Home Office

NON-TECHNICAL SUMMARY

# Role of Arp2/3 isoforms in mouse development and tissue homeostasis

**Project duration**

5 years 0 months

**Project purpose**

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

**Key words**

Actin cytoskeleton, Development, Immunology, Neurology

| Animal types | Life stages |
|---|---|
| Mice | embryo, neonate, juvenile, adult, pregnant, aged |

## 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?**

To understand how the cell's internal skeleton is regulated by specific protein complexes during tissue formation and maintenance, particularly in blood, brain and immune function.

**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?**

Actin, one of the most abundant proteins of the cell, assembles into linear polymers to make up the actin skeleton of the cell. This internal actin skeleton provides the driving force and structural support for the physical integrity of cells and a wide range of essential cellular processes such as cell migration and adhesion. The precise spatial and temporal regulation of the actin cytoskeleton is crucial during the development and throughout the lifetime of multi-cellular organisms. Consequently, dysregulation of the actin cytoskeleton impacts tissue and organ function including the brain and the immune system, and in the worst case, the development and lifespan of an organism. Studying how the Arp2/3 complex which is unique in the cell in promoting the formation of branched actin polymers to regulate the function of the actin cytoskeleton is therefore key to understanding human development and tissue maintenance to uncover the basis of a wide variety of human diseases such as hereditary ataxia and combined immunodeficiency .

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

The main object of our work program is to obtain a detailed understanding of the role of Arp2/3 complexes in regulating cellular processes controlling the development and function of organs and tissues. The outputs of our project will be mainly in the form of publications in peer-reviewed articles and presentations at scientific conferences. This project will create novel mouse strains, which will be available for the wider research community. It will also provide insights into the basis of human conditions and diseases such as neurodegeneration, inflammation, and immunodeficiency.

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

Emerging genetic evidence in humans has already established the importance of actin cytoskeleton and the Arp2/3 complex in human development and health. For example, loss of function mutations in human ARPC1B, the gene encoding one of the proteins within the Arp2/3 complex, leads to Wiskott-Aldrich syndrome-like symptoms including severe inflammation and immunodeficiency. Mutations in the genes encoding the other proteins of the Arp2/3 complex are also predicted to impact human health. Therefore, to fully understand the function of the actin cytoskeleton, we will need to study how it is regulated by the Arp2/3 complex in living animals.

In the short term, the new scientific insights obtained from our work will be beneficial for other scientists including developmental and cell biologists investigating how regulation of the actin cytoskeleton controls the form and function of cells and tissues during development and adult life. In the long-term, the phenotypes and molecular mechanisms we uncover will also benefit scientists and clinicians trying to understand the underlying basis for pathogenic and genetic conditions of the nervous and immune systems that lead to disease or dysfunction. Basic scientists and clinicians will also benefit from the mouse strains we will generate during this project.

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

We will share our data, analysis tools, and resources with other scientists, using scientific communications and collaborations, repositories, and our lab webpage, which will reduce the number of replicated experiments in the field. We will disseminate our research by publishing results in peer-reviewed journals. We will also present our work to academic peers at scientific conferences and engage with public partners to disseminate our results.  The mouse strains we generate will be shared with collaborators and the wider community (scientists and clinicians).

**Species and numbers of animals expected to be used**

- Mice: 28000

# 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 will investigate how Arp2/3 complexes regulate mammalian development and tissue maintenance, consequently, mice represent the best available animal model given the many similarities with human development and availability of genetic tools.  As we are investigating the changes that occur during animal foetal development and throughout their lifetime, it is necessary to use embryonic, postnatal, adult, and aged mice.

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

The majority of our experiments will involve the generation and breeding of genetically altered mice. Embryos and animals may also receive antibiotics and other drugs such as Doxycycline, Tetracycline and Tamoxifen through injection or via their diet and water in order to induce gene deletion or activation. Animals will be tested for gait, cognitive development, and social behaviour to evaluate their neuronal function. To examine the changes in the actin cytoskeleton and the Arp2/3 complex during the normal aging processes, animals with no phenotypes may be kept for up to 16 months and animals with adverse effects for up to maximum 12 months, respectively. When needed animals will be housed in the Digital Ventilated Cage (DVC) for 24/7 monitoring and analysis of animal activity to detect any behavioural changes. Cell labelling reagents such as 2-deoxy-5-ethynyluridine (EdU) and Adeno-

associated virus (AAV) may be introduced via injections prior to the humane killing at later time points. Blood may be withdrawn by microsampling (<1% total blood volume) every 7 days or at maximum <15% total blood volume in 28 day intervals. In some cases, general anaesthesia and contrast imaging agents will be administrated prior to non-invasive imaging up to once a week but no more than 20 times during an animal's lifetime. Generally, administration of substances and behavioural tests will only cause transient discomfort and no lasting harm.

To investigate the role of the actin cytoskeleton in blood and immune cell function, we will immunologically challenge animals using immunity inducers such as egg white, induce colitis for 5 days using DSS or infect animals with intestinal pathogens as these procedures will induce conditions that are similar to that seen in humans with deficiencies in the Arp2/3 complex. The animals will be followed for a maximum of 6 months and may also receive antibiotic treatments before and during the procedures. These treatments will be performed once, after which animals are killed. These analyses will also involve the isolation of immune cells from genetically altered animals, which will then be transplanted into the host animals (commercially available immunodeficient mice or mice treated with whole-body irradiation beforehand). When required, these different procedures will be performed in combination.

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

The majority of genetically modified animals we will generate are expected to display either no phenotypes or mild phenotypes, which do not impact the behaviour, gross appearance or welfare of the animal but are only evident at post-mortem after humane killing. In some cases, a limited number of animals will experience moderate phenotypes: these might include abnormal gait and loss of motor coordination after 3 months as a consequence of neurodegeneration and possibly laboured breathing due to cardiovascular dysfunction as well as limited weight loss, scruffy fur, and hunching in response to infection. Animals with motor coordination defects will be humanely killed before they display clinical signs such as reduced and/or misdirected movement, abdominal discomfort, hunched posture, or ruffled fur.

**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)?**

Based on our experience to date, we are expecting less than 45% of animals to exhibit moderate severity. All other animals will only have mild severity.

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

- Killed

# 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?**

Laboratory-based experiments in the test tube (biochemistry) or in cells in a dish (cell biology) have greatly enhanced our molecular understanding of how the actin cytoskeleton regulates processes that control the form and function of cells. Such analysis may guide but will not fully uncover how the actin cytoskeleton controls mouse development and tissue maintenance. This is because these highly complex processes involve the dynamic interaction of multiple proteins, cell types and physiological systems, that need to work together and change over time. Currently, no cell-based system in a dish can fully replace or replicate all these factors. Therefore, to fully understand the function of the actin cytoskeleton and disease we will need to study how it is regulated in living animals. Our in vivo studies may also expose phenotypes that implicate Arp2/3 isoforms in various idiopathic diseases, thereby opening up new targets in disease treatments.

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

We currently use a variety of biochemical assays with purified proteins as well as cell-based approaches including live-cell imaging to characterise and study the regulation of the actin cytoskeleton. The results from these non-animal-based experiments provide mechanistic and molecular insights into the function and regulation of the actin cytoskeleton. Importantly, this information as well as hypotheses generated from experiments in test tubes and cells on a dish helps us to reduce the number of animal-based experiments by only focusing on the function of Arp2/3 complexes in controlling the actin cytoskeleton in specific tissues.

**Why were they not suitable?**

The isoform-specific Arp2/3 function cannot be investigated in non-animal organisms such as yeast and fruit flies as they do not have these Arp2/3 isoform genes. Biochemical and cell-based approaches are extremely powerful; however, they do not supplant the use of animals to understand the function of Arp2/3 isoforms in regulating the actin cytoskeleton. This is because cells in a dish fail to capture the full complexity of tissue organization which involves the complex and integrated interaction of many different cell types and the communication between different organs and systems (e.g., immune, hormonal, nervous etc) of the animal. We will consider organoid cell culture differentiated from ESC and patient iPSC as non-animal alternatives. Those cells can self-organise tissue-like structures that will help us to refine the design of our animal experiments and reduce animal use.

# 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?**

Our animal use projections are based on three main factors:

1. The number of genetically modified lines we are currently maintaining.

2. The number of genetically modified lines we are planning to create.

3. The complexity of the crossing schemes to generate experimental animals.

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

We use several strategies to limit the number of mice we use including:

1. Where possible we use both sexes for all experiments and always maximise tissue usage and the amount of data we get from each mouse.

2. We will use the NC3R's Experimental Design Assistant or similar software such as G*POWER to perform power calculations for each experiment.

3. We follow PREPARE guidelines and the checklist.

4. Avoid duplication of any work that has already been published by other groups, after extensive literature searches of published work on Pubmed and the BioRxiv pre-print server.

5. Where possible use results from in vitro cell culture models and ex vivo tissue culture models to generate hypotheses to guide our animal-based experiments.

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

In addition to the measures outlined above, we ensure that all lab members minimise animal numbers through the following approaches.

1.  Being fully aware of all aspects related to the 3Rs and using available online resources such as Experimental Design Assistant pages.

2.  Carefully review our mating strategies to ensure we generate the maximum number of animals with the desired genotypes with the minimal number of breeding steps and avoid overbreeding. Where possible, we will set up pairs of homozygous and heterozygous to produce double the number of homozygous offspring when compared to the mating of heterozygous pairs.

3. Regularly review our mouse colony and where possible archive strains by freezing sperm and embryos.

4. When available, we will use in-house strains from our animal facility to promote efficient breeding. Most of our strains except for experimental strains will be maintained by intermittent breeding with in-

house wild-type mice to reduce animal use (generally breeding twice a year rather than constant breeding; NC3Rs guidance for breeding and colony management).

5. Use the minimal number of mice needed for statistical power when testing an experimental hypothesis based upon pilot experiments to inform on the numbers required. Also, use published and unpublished data in the lab to optimise the animal number required.

# 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.**

The mouse is chosen because it is the least sentient mammal that expresses all eight Arp2/3 isoforms that is suitable for efficient genetic modifications. Mouse embryonic development, normal biology, and behaviour are well described, and there are well-established husbandry techniques for this species, including efficient analgesic methods. Many useful tissue-specific or inducible Cre mouse lines already exist that can be crossed with our Arp2/3 isoform mutants, obviating the need to create more transgenic Cre lines. The mouse is also suited to study the physiological regulation of the actin cytoskeleton by the Arp2/3 complex because the biology and pathophysiology of mice are relevant to humans. Mice will therefore serve as an accurate animal model for a wide range of human conditions and pathologies. For example, disease-causing mutations, subsequent pathogenesis, and subtypes of neurodegenerative diseases including Parkinson's disease and spinocerebellar ataxia are well conserved in humans and mice. Most of our studies will be performed on dissected tissue and ex vivo tissue culture from humanely killed animals.

To investigate the function of the actin cytoskeleton in blood and immune cells (i.e. haematological and immunological function), we will use the following methods by injection or intake through food and drinking water: exposure to inducing agents (e.g. OVA and LPS) that are expected to cause no or mild harm regardless of the genotype background, bacterial infections that may cause gastrointestinal discomfort in some mutant strains and DSS-induced colitis model. These models and procedures will allow us to uncover insights into human disease as they induce conditions that are similar to those seen in humans with deficiencies in the Arp2/3 complex. As these methods/approaches are well described, it is straightforward for us to know/use the lowest concentration of agents or pathogens to apply to our mice to ensure their well-being while still achieving our goals.

To investigate the function of the actin cytoskeleton in the nervous system (brain), we will use a genetic approach to ablate Arp2/3 isoforms. In some cases, our genetically altered mice develop abnormal behaviour coincident with progressive loss of neurones and/or their function. These phenotypes, which result in reduced coordination replicate the normal aging processes rather than traumatic brain injuries. To further minimise suffering and harm to animals, we will modify genes only in neuronal cell types and/or temporally rather than in the whole animal and permanently where possible. We will assess

animal gait, cognitive development, and social behaviour by the established behavioural tests that do not lead to pain or distress to ensure animal well-being during analyses.

**Why can’t you use animals that are less sentient?**

The major development of the nervous and immune systems happens after birth even though those systems first appear during foetal development. Given this, we cannot use embryos to study the physiological regulation of actin cytoskeleton during tissue development and maintenance, with emphasis on blood, brain, and immune cells. Where possible, ex vivo tissue culture from animals in the early stage of life will be used to uncover phenotypes to reduce animal numbers and suffering. The mouse rather than another species such as fruit flies or worms is used because they are the least sentient mammal that expresses the eight different protein complexes that we are studying.

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

Animals with neurological phenotypes and impaired movement by genetic alteration or aging may result in a reduction in feeding and water intake. Given this possibility, in addition to weekly weighing, these animals will be offered increased monitoring according to a scoring sheet that includes the following parameters: activity; posture; movement/gait; coat condition; breathing, and dehydration (as per Wilkinson et al., 2019; DOI:10.1177/0023677219865291). Experimental animals are weighed weekly and extensively monitored for pre-/post-procedure health and good welfare on a daily basis according to The Mouse Grimace Scale available at NC3Rs. Animals will be offered wet food as well as additional bedding and cage enrichment if necessary. Animals exhibiting detrimental phenotypes that are not common in our project will be immediately humanely killed. Pilot studies guided by previous observations on DSS-induced models in the establishment will be performed to determine the minimal dose of DSS required to induce colitis in our mice.

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

We will follow the NC3Rs website for the general principles of blood sampling and substance injection. We will follow PREPARE guidelines for experimental design, sample size, and ARRIVE for reporting our results. In general, we will follow guidance from “Refining procedures for the administration of substances” (Morton et al., 2001; DOI:10.1258/0023677011911).

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

We will stay informed of best practices, 3Rs and any implementation in animal handling through newsletters and communications on slack regularly published from our animal facility. We will seek further information from the NC3Rs website and our NC3Rs programme manager. This includes by finding NC3Rs projects relevant to our studies particularly in the brain and immunological research at NC3Rs our portfolio page as well as attending 3R Online-Seminar series “Alternatives to animal use in research and education – Refine, Reduce & Replace“, provided by Berlin-Brandenburg research platform BB3R.