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NON-TECHNICAL SUMMARY

Mechanisms controlling cell division and cell identity during development, reprogramming and disease.

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

cell division, cell identity, development, reprogramming, frog

Animal types

Xenopus laevis

Life stages

adult

Retrospective assessment

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

Objectives and benefits

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

What's the aim of this project?

The aim of this project is to understand the rules that cells use to control division and to specialize into particular types, such as nerve or muscle cells, during development and in response to reprogramming and disease.

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.

Why is it important to undertake this work?

One of the most important decisions a cell makes is to choose its fate. All aspects of a cell are defined by its identity: its shape and size, its migration and choice of neighbours, when it divides, and its ability to do specialized functions. For example, muscle cells are specialized for contraction, neurons for electrical activity, and red blood cells to carry oxygen.

Establishing and then maintaining the correct cell identity throughout a lifetime is fundamental for health. In several diseases, such as cancers and Parkinson's disease, mature cells have difficulty maintaining their specification. Most cells gain their identity during development and understanding these rules should help us to uncover how disruption of a cell's identity can cause disease. The knowledge from this work may also help to advance regenerative therapies that engineer cells to restore a normal function in the body, for example to replace the special nerve cells that are lost in Parkinson's disease.

What outputs do you think you will see at the end of this project?

This project is expected to discover novel fundamental insights into how cells control their identity. This will be during normal development of the embryo, and when cells are challenged to alter their fate during reprogramming or in diseases such as cancer. We will report our findings in publications in peer-reviewed scientific journals, and at scientific conferences.

Who or what will benefit from these outputs, and how?

In the short/medium term, our research and data are of potential interest to other scientists studying similar topics, such as developmental biology, mechanisms controlling gene expression, and reprogramming cells.

In the longer term, we anticipate that our work may be useful to researchers studying diseases. For example, understanding how cell identity can contribute to diseases may help in the discovery of new treatments. Understanding the rules of cell identity should also help scientists to develop techniques to

effectively reprogram cells into specific types. Such cells could then be used in cell therapy, a promising treatment in which new healthy cells are used to replace diseased or damaged ones.

How will you look to maximise the outputs of this work?

We value collaboration with other scientists and research groups, as a means of sharing skills and discussing ideas. For example, we will continue to collaborate with experts in reprogramming using frogs.

Our findings will be published open access so that they are available to all at no cost. If appropriate, we will make our manuscripts available on a preprint server such as bioRxiv. We will consider publishing unsuccessful approaches, for example as supplementary information in our manuscripts. The project may generate large datasets, for example from sequencing experiments, and we will deposit these in publicly accessible repositories for use by other scientists.

Species and numbers of animals expected to be used

- *Xenopus laevis*: 1000

Predicted harms

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

Explain why you are using these types of animals and your choice of life stages.

We use adult *Xenopus*, the clawed frog, and their embryos at early stages before they can feed independently. Adult females are used to obtain oocytes (immature eggs) and, after hormone-induced ovulation, eggs. The eggs can then be fertilized in vitro (i.e. in a culture dish or tube) to generate the embryos.

Frog embryos are an ideal model to answer our questions about how cells control when they divide and how they choose to mature into specialised types of cell. Practically, amphibian embryos have several advantageous features, such as external development and large size; they are robust, easy to culture and are amenable to various experimental manipulations. There are *Xenopus* Resource Centres that facilitate the production and distribution of genetically altered animals that we can use to address our aims. Moreover, there is extensive knowledge of *Xenopus* development, which we can use to understand our discoveries.

Reprogramming by transplanting nuclei into frog eggs or oocytes is a well-established procedure. We use this technique to investigate the mechanisms that allow or prevent the fate of a cell to be changed.

Finally, we use frog eggs/oocytes or embryos to make extracts that allow us to study the molecular machinery that controls cell division and cell fate decisions.

Typically, what will be done to an animal used in your project?

This is the typical procedure that will be done to a frog in this project: An adult female frog is injected with hormones to induce it to lay eggs. The female is injected under the skin at the base of the back into a lymph sac, which helps to spread the hormone throughout the body. Typically, females are given two hormone injections. The first is to encourage the female to produce a high number of mature eggs. The second injection, given approx. 2 to 14 days later, is to make the female lay the eggs. The female is placed in a salt water solution to lay the eggs, typically for 2 to 8 hours. This salt water mimics the condition inside the female's body to keep the laid eggs 'fresh' so we can use them for several hours after laying. Females are then returned to tank water. After a rest of no less than 3 months, females can undergo this procedure again.

What are the expected impacts and/or adverse effects for the animals during your project?

The female is expected to experience transient mild discomfort caused by the injection.

Rarely, in fewer than 2% of procedures, females do not lay their eggs properly and become bloated and lethargic. These animals will be killed unless there are signs of recovery within 24 hours.

Expected severity categories and the proportion of animals in each category, per species.**What are the expected severities and the proportion of animals in each category (per animal type)?**

Species: Xenopus

Expected severity: mild

Proportion of animals: 100%

What will happen to animals at the end of this project?

- Killed
- Used in other projects

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The development of an organism from a single cell is an orchestration of biological processes that vary intricately across space and time. This complex process cannot be replicated by cells in culture, and so we need to use embryos. The large size and rapid external development of frog embryos allows us to

study cell fate most easily and efficiently. Moreover, many mechanisms controlling development in frog are conserved in mammals.

We need to use oocytes/eggs for our reprogramming experiments, for example to investigate the features and factors that allow the identity of a cell to be changed. There is currently no alternative technique that will allow us to directly answer some of the questions in our project.

Which non-animal alternatives did you consider for use in this project?

We considered using tissue culture cells as a non-animal alternative.

Why were they not suitable?

This project will also involve complementary tissue culture experiments, for example using stem cells. We can only use tissue culture cells to address some of our questions because cells grown in dishes cannot recapitulate the development of an embryo nor provide the reprogramming environment of oocytes and eggs.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

We have estimated the number of animals we will need to use in this project based on our planned work and our previous use of frogs for similar experiments.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Female frogs can lay hundreds of eggs per ovulation. We keep females in a salt solution during the day as they are laying the eggs. This means we can collect large numbers of eggs, for example to prepare egg extracts. It also allows us to continue to use eggs throughout the day, for example to generate small batches of embryos as they are needed.

We re-use females for superovulation multiple times, as is standard practice in the *Xenopus* research community to reduce the number of animals used. We do not set an arbitrary maximum number of times we superovulate (administer hormones to stimulate egg production) each individual female. The eggs need to be of sufficient quality to be useful in our experiments. So, we monitor the quality of eggs produced to identify females that are 'good layers' suitable for re-use.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

The quality and yield of eggs varies between individual superovulated females. When possible, we share 'good quality' eggs with other researchers.

The double ovulation procedure (in which an individual female lays eggs twice with a 7-14 day rest between each ovulation) is being used more commonly in the Xenopus research community to reduce the number of animals. We will trial double ovulation, if appropriate for our experimental needs and if the procedure does not increase the harm each female experiences.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

We will use an amphibian model Xenopus, the clawed frog, in this project. Some of the frogs may be genetically altered, but the genetic modification is not expected to cause any harm.

We inject adult females with hormones to induce them to lay eggs. We may induce ovulation in the same female on multiple occasions. This is similar to what occurs in the wild, where Xenopus females ovulate multiple times in a season.

The eggs and immature embryos we will use in our experiments are not protected animals (under the Animals (Scientific Procedures) Act). They will be used in several methods. Early stage embryos, produced by in vitro fertilization of the eggs, can be manipulated using micro-dissection and micro-injection of various reagents, for example to change the expression of specific genes. Oocytes / eggs can be injected with nuclei to assess reprogramming. Eggs /embryos can be crushed to make extracts.

Why can't you use animals that are less sentient?

The majority of our experiments use eggs and immature frog embryos. We are using frogs because they are an excellent model for vertebrate embryo development and the large size of the embryos allows us to easily micro-manipulate them. We cannot use less sentient models, such as Drosophila, as they do not have these advantages.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We will consult our specialist Xenopus NACWO who is proactive about improving the welfare of our Xenopus colonies. We will continue to follow improvements in colony care, for example individual frogs are photographed for identification, which is a minimally invasive ID method and improves clinical monitoring. Animals are kept in tanks with dark backgrounds and refuge tubes, which reduces stress. We have observed that females maintained in such enriched environments produce better quality eggs. We will source our animals from in-house breeding when possible. This reduces the need to import animals from other facilities, which not only removes the stress associated with transport but also reduces the risk of introducing pathogens into the colony.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will follow guidelines for planning and reporting research involving animals, for example the checklist in PREPARE (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence) and ARRIVE (Animal Research: Reporting of In Vivo Experiments). <https://norecopa.no/prepare>; <https://arriveguidelines.org/>.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We discuss best practice with colleagues in the Xenopus research community and with the Chief Aquatic Technician at our facility. We will consult and consider guidance from the Named Animal Care and Welfare Officer and the Named Veterinary Surgeon. We will also refer to material provided on the NC3Rs website (<https://www.nc3rs.org.uk/3rs-resources>). I am currently on the European Xenopus Resource Steering Committee where new developments in frog husbandry are often discussed.