin colour of Europeans and the darker skin colour of Africans [1].

Further information:

[1] Lamason RL, Mohideen MA, Mest JR, Wong AC, Norton HL, et al. SLC24A5, a putative cation exchanger, affects pigmentation in zebrafish and humans. Science 2005; 310 (5755): 1782-1786. Available at: http://science.sciencemag.org/content/310/5755/1782

News article on this paper: www.scientificamerican.com/article/researchers-identify-huma/

Image 3: Nebulin mutation



Elisabeth Busch, Wellcome Trust Sanger Institute.

What's the difference?

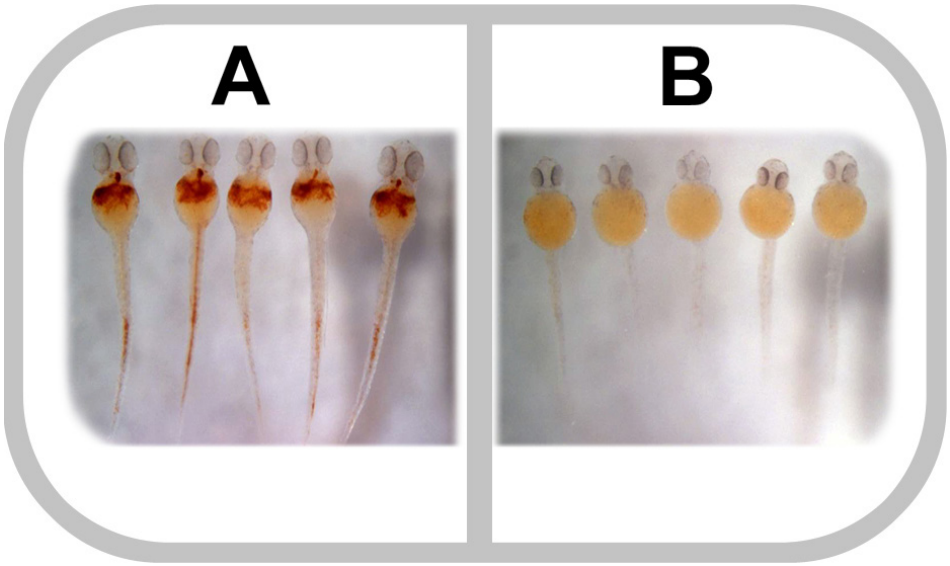
The picture shows two young zebrafish, known as fry. The body of zebrafish fry B is curved. If you look closely you'll also see that its mouth is open. This is because it is unable to fully close its mouth as its muscles are too weak.

Why is this relevant to us?

Zebrafish fry B is showing symptoms of a condition called nemaline myopathy. This is a genetic disease where muscles fibres do not form and function properly. This leads to poor muscle tone and weak muscles. Infants with this condition usually have problems with breathing and feeding as their muscles are not strong enough to support the body. This can also lead to skeletal problems such as curvature of the spine (scoliosis).

Genes that are associated with this condition are present in both zebrafish and human genomes. Observing the physical and genetic changes in zebrafish with nemaline myopathy can help us understand more about the human condition and potentially lead to new and better treatments for the disease.

Image 4: Red blood cell formation



Anna Cvejic, Wellcome Trust Sanger Institute.

What's the difference?

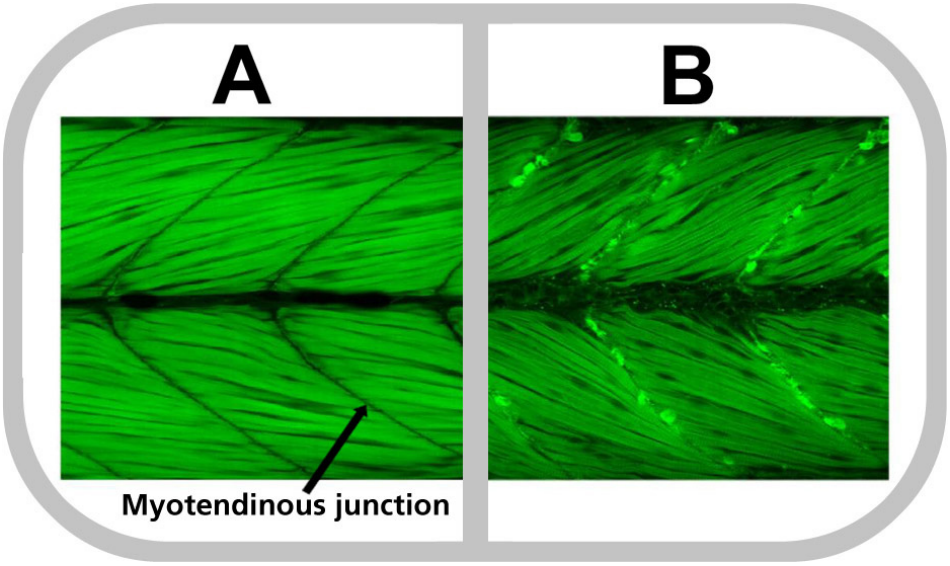
The zebrafish embryos in picture B look paler and are not stained red.

These embryos have been stained for the presence of haemoglobin, the protein found inside red blood cells that carries oxygen. Picture A shows normal embryos containing red blood cells with haemoglobin. The embryos in picture B have been injected with a substance that blocks a gene important for red blood cell formation. As a result these embryos are exhibiting severe anaemia with very few mature red blood cells. This is why they do not stain red for haemoglobin.

Why is this relevant to us?

Red blood cell formation and iron uptake has been found to be linked to a gene called *ARHGEF3* that is found in both humans and zebrafish. This gene encodes an exchange factor which activates other genes along a cell signaling pathway. When the function of this gene and its target genes is blocked it leads to a severe decrease in mature red blood cells. This type of research can give us a better understanding of the function of the genes and the roles that they play in iron metabolism and the development of red blood cells and blood vessels.

Image 5: Nebulin deficiency



Elisabeth Busch, Wellcome Trust Sanger Institute.

What's the difference?

There are bright green blobs in picture B.

These pictures show a close up of muscle fibres in two zebrafish. These muscle fibres attach to the skeleton of the fish via cartilage-like structures called myotendinous junctions. In both pictures the muscle has been stained green to highlight a muscle protein called actin. Picture A shows normal muscle fibre arrangement so this fish would be able to swim normally. Picture B shows a build up of clumps of actin in the areas where the muscle attaches to tendons. This fish would not be able to swim well as its muscle would be a lot weaker and unable to function properly.

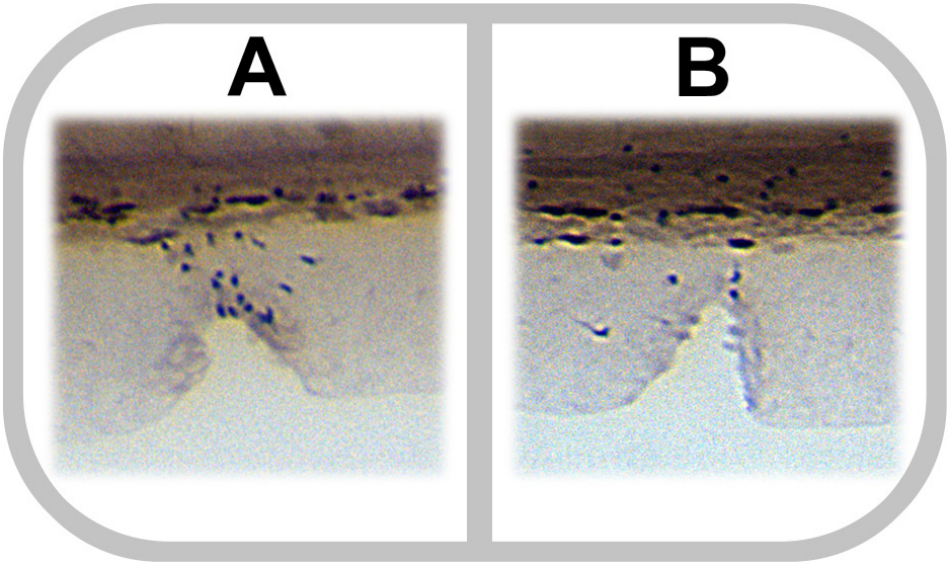
Why is this relevant to us?

The build-up of actin in the muscle block is one of the features of a zebrafish that lacks nebulin, a protein that binds to actin. Nebulin deficiency is associated with the genetic condition nemaline myopathy, a disease that leads to muscle weakness. Understanding more about the genetic changes involved in nemaline myopathy may eventually lead to better treatment of the disease in humans.

Further information

Research at the Wellcome Trust Sanger Institute is looking at the mechanisms responsible for muscular dystrophies and myopathies by looking at muscle development in zebrafish.
More information about their work is available at:
www.sanger.ac.uk/science/groups/vertebrate-genetics-and-genomics
www.sanger.ac.uk/science/collaboration/zebrafish-mutation-project

Image 6: Immune response



Anna Cvejic, Wellcome Trust Sanger Institute.

What's the difference?

Embryo A has more blue dots than embryo B.

The image shows a close up of the fins of two zebrafish embryos. Both embryos have had a small nick cut into their fin. The blue dots are neutrophils (a type of white blood cell involved in the immune response to injury) that have been stained with a dye. The wound on embryo A (wild type) has a lot more neutrophils than the wound on embryo B (mutant). This is because the *WASp* gene has been "knocked down" in embryo B. This means gene expression was inhibited by a chemical and the protein product Wiskott-Aldrich syndrome protein (WASp) was not produced. The result of this gene knock down is reduced movement of neutrophils to the wound site. This suggests the *WASp* gene and its protein product play a role in the navigation of neutrophils towards chemical cues coming from wound sites or sites of infection. This is an essential part of the body's immune response.

Why is this relevant to us?

The Wiskott-Aldrich syndrome protein (WASp) is associated with the X-linked immunodeficiency disease, Wiskott-Aldrich syndrome. This is a severe condition that is characterised by regular infections, eczema and a low platelet count. This condition can cause death through severe infections or haemorrhaging.

Understanding the mechanisms of the *WASp* gene can help researchers understand more about the body's immune response and identify the best treatments for Wiskott-Aldrich syndrome as well other immune and inflammatory diseases.

SUPPORTING NOTES FOR TEACHERS

What is a model organism?

The term model organism is used to describe any non-human species that is used in scientific research to better understand the causes and potential treatments of human diseases. Model organisms are used for experiments that would be considered unfeasible or unethical to carry out with humans.

What is a zebrafish and why is it used in research?

The zebrafish is a small freshwater fish that is approximately 4 cm long. It originates from South Eastern Himalayan regions including India, Pakistan and Bangladesh. It is now a popular aquarium fish and you are very likely to see one in your local garden centre or pet shop. They are very robust and easy to look after in a small aquarium tank which makes them popular pets.

There are several reasons why the zebrafish is considered an ideal model organism:

- It is small and therefore does not require large amounts of space.
- It develops quickly; all of the five major organs are present five days post fertilisation.
- It has a short generation time with sexual maturity being reached when fish are 3-4 months old.
- They produce a lot of eggs; a single female can produce over 300 eggs every two weeks.
- The eggs are fertilised externally and the embryos develop outside of the female (*ex utero*) making them easy to study.
- Zebrafish embryos are translucent enabling researchers to watch the tissues develop under a light microscope.

Another major reason why zebrafish are increasingly being used in research is because there are a lot of genome resources available to support studies. This includes the zebrafish reference genome sequence and a database of information on zebrafish mutants called the Zebrafish Model Organism Database or ZFIN.

What diseases have zebrafish been used to investigate?

Zebrafish models have been used to investigate a range of different human diseases and disorders, some inherited and some acquired. These include:

- Alzheimer's disease
- Congenital heart disease
- Polycystic kidney disease
- Duchenne muscular dystrophy
- Malignant melanoma
- Leukaemia

Zebrafish can also be used to test candidate drugs for these diseases making them a valuable tool in the development of treatments for human diseases.

RECOMMENDED SUPPORT RESOURCES

Websites

The Vertebrate Genetics and Genomics group at the Wellcome Trust Sanger Institute carries out research to understand biological processes of potential importance to human development and health.
Further information: www.sanger.ac.uk/science/groups/vertebrate-genetics-and-genomics

The Zebrafish Mutation Project at the Wellcome Trust Sanger Institute aims to create a knockout allele in every protein coding gene in the zebrafish genome.
Further information: www.sanger.ac.uk/science/collaboration/zebrafish-mutation-project

Recent news

Heart failure research to find cure using zebrafish – BBC News 1st February 2011.
www.bbc.co.uk/news/health-12322819

Fin to limb evolution clue found – BBC News 24th June 2010.
www.bbc.co.uk/news/10396532

Videos

Zebrafish embryo development - 24 hours in 46 seconds:
https://youtu.be/eQNxHGpK7Wc

Glowing heart and blood in a zebrafish:
https://youtu.be/84HbKeCecOI

FURTHER READING

Giacomotto J and Ségalat L. High throughput screening and small animal models, where are we? British Journal of Pharmacology 2010; 160: 204–216.
Available at: http://onlinelibrary.wiley.com/doi/10.1111/j.1476-5381.2010.00725.x/pdf

Hariharan IK and Haber DA. Yeast, Flies, Worms and Fish in the Study of Human Disease. The New England Journal of Medicine 2003; 348 (24): 2457-2463.

Available at: www.ohsu.edu/nod/documents/2009/04-20/Hariharan%202003.pdf