Home Office

NON-TECHNICAL SUMMARY

Understanding the dynamics of cell fate decisions during zebrafish embryogenesis

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Cell differentiation, Embryology, Stem cells

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

During the development of the embryo, cells have to decide on becoming all the different cell types that make up the adult body. They also have to do this in a very coordinated way, with tens of thousands of cells all deciding which part of the body they are going to generate. How this coordination is achieved

is one of the great mysteries of biology. Our current understanding is based on the idea that long-range signals travel across the embryo to instruct cells about which cell type to become. Cells close to the source of the signal would receive a lot of it and therefore turn into one cell type, while those further away would receive less and therefore decide on a different fate. However, this only works in some cases, as the cells themselves are moving around very rapidly. Imagine a headmaster shouting instructions to an entire school of children playing outside. Whatever initial pattern that might be set up is going to be rapidly destroyed as they move around the playing field.

Another way is to make use of the fact that cells travel within distinct cell layers and are constrained by mechanical forces to move in streams. It's a bit like having the children back inside the school moving between different classrooms. Now the teacher can move through the corridors and give out specific instructions as the children move past. In the same way, specific signalling cells move through the embryo and pass on instructions to other cells as they move past. In reality, many teachers are required to walk the corridors of the school to make sure everything is kept in check. Note that in this model, it's the timing of when pupils meet the teachers that will determine how patterns form, rather than their spatial position as in the playground idea.

To understand this complex process, we collect information from each cell and its state (or each pupil and whether or not they have their shirt hanging out!) and track their movements as they move through the embryo. At the same time, we collect information about the instructions each cell is receiving. This is a huge amount of information that requires computer power to simulate the outcome. But once this is achieved, we can ask questions about what the minimal set of instructions are that are required to generate a well-coordinated pattern of cell differentiation.

Zebrafish embryos are ideal for this sort of large-scale experiment, for several reasons. Firstly, their embryos are visually transparent, meaning that we can image all the cells in the embryo at once. Secondly, they develop external outside of the mother and require only a simple temperature control for them to develop normally, meaning that we can culture them under a range of different microscopes and follow their development by time-lapse microscopy. Finally, we are able to insert new DNA sequences into their genomes make fluorescent proteins to light up when certain genes are being switched on or off, allowing us to follow where specific signals are being received by cells as they travel through the embryo. Together, this means we can collect all the information required to watch how patterns in cell type differentiation occur in real-time, and across the whole embryo.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

This project aims to reveal some fundamental principles about how cells make collective decisions during early development to become specific cell types. In healthy situations, cells will retain these specific cell types throughout an organism's entire lifetime. However, in disease states such as cancer, cells reverse the programming received during development and re-enter a state that leads them to divide excessively, generate alternate cell types, or migrate into the body and establish new tumours elsewhere. Therefore, the more that we understand the fundamental process involved in development, the better we can understand what goes wrong in cancer.

Stem cells are cells that, like very early embryonic cells, can generate all cell types of the body. Recent work has enabled researchers to generate stem cells from cells of adults, that can then be redirected to many other different cell types. A major hope for the treatment of many diseases is through the use of stem cells to generate replacement cells, tissues or even organs. In addition, the ability to generate multiple cell types from an individual patient is revolutionising the study of disease and opening up the possibility of patient-specific medicine. For any of these possibilities to realise their full potential, we need to direct stem cells in a very well controlled way, without the risk of cells taking decisions that would be harmful such as entering a cancer-like cell state. By investigating the fundamental processes coordinating cell fate decisions in early development, we hope to inform researchers working in the development of stem cell differentiation protocols and regenerative medicine. For this reason, we directly collaborate with stem cell researchers on a number of different projects and even have some in the lab.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

Approximately 17,000 adult zebrafish will be used over the 5 years of the project. Their role in the project is to provide embryos for experiments at non-regulated stages.

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

The adult zebrafish will be housed in a dedicated aquarium within the department, run by trained staff. We will generate genetically altered zebrafish by introducing modified genetic material at the 1 cell embryo stage and growing these embryos to adulthood. We do not expect there to be adverse effects to adults from these alterations but sometimes younger larvae fail to thrive for unknown reasons following introduction of genetic material and very occasionally fish can show signs of being unwell only at older stages. If the larvae or adult fish appear unwell at any stage we will humanely kill them. We do not expect there to be any adverse effects from breeding the zebrafish.

In order to know which fish contain genetic alterations we sometimes need to cut a small portion of the fish’s tail fin under general anaesthetic and analyse the genetic code inside this tissue. The fish is then kept in a separate tank with fresh water and the fin then regrows relatively quickly. The severity level of this procedure is expected to be mild. It is unlikely but possible that fish might develop an infection following removal of a small part of the tail fin, in which case we will either humanely kill the fish or treat it appropriately under the guidance the Named Veterinary Surgeon.

We very occasionally need to anaesthetise fish for in vitro fertilisation (IVF) and the expected severity

level is also mild. For both of these procedures it is possible that fish may not recover from anaesthesia but this is very unusual (less than 1%). At the end of the protocols fish will be humanely killed or supplied to other project licences or recognised establishments with the authority to breed and maintain genetically altered zebrafish of this type.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

The central aim of the project is to follow cells as they make decisions in their normal environment. Therefore, these experiments can only be performed in an animal. However, all experiments are performed at an early stage of life development (zebrafish embryos younger than 5 days old) which do not require protection in law.

An additional way to replace a large number of animals for scientific research, is to make use of mathematical models and computer simulations to ask whether a certain set of observations are sufficient to generate the biological process we are interested in. This helps us to define more precisely the experiments that are most scientifically interesting and of relevance to perform in the embryos on this project and will ultimately reduce the number of animals required for the project.

Reduction

Explain how you will assure the use of minimum numbers of animals.

All of our experimental work will be carried out in zebrafish embryos younger than 5 days old (which are not protected under The Animals (Scientific Procedures) Act 1986. Animals older than 5 days old will only be used for establishing genetically altered zebrafish for subsequent breeding. The number of adult animals used is therefore solely related to the numbers required to maintain sufficient breeding stocks of animals. We are using several methods to reduce the numbers of adult animals used. First, we will share relevant fish stocks with other users within the facility. Second, we will try to limit repeated breeding to once per week to optimise breeding performance. Third, we will minimize the generation of embryos wherever possible for our experiments. Fourth, we will freeze sperm from genetically altered lines of zebrafish for longer-term storage.

We will carefully design our experiments so that we use appropriate numbers of embryos for each experiment. Where necessary and possible, we will carry out pilot studies to determine the number of embryos required to achieve robust statistical analysis. If we require assistance in our experimental design, we will consult with a statistical expert. We will ensure that our publications conform to the ARRIVE guidelines: https://www.nc3rs.org.uk/arrive-guidelines.

To make our experiments robust, we will control for variability in the following ways:

We will reduce environmental variability by carefully housing breeding adult fish in the dedicated zebrafish facility and by keeping genetic background constant within each genetically modified line of fish.

We will assess normal levels of variability within experiments via pilot experiments, allowing us to select appropriate statistical methods and number of embryos

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

We use the zebrafish because (1) its anatomy and genetics are a good model for other vertebrate species; (2) its embryos are externally fertilized and can be obtained without harm to the mother; (3) they are large and near transparent, facilitating imaging studies.

We don’t envisage any suffering in licenced animals beyond the mild procedures described above. We will only use zebrafish embryos younger than 5 days old for our experiments, which are not yet capable of independent feeding or complex cognitive behaviours. We will aim to reduce any potential suffering of these embryos by promptly killing them using a humane, approved method at the end of the experiments and, where possible, by anaesthetising embryos that are sufficiently developed to be capable of initiating movement during imaging (those above 18 hours old).

Adult fish will be housed in a dedicated centralised zebrafish facility, where they will be looked after by full time staff, who will ensure their welfare. Numbers of fish per tank, water quality and food quality and quantity will be optimised andcarefully controlled.