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Fishing for clues

Zebrafish as a model for human disease

Key words
zebrafish
genetics
development
model systems

Understanding how our bodies work and what causes human disease is the key to future medical breakthroughs. In this article, Caroline Parkin of Sheffield university describes how fishes can help us to learn more about our own biology.

Most discoveries in medical science are a result of experiments that cannot be performed on humans. We can use animal models to obtain vital clues about the causes and progression of human diseases. There are a range of model organisms to suit different topics; at the University of Sheffield we use zebrafish to model human disease.

Can we really study human disease in a fish?

Yes! Fish and humans are not so very different when you look closely - especially when you look at their blue-print for building their bodies - their DNA, or genetic code. By sequencing the DNA, we now know that there is a remarkable similarity between the genetic codes of different species, including zebrafish and humans.

Although you and I may appear to be very different to fish, the reality is that we're not that different at all. We have two eyes, a mouth, and a gut, for a start. And when we look closer - at all the cells inside them – there really are more similarities than differences.

This similarity between the genes of different organisms, which scientists call conservation, or genetic homology (from an ancient Greek word meaning 'to agree'), is the fundamental reason why we can use fish to learn about human diseases.

Zebrafish in one of the tanks at the Sheffield aquarium.

The genetic 'recipes' (genes) used to build particular body parts have often been conserved between species. For instance, the information to make an eye is stored in many different genes, but since human eyes and those of fish are built, and work, in much the same way, the 'recipes' are similar.

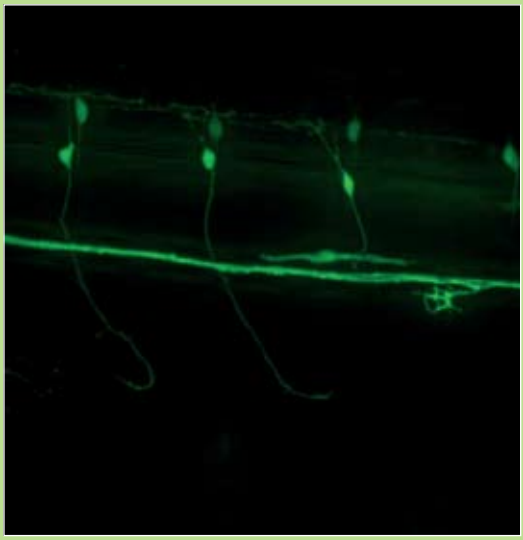
Why zebrafish?

Zebrafish are tropical fresh water fish from the minnow family. They are popular in home aquariums and you'll find them in many pet shops under the name 'Danios' (from their scientific name – *Danio rerio*, see Catalyst Vol 19, Issue 3, pp 7-8, What's in a Name?).

Most research is carried out using zebrafish embryos. The transparency of zebrafish embryos has become one of the leading reasons for using them for scientific research. Being able to see through the embryos allows us to watch changes that occur, both in normal development and when things start to go wrong. We can see cells move around, the heart as it beats, and the gut undergoing peristalsis, all in the living embryo. This transparency also means we can make use of a naturally fluorescing protein called GFP (Green Fluorescent Protein) – that can be used to label individual organs, cells or even compartments within a cell.

Green Fluorescent Protein

GFP is a protein, originally extracted from a species of jellyfish, which has proved vital in many fields of biological research. *Fluorescent* describes a substance which glows with visible light when another colour of light is shone on it. For example, white clothing may glow purple when (invisible) ultraviolet light is shone on it – you may have seen this in a night club. GFP glows green under blue light. The importance of GFP in Biology is that molecules of GFP can be used to show up particular organs, or to show that a gene has been successfully transferred into an organism. In 2008, three researchers shared the Nobel Prize for Chemistry for their discovery and development of GFP.



A muscle fibre (across the picture) with the motor neurons (nerve cells, above the fibre) which will control its operation, seen using GFP.

From genes to function... and back

When human genes involved in disease are identified, we are able to examine the function of the homologous genes in zebrafish. The zebrafish model also allows us to look at the disease processes more directly, for instance understanding the progression of a disease, or the precise cell or protein biology that underlies a disorder.

In Sheffield collaborations between medical doctors and scientists have helped establish a number of diseases models in zebrafish.

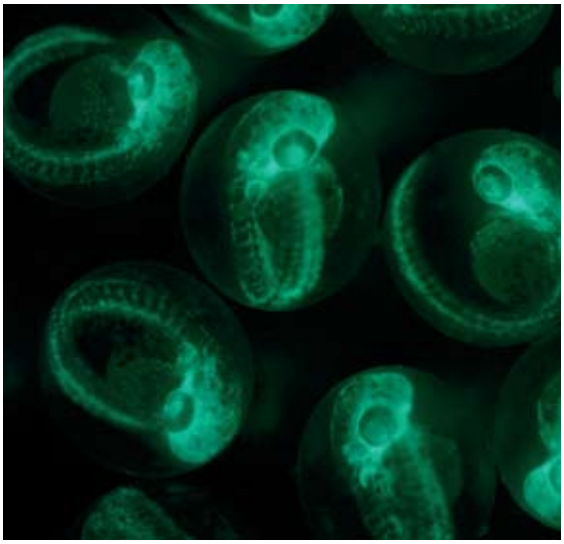
Using zebrafish to study hypoxic signalling and cancer

Cancer occurs when cells grow and divide out of control, even invading surrounding tissues and spreading to other areas of the body (metastasis). As tumour cells grow, they need oxygen to supply their needs.

A signalling pathway called the hypoxic signalling pathway allows tumour cells to survive under conditions of low oxygen supply ('hypoxia'), stimulating new blood vessel growth to the tumour. In this way, tumours become ever more dangerous, since they can get more oxygen and keep growing.

In addition, the new blood vessels can transport tumour cells to other sites in the body, where they can form metastases (new cancerous tumours).

We are using zebrafish larvae to study these processes. GFP allows us to follow blood vessel development in live fish larvae. We can also use GFP to show up the activity of the hypoxic signalling pathway in a living zebrafish larva and therefore study these processes as they are happening. Once we understand the normal events we can modify the environment around the larva in different ways, such as by changing the oxygen levels, introducing drugs that may affect blood vessel growth and we can manipulate genes to see what affect they have on blood vessel growth or oxygen sensing. In addition, we can search for chemicals that activate or inactivate the low-oxygen response.



These zebrafish embryos fluoresce because they contain GFP.

Understanding disorders of the nervous system

The central nervous system (CNS) coordinates the activity of the body. It includes the brain and spinal cord.

Neuronal network in a zebrafish larva.

Disorders of the CNS are severely debilitating and sufferers can be affected in profoundly different ways, through diseases such as Alzheimer's disease, Parkinson's disease, motor neuron disease and multiple sclerosis, or psychiatric disorders such as schizophrenia and bipolar disorder.

Work with zebrafish is bringing greater understanding of diseases of the central nervous system, bringing the hope of better treatments for patients such as this elderly Alzheimer's disease patient.

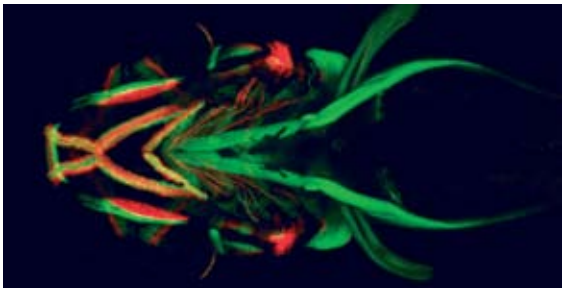
The zebrafish brain and spinal cord are very similar to those of a human. They develop in much the same way and require almost identical genes to make them. The genetic basis for some CNS diseases is only just beginning to be understood, and we can use zebrafish to understand much more about what goes wrong when particular genes are mutated.

But zebrafish can be even more useful, because we can use the fish to find new genes and then explore their roles in human disease. Another important use of zebrafish is to look at the effect of environmental factors, such as chemicals and toxins, on the development of neurodegenerative diseases.

Muscle disease and development

Muscular dystrophy is the collective name for a group of genetic diseases that cause the progressive breakdown of muscle in the body, which leads to weakness and subsequent loss of mobility. There is no known cure and treatment is limited.

We can use zebrafish models to investigate the genetic causes of muscular dystrophy and try to determine what changes are occurring in the cells when certain genes are mutated.



GFP shows up muscles in the head of a zebrafish embryo.

Duchenne Muscular Dystrophy, DMD, is caused by a defect in the gene coding for Dystrophin, a structural protein that provides support in the muscle cells. When the *dystrophin* gene is mutated the resulting protein is no longer fully functional. Over time the cells collapse and die. This leads to the muscle wasting characteristics of DMD.

We can model DMD and other muscular dystrophies by looking at fish that carry similar mutations to those found in humans. One such mutant is called *sapje* and has a mutation in the *dystrophin* gene. As in the human patients the muscle in the fish degenerates and dies.

To understand diseases such as muscular dystrophy we need to know how muscle normally develops in an embryo and how it functions in a living organism. Zebrafish have been used extensively to understand the development of muscle from single cells (called myocytes) into large bundles of contractile muscle fibres that allow a fish to swim or you and I to walk, run and even swim.

Muscle fusion and repair

To make large muscle fibres many single myocyte cells must fuse together to create what is called a syncytium, which may consist of thousands of individual cells. The syncytium is important as it allows coordinated contraction across the whole muscle.

Muscles don't only fuse during embryogenesis. When muscle is damaged, either through injury or normal wear and tear (think of how your muscles ache after lots of exercise), new muscle cells are able to fuse with the existing muscles to repair them. By understanding fusion in both adults and embryos it is hoped we can enhance the restoration of damaged muscle following disease or trauma.

Conclusion

The usefulness of this tiny tropical fish cannot be overstated. It has already provided many breakthroughs and expanded our knowledge of genetic interactions in the normal and diseased state. New techniques are constantly being developed that will enhance the strength of this model organism in our quest to understand and develop treatments for human diseases.

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For more information about zebrafish and to see some great movies of the work in Sheffield, visit www.fishforscience.com