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Project summaries granted during 2013

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  Foodborne bacteria Escherichia co/i Campylobacter Food safety Food security
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- **Targeted anti-cancer therapies**

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- **Investigating cell viability/integrity in vivo**

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  adipocytes, diabetes, miRNAs, obesity, microbiota
- **Nervous System Injury and Repair**

  Spinal cord injury, traumatic brain injury, stem cells, gene therapy, regeneration

Using Xenopus and zebrafish, to study development

The purpose of the project is to increase our understanding of embryonic development. In order to do this, we use frogs (Xenopus laevis and Xenopus tropicalis) and zebrafish (Danio rerio), which provide an excellent source of large, accessible embryos.

During embryonic development, a single cell (the fertilised egg) gives rise to many different kinds of cells, each with their own function (for example, red blood cells, which carry oxygen around the body and muscle cells, which contract). One of the major questions in biology is how this process of differentiation occurs. It has become widely accepted amongst developmental biologists that cells in the embryo communicate with each other through cell-cell signalling such that when a cell receives a message from another cell it responds by turning certain genes on or off. This differential gene expression is thought to underpin the establishment of different types of cells and tissues.

Both *Xenopus* and zebrafish provide very good experimental systems for studying embryonic development. The eggs are relatively large and, since they develop outside the mother, they can be observed directly throughout their development. This makes getting information on Xenopus and zebrafish embryos relatively straight forward as they are accessible from the single cell stage, and protocols to manipulate genes and to create reporter and mutant lines are available.

The decisions about what kind of cell to become occur very early in development (within the first day or two following fertilisation), so it is not necessary to keep the embryos for more than three days. *Xenopus* tadpoles do not start to feed until after 7 days of development, and zebrafish feed after 5 days; up to this point, the embryos are not included in the Home Office Animals (Scientific Procedures) Act 1986. However, the adult frogs that provide the eggs are protected under the Act.

To obtain eggs, a female frog is injected under the skin on the back with a hormone to induce her to release eggs. The hormone has no ill-effect on the frog, and only accelerates the natural process of ovulation. The females are injected by licensed researchers, experienced in handling frogs. Each female lays hundreds of eggs, providing a large quantity of experimental material. Injections are carried out once every four months, which allows the frogs a rest period during which they produce more eggs. As long as the female frogs stay healthy they can live for many years in a laboratory colony. Zebrafish will produce embryos in the morning, if a male and female have been placed together the evening before. There is no need to induce fish to lay eggs.

Once we have generated embryos we use them to find out what particular genes do in development: What tells a cell to become a muscle cell? How do cells talk to each other? How do cells moderate their responses? We address these questions by either causing an embryo to have too much or too little of a specific gene. Most of the time we can learn what we need to using the embryos prior to their becoming protected. Sometimes we need to raise lines of fish or frogs that are genetically altered. We do this to produce fish or frogs that have sets of neurons or muscle cells that are fluorescent. Using special microscopes, the development of these cells can be watched *in vivo* providing insight into developmental processes. Generating a mutation in a specific gene will prevent it from working. Analysing animals that have been targeted such that specific genes do not work (mutants) provide

valuable insight into the normal function of that gene. These mutant and transgenic lines of frogs and fish are also protected and we need to justify generating and maintaining these animals.

Developmental mechanisms are very similar between frogs, fish and mammals, including humans. This means that what we learn about the genes that regulate frog and fish development is very likely to be applicable to human development. Notably, it is often these same genes important for embryonic development that malfunction in cells that become cancerous. In other words, better understanding of the mechanisms of development gained by the basic research undertaken under this project licence could ultimately underpin the development of new treatments for human disease.

Environmental effects on the physiology and behaviour of fishes

This project will examine the effects of environmental conditions on aspects of behaviour and physiology in fish, and in particular how those traits that affect performance and fitness. During the last several decades there has been increasing concern that human activities may be having a negative effect on the natural environment. Aquatic habitats in particular have displayed rapid shifts in temperature, oxygenation, and carbon dioxide levels. Changes in the abundances and distributions of several aquatic species have been linked to these environmental changes, but there is a distinct lack of information on the physiological mechanisms that influence the response of animals to aspects of environmental change.

It is critical that we obtain this information so we can understand how animal populations will be able to respond in the face of a rapidly changing natural environment. The work must include live animals since the aim is to examine complex and long-term effects on performance: as yet our understanding of the processes involved is too superficial to allow us to predict any such effects from theoretical models or *in vitro* experiments. The work will use approximately 5000 fish over the duration of the five year project.

The experiments will use a range of live freshwater and marine fish species. Fish from freshwater and marine habitats each experience unique environmental challenges that can affect aspects of their physiology and behaviour. The planned series of experiments will involve manipulating key features of the aquatic environment in laboratory aquarium tanks (e.g. the temperature, dissolved oxygen or carbon dioxide levels, food availability) and testing the effects on a range of traits including social behaviour, foraging, predator avoidance, mating behaviours, growth patterns, metabolic expenditure, and swimming performance. Holding tanks and experimental arenas for observing behaviour will replicate key features of the natural environment (e.g. by using aquatic plants, low light levels, tanks where appropriate).

Most interventions will be mild and so it will be possible to minimise the number of animals used by measuring a range of such traits in the same individuals; this will also allow us to test how different traits are related and whether there are synergistic effects. Experimental fish will often be unaffected by the procedures and so, where possible, they will be discharged from the control of the Act at the end of the experiments, either back to a breeding stock population or (if appropriate) released to the wild.

The results will reveal the key environmental features that influence the viability of marine and freshwater fish, and so will help in the conservation and management of these animals.

Tumour targeted drug delivery using FUS under MRI guidance

Our main objective is to develop new solutions to overcome the current challenges of cancer chemotherapy by using ultra-sound as a treatment, in addition to its traditional role as a diagnostic tool. We also wish to develop systems that will allow the delivery of new treatments by minimally-invasive means under the guidance of live images obtained in a magnetic resonance scanner.

Our initial work is aimed at understanding the different ways in which focused high- intensity ultrasound can work in delivering therapy to cancers. Firstly, ultra-sound can cause local heating of the targeted tissues. Medium-intensity ultra-sound is already used for this purpose to deliver gentle heat to deep tissues in physiotherapy, for example. At high intensities, the local heating can be sufficient to cause cell death and, if targeted precisely at tumours, a potentially safe and effective therapeutic effect.

Secondly, ultra-sound can cause the generation and then the destruction of tiny bubbles in the targeted volume. The collapse of these bubbles, so-called “cavitation” can weaken the membranes of nearby cells, causing them to leak and die. A lower level of damage can result in better penetration of therapeutic drugs to the targeted tissues, resulting in a highly local but effective treatment. This could be enormously beneficial in cancer therapy, where the available drugs are often fairly toxic and are associated with side-effects that can limit the doses that can be delivered. Any improvement in the local uptake of these agents specifically in the tumour could therefore result in a better outcome from the disease, fewer side-effects, or possibly both.

Cavitation can be greatly enhanced by administering pre-formed micro-bubbles, tiny spheres filled with an inert gas. Local delivery of the ultrasound to the tumour again ensures that destructive cavitation, or enhanced delivery of drugs, only happen where they are expected to be beneficial.

Another use of ultrasound in cancer therapy is to cause the local release of a toxic drug from a ‘carrier’ molecule, which otherwise ‘hides’ its toxicity from the rest of the body. The complex of the drug and its carrier can be administered and will circulate with essentially no effect. However, if ultrasound is focused at the tumour, then the active drug is released from its carrier. This should result in very high concentrations of the active drug being achieved in the tumour, but much smaller levels elsewhere. Any drug-carrier complex remaining in the system can be harmlessly excreted.

All of these potential treatments require further development in intact animals before they can be tested in people. It is important that the tumour is three-dimensional, that drugs and other agents are delivered to it via a bloodstream and that the risk of damage to other structures can be shown to be minimal.

None of these requirements can be assessed without using living systems.
We shall start with a well-established animal model of human cancer, in which tumours are implanted just beneath the skin of mice or rats. The tumours are not expected to cause the animals any significant harm and can readily be measured before and after treatment. Because of our expertise in imaging, we can also obtain detailed pictures of the interior of the tumours, entirely non-invasively.

However, although surface tumours like these are easy to work with and measure, they do not represent the same therapeutic challenges as the majority of solid cancers in humans do, where the tumours are usually within internal organs. We therefore intend to develop new animal model systems for human cancer, in which tumour cells are implanted within the body, using a needle steered accurately to its target under image-guided control. Such model systems are already in use in laboratories around the world, but they required a surgical procedure to implant the tumour cells.

We believe that our minimally-invasive approach will produce equally useful systems in which to develop better cancer treatments, but avoid the need for animal surgery.

Cardiovascular device development

Open heart surgery remains a complex procedure in which devices replace the function of the heart and lungs for a period of time. Although generally safe, these devices may cause some injury to patients which can have significant impacts on health. One focus of our work is to understand the mechanisms of this injury, and to develop devices and techniques to improve this procedure in patients. A second is to develop new devices which can be used to repair the heart and circulation of babies born with defects. Artificial blood vessels will be developed that will grow as the child grows, thus eliminating the need for repeated surgeries to compensate for growth.

During open heart surgery, damage can be caused to the blood, tissues and organs of the patient; the result of a complex interaction between the patient and the life support device. This complexity means that the mechanisms of injury cannot be studied other than in intact animals, with examination of organs, tissues and blood chemistry key to understanding. In developing implantable devices for treating children with complex heart defects, the understanding of the interaction between the device and the surrounding tissue is the key to producing devices that grow at the same rate as the child.

The simulation of child growth cannot be done in the laboratory and can only be achieved using animal models. Much of the development of the proposed devices will be done using sophisticated computer modelling techniques and extensive laboratory prototyping and testing, and no animal experiments will be carried out until enough supportive evidence is gathered from the preceding steps. The number of animals is therefore minimised by ensuring that the devices are sufficiently well developed to provide maximum success from each animal experiment.

Rats and pigs are to be used in the proposed experiments, because these animals have very similar heart and blood vessel anatomy to us and respond in a similar way to open heart surgery. A lot of scientific literature already exists about the use of these animals models, and this background data will inform the experimental setup and maximise understanding of our results.

Animals will undergo open heart procedures that are almost identical to those carried out on patients in hospital operating theatres, including anaesthesia, life support and surgical procedures on the heart. There is a small risk of a rupture of blood vessels resulting in a large loss of blood, under which circumstances the animal would be humanely killed. There may be some pain and discomfort during the recovery phase. This will always be managed by the administration of pain control drugs.

This research will benefit adults and children through an improved understanding of the mechanisms of injury associated with open-heart surgery, and development of improved treatments and devices, It will also benefit children who require implantable devices to repair complex heart defects, by reducing the number of surgeries they would otherwise have to undergo to replace devices that they have outgrown with larger sized ones.

Mechanisms and strategies for brain damage and repair

This work investigates mechanisms and strategies for limiting damage and improving recovery after stroke and brain damage. We will use several strategies to limit damage and promote recovery including improving the success of stem cells and taking advantage of the inflammatory responses.

Stroke is the single most common cause of neurological disability in the UK. Every year, over 150,000 people in the UK have a stroke with most people being over 65. One in four who live to be 85 years of age will suffer a stroke. In addition, older people experience more severe strokes, with worsened outcome. Hence stroke is one of the main causes of the decreased functional capacity and increased disorder and impairment associated with the ageing process and causes a major threat to independence and life quality in older people. Despite this there is a paucity of effective therapies for stroke. With the ever increasing ageing population, the burden to stroke patients, their families and friends let alone the NHS is only going to increase unless successful interventions are developed to limit damage and/or improve recovery after stroke.

Stroke is a blood—flow related phenomenon that causes a complex cascade of events in compromised tissue and can not be fully replicated in non-animal studies. The brain has a complex cellular structure involving different cell types and different cellular compartments and brain damage and repair involves interplay between these cell types, components and compartments. Non-animal studies have their limitations for modelling stroke and do not allow behavioural and cognitive assessments of outcome. In addition there is an additional step of translation from non-animal to animal to human. Animal models of stroke produce reproducible lesions under controlled environments with reliable means of quantifying brain damage and provide the best means for determining insight into key mediators of damage and repair. However, where ever possible, non—animal studies are used. For example we optimise cell systems using non-animal studies prior to animal use and also are able to gain information on cell function from non-animal studies and are integrated with the animal studies.

We aim to maximise signal to noise ratio in our animal experiments by controling variability within groups, improving power of the experiments and reducing numbers used. This is done by performing pilot studies to improve study design and success, controlling conditions under which animals are kept, acquiring statistical input in all studies. In addition we have developed protocols that allow assessment of several outcome measures in the same animals. This work has been designed so that any distress or discomfort that might be caused to the animal is kept to a minimum. All techniques outlined in this application have been refined as much as possible to achieve the least suffering of the animals yet to achieve meaningful satisfactory scientific results which will benefit the progress of stroke research. We are continuously monitoring, review and refining our protocols. All animals are monitored closely postoperatively as required.

Vet advice is sought as required and we have good knowledge of the indicators of pain and distress or disturbances of feeding and drinking and clear endpoints are defined for termination of the experiment. In addition we use non-invasive imaging tools for internal controls making the data more robust. Rats and mice have similar cerebral architecture to humans (circle of Willis) and are the least neuroscentient animals possible that can be used for this work.

The procedures used involve inducing a lesion in the brain. This is to mimic what happens during a stroke. The adverse effects that can occur include swelling of the brain. This usually

occurs within the first 3 days and the animals are heavily monitored during this period and as described above we have clear endpoints for termination to avoid animal distress. The results generated will be used to inform on clinical trial design and help direct future strategies for improving recovery after stroke.

Enteric pathogens — pathogenesis and control

The topic of this project is a group of bacterial pathogens that can infect multiple host tissues and/or multiple host species, often with differing consequences in these different situations. The bacteria targeted in this Project are particularly associated with livestock species and include several zoonotic agents that can infect humans through contact with contaminated food, water or environment. The impact of diseases caused by these bacteria on animal and human health and welfare is high although many of these diseases may be preventable through adoption of appropriate control measures.

It is increasingly apparent that bacteria and their hosts interact through multiple determinants which, in combination, direct the outcome of infection.

These bacterial and host factors can make different contributions during infection in different tissues/species and a thorough understanding of their contribution during infection in different niches is required in order to advance understanding and develop effective interventions and control. The overall aim of this project is to characterise microbial or host factors that confer, promote, diminish or prevent colonisation, persistence and disease in order to develop optimal methods for diagnosis and control for welfare, safety and economic benefits.

To do this requires use of a range of experimental approaches including studies on isolated pathogens and laboratory-based infection systems using cell culture systems; investigation in animals is unavoidable since these pathogens inhabit mammals as part of their normal lifestyle. Natural infection with these pathogens occurs in a range of large and small animal hosts, and the selection of animals for the investigations proposed reflects this diversity and will vary depending upon the pathogen-host system. A range of animal species is incorporated into the project proposal including livestock species and rodents — the latter are required for comparative purposes and, more particularly, to elucidate how and which host responses promote resistance/protection against infections.

For the pathogens under investigation, determinants that confer infection in different hosts are not fully defined and there is a need to undertake these further studies, particularly in livestock species, to characterise virulence factors, disease mechanisms and immunity. By doing so, effective means for preventing or otherwise controlling these infections in animals and/or their onward transmission into human populations can be developed and assessed. Wherever possible, materials from animals will be analysed for multiple purposes at the time of investigation and will also be archived to provide a resource to promote further analyses at later times. Procedures utilised are well-established and will be undertaken by highly experienced veterinary and animal handling staff to ensure that animals receive the highest standard of clinical care and attention.

Throughout the project, animal numbers will be kept to a minimum necessary for the specific questions addressed — each proposal will be individually appraised through a procedure including thorough statistical and ethical assessment as part of the approval process.

The Automatic nervous system in health & disease

Summary
Alpha-blockers are drugs that can be used to treat cardiovascular and urological problems in humans. We wish to continue breeding mice in which the target proteins of the alpha-blockers (alpha-receptors) are missing and thus determine the importance of these receptors and the effect this deletion has on other related receptors which are also therapeutic targets in humans.

Why?
There are 6 different alpha-receptor targets (subtypes) that alpha-blocker drugs can act at. However, at present, we do not have a set of drugs that can adequately block individual subtypes. This has resulted in an incomplete understanding of how blood vessel diameter is controlled (via the alpha-receptors) by the nervous system. There are other receptors on blood vessels that are also involved in the control of blood pressure but we do not know how these interact with the dominant alpha-receptors. By genetically removing 1 group of receptors (alpha 1-receptors) we can determine the effect this has on the remaining receptors (e.g. alpha2-receptors, beta-receptors, ATP-receptors, angiotensin-receptors). This improved understanding of the mechanism of nervous control of blood vessels will lead to the development of better drugs for treating hypertension.

Project plan
Blood vessels are removed from normal mice and mice lacking all 3 types of alpha l-receptor. The blood vessels are then examined in an apparatus that enables stimulation of the surrounding nerves. The response to nerve stimulation in the presence and absence of various drugs provides information on the function of the various receptors. Microscope-based studies using fluorescent drugs report on the cellular distribution of receptors within the blood vessel wall.

Use of Mice
Early developmental work focussed on the use of artificial cells which can be forced to express a particular receptor type. However, single cell work does not permit the study of the nerve-muscle interaction which is central to the control of blood pressure. Unfortunately, there is no alternative to taking a complete blood vessel from an animal. Our statistical power calculations indicate that, for genetically identical animals, we require to use 4-6 mice per experimental procedure. Therefore our maximum usage would be 276 males per year. However, we estimate a need for around 500 per year in order to maintain a steady supply of males of an appropriate age. We choose to use the mouse due to the ease with which the genetic mutations can be made.

Suffering
The proposed work will be done on vessels taken from mice following a humane kill. The only procedure to be performed on live mice will be monitoring of blood pressure which is regarded as a non-invasive and non-harmful. This procedure involves places the mouse in a restraining tube and attaching a tail cuff. The entire procedure takes no more than 30 minutes. No adverse effects are expected.

Benefits.
A complete understanding of the way in which blood vessel diameter is controlled by the nervous system will ultimately lead to the design of much better (more specific) drugs to treat

diseases of the cardiovascular system. The project will make a significant contribution to this understanding.

Targeted anti-cancer therapies

Aim
The aim of this work is to develop new delivery systems able to carry anti-cancer therapeutic DNA and drugs specifically to the tumours, without secondary effects to normal tissues.

Reasons for this work
At least 1 in 3 people in the UK will be diagnosed with cancer during their lifetime. Therefore improved treatment of cancer would greatly reduce suffering and save many lives.

The efficacy of conventional therapy is often limited by its difficulty to selectively reach tumours after intravenous administration, without secondary effects to normal tissues.

Novel targeted delivery systems have the potential to be highly selective for the tumour and thus to significantly improve therapeutic responses after intravenous administration of anti-cancer treatment.

To date, these novel therapies have been tested on cancer cells *in vitro* with encouraging results. However a whole organism bearing a tumour is necessary to verify the delivery of these therapeutics to the tumour and the absence of any side effects.

Mice are the species of choice for these studies because they demonstrate many features of the human diseases and the genes involved are common to both species.

Project plan
New delivery systems will be extensively tested in cell culture and only those of proven efficacy are advanced to *in vivo* studies, first to establish suitable dosing, then to evaluate their biodistribution. The delivery systems showing suitable biodistribution will then be tested for efficacy in a tumour-bearing animal. Efficacy will be measured as tumour growth delay, by calliper measurement of subcutaneous tumours. A pilot study with just a few animals will indicate if further work would be appropriate.

We expect to use a maximum of 1180 rodents per year. The number of animals used is the minimum that will give any statistically significant results. If fewer mice are needed to get the results required then lower numbers of mice will be used.

Animals will be housed in groups in cages with soft bedding and environmental enrichment (i.e. plastic houses). Good husbandry, daily monitoring and care by a team of well-trained animal technicians will ensure that animal welfare is paramount.

In all experiments the mice will be monitored closely to ensure that no unforeseen adverse reactions cause distress to the animals. Tumours will be established in mice by a single injection of cancer cells and allowed to grow until they reach a suitable size for distribution and therapy studies. Tumour growth will be measured before and after treatment to determine the response to the novel therapy. The mice will be continually assessed for any (rare) signs of distress. We will take every measure to avoid any animal suffering. Following humane killing of the animals, tissues such as liver, lung and tumours will be removed for analysis.

Expected benefits
Developing novel targeted treatments for cancer will not only kill the tumour cells but minimise

the death of normal cells in the body. They will therefore reduce the painful side effects associated with conventional therapies and improve the likelihood of patient survival.

Investigating cell viability/integrity in vivo

The aim of this project is to use mouse models of human cancer to understand the role played by factors and pathways that regulate tumour cell viability and integrity during tumour development and in cancer therapy.
Cancer is a multistep disease. A fundamental step during tumour development is inactivation of pathways regulating cell viability and integrity which normally serve to protect against the development of malignant disease. Inactivation of these pathways also causes problems for many therapies which utilize the same pathways to eradicate tumour cells from our body. Depending on the stage of cancer development, tumour cell behaviour can be modulated by a variety of processes which regulate cell viability and integrity. For some time the Applicant's laboratory has been working on these processes *in vitro.* This licence will allow us to extrapolate our findings to the *in vivo* context by investigating how specific factors and pathways controlling cell viability and integrity affect tumour cell behaviour in a natural environment involving normal cells and cells of the immune system; studies which can only be fully addressed in a living organism.

These studies will ultimately yield insights into what stage these pathways regulate tumour development and how they can then potentially be exploited for tumour therapy. To achieve this goal, we will breed compound GM mouse models carrying germline mutations mimicking those found in human cancer and occasionally use chemical carcinogens to induce specific gene mutations to understand how these pathways are modulated during tumour development. We will also cross these models to mice which contain tissue-targeted or whole-body, activating or inactivating lesions in components of pathways which regulate cell viability and/or integrity. These models will then act as pre-clinical models to monitor responses and mode of action of therapeutic agents. In some studies we will study the action and response of cancer cell lines *in vivo* using xenograft/allograft models which may be as informative yet be a milder procedure than using compound GM lines.

A proportion of animals used in this study will spontaneously form tumours for which recognised monitoring signs and established endpoints are established. All experimental mice will be monitored closely and humanely killed if they show sign of suffering or distress. We will carefully calculate the minimum number of mice needed to obtain statistically significant results for each experiment and all experimental designs will adhere to the guidelines for the welfare and use of animals in cancer research (Workman et al 2010). A maximum of 45,000 mice will be used in this study and we will continually consult NC3R guidelines to reduce animal numbers and suffering wherever possible. For example, we will use non-invasive imaging techniques to monitor disease progression which reduces both severity and numbers of animals required.

Increasing our knowledge of the underlying causes of cancer will dramatically improve our ability to diagnose, treat and prevent this disease which affects one in three of the human population. The use of mouse models has significantly contributed to this aim and will continue to do so.

Breeding and maintenance of genetically altered animals for transplant immunology research

The purpose of this Licence is to permit us to continue to breed and maintain several colonies of genetically altered mice currently being bred and maintained for use in transplantation immunology research, under existing licences expires in a few months and this Licence will permit us to continue to breed and maintain our existing colonies while we seek permission to continue our program of transplant immunology research under a new Project Licence, which will include breeding and maintenance of genetically altered animals. This will avoid the need to kill the mice and to subsequently restock, thus avoiding wastage of animals, time and money.

Fetal programming & transgenerational inheritance

Adverse environments during pregnancy can predispose the next generation to developing various diseases in adult life. More surprisingly, it seems that predisposition to disease can be transmitted to future generations e.g. grandparental nutrition can influence the health of the grandchild. One example is dietary protein levels. In human populations, exposure to low-protein levels during pregnancy may be due to economic or cultural reasons, and is associated with adult-onset diseases such as diabetes. The underlying mechanisms involved are currently unknown. Here, we will feed genetically identical, pregnant mice either normal food or a diet with reduced protein content. The offspring of these females will be returned to a normal diet. Yet, remain smaller and show metabolic changes as adults. Male offspring will be bred to give rise to the next generation, which, despite only ever having a normal protein diet, also show metabolic changes. How then, does the grandmaternal diet produce metabolic alterations in the grandoffspring?

In this project we have several objectives. Firstly, we want to see if there are gene expression changes as a result of grandmaternal diet very early in development, prior to the formation of the first tissues within the embryo. We will also try to create the first cell-based model for these kinds of non-genetic transgenerational effects. This would allow us to perform many more experiments than otherwise possible and minimise animal use. After identifying the genes that are switched on or off, we will look in the sperm of the fathers and see if we can identify any changes in the proteins which are associated with these particular genes, in an attempt to identify a) what in the sperm is altered by maternal low protein, and b) if this is linked to changes in gene function in the next generation.

In addition, we hypothesise that the metabolic disturbances in the adult grandoffspring result from altered development of brain regions that regulate particular behaviours, such as feeding and sleep/wake cycles. We will look at whether this is reflected by changes in particular cell types within these brain regions and in the genes expressed, e.g. hormones that regulate appetite. We will also test at what point in the animals' life these changes become apparent and whether they precede the onset of metabolic disease. To study inherited predisposition, live, breeding animals are required. Mice are genetically similar to humans and there have been many dietary studies performed, making them the ideal model organism for our study.

Over the five years of this project, we will use —500 or less mice. Animals will be maintained in an enriched environment, with free access to food and water. The procedures involved are limited to dietary protein restriction, artificial insemination and humane killing for subsequent investigations. All are regarded as causing no or only mild adverse effects. This work will increase our understanding of how environmental exposures contribute to complex disease risk, even for unexposed descendents. This knowledge may translate to better disease prevention in a clinical setting.

Evolution of Regeneration ability in teleost

In this project I will use the unique characteristics of the *Astyanax mexicanus* surface fish and cavefish to understand the molecular mechanisms and evolutionary adaptation underlying tissue regeneration and the morphological and physiological differences generated during evolution.

Despite the recent advances iii mammalian stem cell biology and regenerative medicine, we still do not understand how cells are organized to form our bodies and why we have a particular size, morphology, and longevity. If we could unveil universal machinery for tissue preservation in “superhealer” species such as fish and urodeles, it would open a way to re-form mammalian organs by enhancing our endogenous ability for tissue regeneration.
Pilot experiments have shown that *Astyanax mexicanus* surface fish tissue regeneration occurs at a much higher speed than in cavefish. Our main objectives in this research project are to find the underlying mechanisms causing the difference in tissue regeneration capacity between surface fish and cavefish and to research the influence of genetic and physiological changes during cavefish adaptive evolution on tissue regeneration capacity. We have chosen for the *Astyanax* fish model as for its unique difference in regeneration capacity within one fish species.

As fish like zebrafish and *Astyanax* have the unique ability to regenerate their tissue, this research cannot solely be done in vitro or in computer models. However, a great deal of work can be done on computers to help us to improve the design of our experiments. To reduce the number of animals used we will use the lowest number of animals necessary for statistical significance and provide embryos and tissue samples to appropriate personal and project licensed holders in UK and other European countries so that there is minimisation of the stocks of fish maintained in EU facilities.

All protocols used involve minimal suffering of the fish and the fish will be monitored closely, so that when suffering occurs, the fish is removed from the experiment. We will remove small pieces of tissue from the fish and analyse the regeneration potential. To analyse the underlying cause of the difference in tissue regeneration capacity, our protocols are designed to analyse physiological differences between the surface fish and cavefish. There are no excepted adverse effects, but when they do occur, the fish will be removed from the experiment.

Understanding the molecular mechanisms underlying tissue regeneration in *Astyanax mexicanus* will help to develop new therapeutic methods improving wound healing in patients. Finding the genetic and physiologic changes required for living in the extreme cave environment could contribute to find strategies for tissue regeneration.

Genetics of vertebrate development and disease

This project licence is required to carry out a program of work to determine the role of most protein coding genes in zebrafish and *Xenopus* frogs, which share many genetic features of humans. The project described in this application is designed to take advantage of whole-genome sequence information and to test the function of genes that have been identified. To do this we will apply new technologies that allow us to understand the function of genes while minimising the number of protected animals used.

Generally we will be using efficient methods to induce and detect mutations, followed by breeding to reveal the function of those mutated genes. We will analyse the consequence of induced mutations primarily during early development. Importantly, experience shows that the vast majority of mutations have no or minimal effect on the animals.

The project will address the relationship between tissue formation and the genes that control the underlying developmental events. One major focus will be to understand how loss of genes function leads to pathological states similar to human genetic disease. This could lead to new avenues of diagnosis and treatment for human developmental diseases. Indeed, work done under previous project licences following directly from our studies of embryonic organ formation led us to a new model system for the study of muscular dystrophy using zebrafish embryos.

The program of work is divided into two major divisions. The first goal of the project is to identify additional mutations affecting early development. This aspect of the project is largely motivated by results of previous work with zebrafish and our recent success in high-throughput mutation discovery and analysis of observable abnormal traits (http://www.sanger.ac.uk/Projects/D_rerio/zmp/).

The second goal of the project is to clone the genes we have previously identified. This aspect of the project is almost entirely a molecular biological effort and will affect the zebrafish only to the extent that they need to be bred to carry the mutations. It is important to note that the mutations to be used in this project are such that an adult carrier is not affected by the mutation in any way. The mutations only produce phenotypes in 25% of the offspring produced by two carriers.

The protocols to be used are all standard methods in zebrafish and frog research. All treatments, with the exception of the chemical mutagenesis treatment, have mild severity limits. Producing mutations by using chemicals will be rarely performed, but require a moderate severity limit. With the exception of the minor surgery of regenerating tissues all of the treatments are completely non-surgical.

Regulation of energy homeostasis in obesity

Mammals have two types of fat: brown and white, with opposing functions. The white fat is an important regulator of the whole body homeostasis that also serves to store energy. The main function of the brown fat is to burn lipids in order to produce heat, a function that can be induced by cold exposure or diet. Increased brown fat development leads to increased energy expenditure without causing dysfunction in other tissues, and is associated with a lean and healthy phenotype, outlining the manipulation of the fat stores as an obvious therapeutic objective. With the proposed research we will identify genes that regulate brown and white fat differentiation and function, and chracterise the molecular mechanisms of their action. We will distinguish the genes that specifically regulate brown or white fat generation and are expressed in their respective precursors, and establish them as signatures for either cell type. We will determine the origin of the brown fat cells, which also exist within the white fat, establish their importance in the regulation of metabolism in vivo, and develop novel strategies to induce the brown fat differentiation and function.

For all these goals we will be using in vitro and in vivo systems, image tracing studies, and transgenic animals. Mice are commonly used animal models for research of various metabolic diseases, including insulin resistance, diabetes and obesity. They contain both white and brown adipose tissues, and there are standardized protocols for induction of brown fat differentiation and function upon cold exposure. There are readily available, well-characterized mouse and rat strains that develop genetically induced obesity (eg. ob/ob, db/db, ZDF), and there are well-established protocols for inducing diet-induced obesity (diet induced obese (D1O) C57B116 mice). These facts, the similarities to humans, and the available tools to genetically modify them, makes mice a perfect animal models for studying the brown and white adipose tissue differentiation and function in regulation of metabolism in vivo. The use of transgenic animals is essential for the image tracing studies, as well as for the precursor cells depletion experiments, possible only in vivo.

The gut microbiota is involved in the regulation of multiple host metabolic pathways, giving rise to interactive host-microbiota metabolic, signaling, and immune-inflammatory axes that physiologically connect the gut, liver, fat, muscle, and brain. Colonization of a germ-free mouse (which is microbiologically pure) with the intestinal microbiota from an obese mouse donor induced a body weight gain, dyslipidaemia and central insulin resistance that was more substantial than when the microbiota from a lean mouse was transferred, providing striking first insights into the potential contribution of the microbiota to the obesity. It is necessary to use germ free animals in order to monitor the changes induced by alterations in their gut microbiota by populating them with microbiotal content from obese or lean donors. No other model can be adequately used to address these very important aspects of the development of obesity and diabetes, which are main health burdens in the modern societies.

By addressing these points, and by discovering ways to exclusively silence genes in the brown fat will that will allow us not only to investigate the miRNAs function, but also to develop new strategies for treatment of dyslipedaemia, diabetes and obesity.

Nervous System Injury and Repair

The goal of this project is to develop and test treatments to repair damage to the nervous system by promoting nerve fibre regrowth from injured fibres (regeneration) and/or fibre sprouting from existing or uninjured fibres (plasticity). In addition, we will evaluate the robust growth response which occurs in the visual and peripheral nervous systems (PNS) relative to the minimal growth response occurring after central nervous system (CNS) injury to further our understanding of these differences and determine how they may be utilised to enhance CNS repair.

CNS injury can be acute, as in stroke or traumatic brain and spinal cord injury, or chronic as in neurodegenerative diseases, leading to loss of motor and sensory functions, potentially resulting in paralysis and/or loss of sensation. Both brain and spinal cord injury are crippling conditions due to the severance of nerve fibres that connect the brain with the spinal cord and body. Repairing nervous system damage involves inducing cut nerve fibres to regenerate across the injury and to make connections below it. Additionally, undamaged nerve fibres remaining after injury can be made to return some function through stimulation of plasticity, bypassing the lesion.

Treatments for nervous system damage aim (1) to block the degeneration process around the lesion after acute damage, or to inhibit the gradual loss of cellular (both neuronal and/or supporting CNS cells known as glia) function in chronic neurodegeneration, (2) to repair the lesion, by inducing axon regeneration or reactivating plasticity in the brain andlor spinal cord. This project will explore the normal injury responses anatomically within the CNS and PNS to better understand endogenous malfunctions contributing to the lack of repair and will examine different repair strategies for neurorepair and neuroprotection focusing on modification of endogenous cells through gene therapy and cell replacement therapies (including stem cells).

The proposed experiments will be performed in rats and mice, in which the biology of nervous system is similar to humans. Many of the animals will be genetically altered and a few thousand over *5* years will be used to obtain the genotypes we need to test our hypotheses. A few hundred animals will be used for tissues only. Our models of brain and spinal cord injury will be performed under general anaesthesia, with additional analgesia being given pen-operatively so the animal does not suffer during or after the procedure. Our lesions will be of moderate severity, whereas within a week post-surgery, the animals will have returned to a near normal condition with very mild deficits only being apparent through specific behavioural and anatomical analysis. Specific humane endpoints will be used to endure that adverse effects do not go beyond the minimum required to achieve the scientific objectives and the numbers of animals will be minimised by careful experimental design. This may involve around 500 a year, but most of these will be the genetically altered types listed above.

Much of the development of our treatments is performed with extensive tissue culture analysis prior to moving to an animal model. However, in order to determine whether these treatments are likely to help human patients, it is vital that they are evaluated in animal models first. For this aim, we will careflully design our experiments so as to use the fewest numbers of animals possible to achieve significance in our results. in addition to validating our novel CNS repair treatments, we will also include therapies already used in the clinic such as rehabilitation and therapies close to clinical trials. These experiments will add to the fundamental knowledge of nervous system injury and impaired regeneration. We will publish our findings in high impact

peer reviewed journals, as well as make it possible to translate viable and novel therapies towards application in human patients.