University of Sheffield

School
Of
Biosciences.

MSc Biomedical Science Handbook

2024/2025

Contents

Welcome

Welcome to the School of Biosciences!

The purpose of this pre-arrival handbook is to provide you with some information regarding the MSc course that you have applied for and guide you through the processes leading up to your arrival in Sheffield.

We hope that you are as exciting as we are to join our MSc programme for the next stage of your scientific career, and we endeavour to help you along the way and make it a truly enjoyable experience that will help you shape your future.

I look forward to meeting you all in due course.

Dr Anne-Gaëlle Borycki & Dr Stephen Brown

Lead of MSc Biomedical Science course

Deputy Director of Post-Graduate Taught courses

School of Biosciences

School of Biosciences

The creation of the School of Biosciences in September 2021 celebrates over 100 years of research and teaching in Biology at the University of Sheffield, previously delivered by the departments of Animal and Plant Sciences, Biomedical Science, and Molecular Biology & Biotechnology. We are a large school comprising around 120 academics, 300 post-graduates (taught and research) and 1500 undergraduates. In addition, a number of technical and ancillary staff, and secretarial staff complete the structure and organisation of the School. In the School, you will find expertise spanning the breadth of bioscience and excellence in both teaching and research. In the most recent review of higher education quality in the UK, our School was awarded top scores for teaching (24/24) and was ranked 4th nationally for research in Biological Sciences in the Research Excellence Framework (REF 2021). If you wish to know more about our ranking in research follow this link:

https://www.sheffield.ac.uk/biosciences/news/biosciences-sheffield-rated-4th-uk-quality-research

We occupy several buildings in the University campus, including the Addison Building, the Florey Building, the Alfred Denny Building and Firth Court.

The Faculty of Science

The Faculty of Science comprises other 2 schools (Mathematics & Physical Sciences, and Psychology) in addition to the School of Biosciences. It oversees all the arrangements that Schools make concerning teaching, examinations and the award of degrees. The Faculty is responsible for ensuring that the University Regulations concerning teaching and examining are upheld and that standards are maintained. Faculty Officers are members of academic staff appointed part-time for a limited period.

Post-Graduate Taught courses within the School of Biosciences

In the School of Biosciences, the PGT (MSc and MRes) courses are grouped under 4 themes: Organisms and Environment, Molecular and Cellular Biology, Biomedicine and finally Communicating science. A dedicated team has oversight of all post-graduate taught courses (including the Masters of research) in the School of Biosciences. It includes the Director of PGT courses (Dr. David Turton), Deputy Director of PGT (Dr. Stephen Brown), a team overseeing the PGT administration (led by James Barker), PGT Tutors (Dr. Marion Germain and Qaiser Sheikh), a PGT recruitment Lead (Dr. Dave Turton) and a PGT assessment Lead (Dr. Henry Roehl).

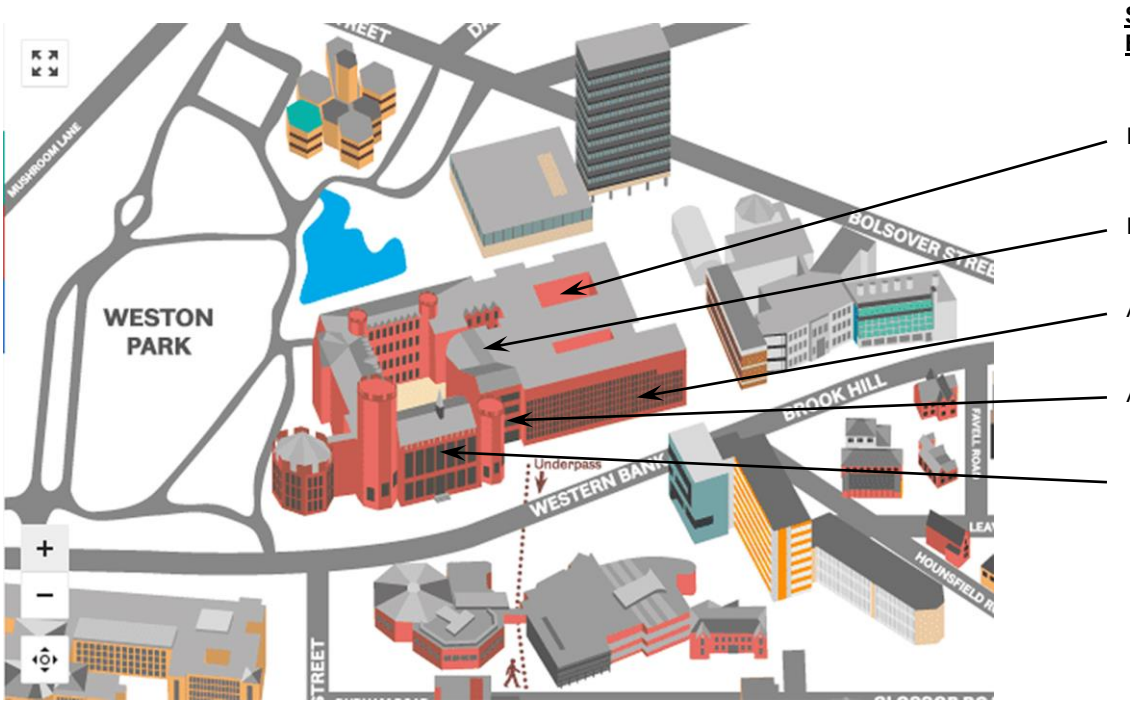
In addition, PGT course Leads manage the structure, organisation, teaching and assessment of each course, and will provide you with guidance throughout your time at the University.

PGT Leads within the Biomedicine MSc course are:

Courses	Leads
Biomedical Science MSc (BIST05)	Dr. Anne-Gaelle Borycki & Dr. Stephen Brown

Map

School of Biosciences Buildings
and others nearby on Western Bank



School of Biosciences Buildings

Perak

Florey

Alfred Denny

Addison

Firth Court

School of Bioscience Office: Room C102, Ground Floor, Firth Court

Student Notice boards: Ground Floor, Florey Building (in base of stairwell).

Useful Contacts for Teaching and Administration

Telephone number and e-mail address for the persons listed below can be found on the School's web page: https://www.sheffield.ac.uk/biosciences/

Teaching:	
Dr. Dave Turton	Head of MSc courses email: david.turton@sheffield.ac.uk
Dr. Stephen Brown*	Biomedical Science MSc CB & DD Pathway Lead
Dr. Anne-Gaelle Borycki*	Biomedical Science MSc SC & RM Pathway Lead
Dr. Dylan Childs	Director of Learning and Teaching, School of Biosciences
Prof. Andrew Beckerman	Director of Learning and Teaching, Faculty of Science
Secretarial:	
James Barker*	Lead of PGT admin team
Dawn Bramham*	PGT admin Officer
Francesca Cliffe*	PGT admin Officer (email: pgtadmin.biosciences@sheffield.ac.uk)
Technical Staff:	
Michelle Durrant	Chief Technician responsible for Teaching Laboratories (email: m.durrant@sheffield.ac.uk)
IT support Staff:	
	Faculty IT technicians (sci-it@sheffield.ac.uk)
Administration:	
Mrs Melanie Hannah	School Manager (technical)
Mr. Matt Wyles*	Deputy School Manager (technical)
Mrs Angelique Mawdsley	School Manager (financial)

* indicates staff with whom you are likely to interact most.

Finding Information

You will find most of the standard information you need to know in the following places:

- This handbook
- The School Postgraduate taught programmes website (more information below)

(https://www.sheffield.ac.uk/postgraduate/taught/courses/2024/biomedical-science-msc)

- Your postgraduate student handbook (https://students.sheffield.ac.uk/biosciences/pgt)

- Email: we will often communicate with you via your University email account once your account is set up. It is your responsibility to check it regularly. **Please note that we can use only your University email address.**
- Blackboard: Blackboard is the University digital platform through which we share teaching materials, deliver online teaching (if needed), share recording of lectures, and upload written assessment. It is important that you familiarise yourself with this platform. We have created a pre-arrival page to help you find information on your course. The direct link to this page is:

https://vle.shef.ac.uk/ultra/organizations/_100413_1/cl/outline

The School of Biosciences PGT website

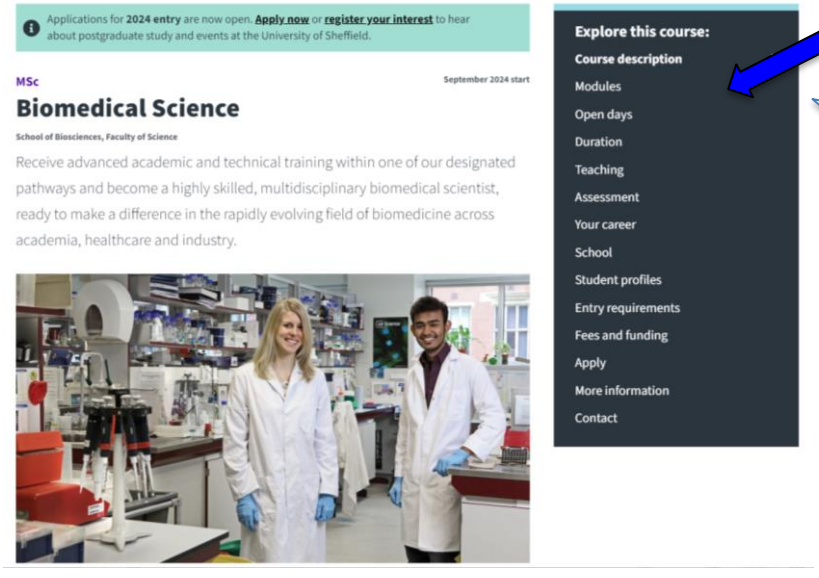
The School's home page web address is https://www.sheffield.ac.uk/biosciences

Information about your particular MSc course is available from this direct link:

https://www.sheffield.ac.uk/postgraduate/taught/courses/2024/biomedical-science-msc

Use the following QR code to access the page directly:

To access specific information about your course, including the list of modules available with a short description, follow the menu on the right of the screen. The list of modules includes core modules common to both pathways and pathway specific modules. Ensure that you have selected your chosen pathway. This list will be useful to help you completing your online module choice.



If you have not already chosen your pathway and wish to discuss it with us, contact Anne-Gaelle Borycki by email (a.g.borycki@sheffield.ac.uk)

Information about PGT modules (module code and title, summary, period of teaching and assessment) can also be found here:

https://sites.google.com/sheffield.ac.uk/biosciences-module-list/biosciences-module-list?pli=1

MUSE and BLACKBOARD

Once you have initiated your registration at the University, you will get access to some of the online resources; the complete package will become available upon completion of your registration.

You will have access to the University's web-based portal service MUSE.

MUSE (My University of Sheffield Environment) allows you to access your email account and other secure web-based services provided by the University and the department, such as library and e-learning resources.

All on-line teaching resources for postgraduate MSc students are posted on **BLACKBOARD**, which can be accessed via the MUSE homepage.

- Note that a specific BLACKBOARD organisation page for pre-registration students has been set up:

  https://vle.shef.ac.uk/ultra/organizations/_100413_1/cl/outline

In the **courses** list, you'll find folders for each of your modules entitled 'module code (Academic year 2024-25)'. Here you can access:

- All on-line materials related to the module, including powerpoint presentations.
- You can also access the virtual meetings (Blackboard Collaborate), and recordings of your lectures (Encore).

Additionally, the **IT** Starter Pack provides you with all the comprehensive information you need to introduce and update yourself to the computing facilities at the University of Sheffield.

The University homepage should be, by default, the start up web page, when you open your browser. The URL on the address bar is:-
http://www.shef.ac.uk/
Biosciences masters can be found by simply adding 'biosciences/postgraduate/masters'.
http://www.shef.ac.uk/biosciences/postgraduate/masters

REGISTRATION EVENTS AND TASKS

All registration events now take place online prior to your arrival at the University. It is important that you familiarise yourself with this process and ensure that you have completed all tasks in order to finalise your registration. Further details are available here: https://www.sheffield.ac.uk/new-students/registration/preparation

If you have any questions or issues, the PGT admin team will be there to help you; they can be contacted at: pgtadmin.biosciences@sheffield.ac.uk

One of the tasks that you need to complete is your module choice. You will choose your module using an online platform (the link will be available in your pre-registration online window), which opens on **4 September 2024**. To help you choosing your modules, we have put together some information in the pages below, including the modules taught in your course, and the timing and assessment for each module. When choosing your module, consider not only your own interest in the subject but also the workload.

If you are worried that you have not made the right choice, there will be an opportunity to change your module choice at the start of the semester through the add-drop module form.

Finally, we encourage you to check the pre-registration Blackboard page that we have set up as it contains additional information, which you may find useful.

Course Information

The Academic Year
The postgraduate taught academic year consists of approximately 46 weeks divided into three semesters each of fifteen weeks. The Autumn semester usually starts in late September and consists of twelve teaching weeks before Christmas and, after the Christmas Vacation, a three-week examination period when the modules taken during the first semester are assessed. The Spring Semester consists of twelve teaching weeks broken into two blocks, one before and one after the Easter Vacation. The spring Semester concludes with a three-week assessment period when the modules taken in this semester are assessed. Postgraduate taught students also continue through the summer vacation with full-time work on their research project with the final examination held towards the end of August.

The dates of the semesters for the coming year are:

Session 2024-2025

Intro Week:	23 September – 27 September 2024
Autumn Semester:	30 September - 20 December 2024
Christmas Vacation:	*23 December 2024 –20 January 2025 (4 weeks)*
Autumn Semester (cont):	20 January – 07 February 2025
Spring Semester:	10 February – 04 April 2025
Easter Vacation:	*08 April – 25 April 2025 (3 weeks)*
Spring Semester (cont):	28 *April*– 23 *May* 2025
Summer Semester (cont)*:*	26 *May* - 06 September 2025

Important dates at the beginning of your course (please note these are in addition to events organised by the central administration of the University):

Meeting the School of Biosciences postgraduate students, *Wednesday 25 September 9.30-11.30, Firth Hall.*
A welcome event with some fun activities to meet other postgraduate students and introduce you to your course leads. This may be followed by a visit of the University and School of Biosciences facilities and lab.

tion MSc induction sessions, *Monday 30 September 9:00-11.00, Arts Tower Lecture theatre 04*
An important series of presentations to introduce your course and other essential University services.

Introduction to ED&I, *Wednesday 02 October 12.00-13.00, Alfred Denny building Lecture theatre 02*
This lecture contains essential information about Equality, Diversity and Inclusion at the University of Sheffield.

Introduction to PGT Employability, *Wednesday 02 October 12.00-13.00, Alfred Denny building Lecture theatre 02*
This lecture provides you with an introduction to the sessions organised by the Employability team aiming at helping you prepare for your further studies and employment at the end of your course.

Introduction to PGT Tutors, *Thursday 03 October 9.00-10.00, Alfred Denny building Lecture theatre 01*
This lecture contains information about support provided by the PGT Tutors throughout your course.

PGT welcome drinks and snacks, *Friday 04 October 15.00-17.00, Alfred Denny building Conference Room.*
We will get together to celebrate the start of your course. Drinks and pizzas (to be confirmed).

COURSE STRUCTURE

The MSc courses in Biomedicine comprise 180 credits. 75 credits are associated with your compulsory research project - 15 credits for the background literature review and research proposal, and 60 credits for the practical work and dissertation. A further 30 credits are associated with each of two other compulsory modules - Critical analysis of current science & Advanced scientific skills. The outlines of these compulsory modules can be found on the website. In addition, the Stem Cell and Regenerative Medicine pathway has the core practical module of Practical Developmental Genetics (BIS454) and the Cell Biology and Drug Development pathway has the core practical module of Practical Cell Biology (BIS453).

In addition to these compulsory elements, you have the choice of four 15-credit modules to make up the remaining 60 credits. The choice of lecture modules and the choice of practical modules (P) are also outlined here and are course-specific. Please note that numbers of participants on some modules may be limited ('capped') in order to maintain an effective learning environment. This will be done on a clear and transparent system based on the application number of each student.

Overall MSc Course Structure

Core modules (these are compulsory)

BIS456 Laboratory research project	60 credits
BIS405 Literature review and research proposal	15 credits
BIS402 Advanced scientific skills	15 credits
BIS445 Critical analysis of current science	15 credits

Stem Cell and Regenerative Medicine pathway

Core module

BIS454 Practical Developmental Genetics (P)	15 credits

Optional modules (choose 60 credits from these options)

Semester 1

BIS447 Stem Cell Biology	15 credits
BIS461 Modelling Human Disease and Dysfunction	15 credits

Semester 2

BIS438 Culture and Differentiation of Human Pluripotent Stem Cell (P)	15 credits
BIS448 Cancer Biology	15 credits
BIS462 Principles of Regenerative Medicine and Tissue Engineering	15 credits

Cell Biology and Drug Development pathway

Core module

BIS453 Practical Cell Biology (P)	15 credits

Optional modules (choose 60 credits from these options)

Semester 1

BIS437 The Biotech and Pharmaceutical Industry	15 credits
BIS449 Membrane Receptors	15 credits

Semester 2

BIS440	Genomic Approaches to Drug Discovery	15 credits
BIS448	Cancer Biology	15 credits
BIS460	Sensory Neuroscience	15 credits
BIS439	Small Molecule and Functional Genomic Screening (P)	15 credits
Total		180 credits

Choosing your modules

When choosing your modules, consider spreading your workload equally between the first and second semesters. Semester 1 contains 45 credits of core modules and semester 2 contains 15 credits of core module (excluding the Research Project). So, ideally you should choose no more than 30 credits of semester 1 modules and no more than 45 credits of semester 2 modules. Your optional modules contain lecture and practical modules, so you can choose to gain more practical skills or more theoretical skills.

Below are various tables and graphic representations of the academic year indicating when modules are taught and when assessment will take place to help you plan and manage workload. Take this into consideration when choosing your modules too.

Note that module choice need to be completed early September as part of your

If you have questions or need help, contact Anne-Gaelle Borycki (a.g.borycki@sheffield.ac.uk) and/or Stephen Brown (Stephen.brown@sheffield.ac.uk)

MSc in Biomedical Science (BMS)
Overview of Timetable

Semester 1

Module	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
BIS402 Advanced scientific skills	BIS402	BIS402	BIS402	BIS402	BIS402	BIS402	BIS402	BIS402	BIS402	BIS402	BIS402	BIS402
BIS405 Literature review and research proposal	BIS405	BIS405	BIS405	BIS405	BIS405	BIS405	BIS405	BIS405	BIS405	BIS405	BIS405	BIS405
BIS445 Critical analysis of current science	BIS445			BIS445	BIS445	BIS445	BIS445	BIS445	BIS445	BIS445		BIS445
BIS456 Laboratory Research Project	BIS456	BIS456	BIS456									
Stem Cell and Regenerative Medicine Pathway												
BIS454 Practical Developmental Genetics (P)					BIS454	BIS454	BIS454					
BIS447 Stem Cell Biology							BIS447	BIS447	BIS447	BIS447	BIS447	BIS447
BIS461 Modelling Human Disease and Dysfunction								BIS461	BIS461	BIS461	BIS461	BIS461
Cell Biology and Drug Development Pathway												
BIS453 Practical Cell Biology (P)	BIS453	BIS453	BIS453	BIS453								
BIS437 The Biotech and Pharmaceutical Industry	BIS437	BIS437	BIS437	BIS437	BIS437	BIS437	BIS437	BIS437				
BIS449 Membrane Receptors	BIS449	BIS449	BIS449	BIS449	BIS449	BIS449						

CORE MODULES OPTIONAL MODULES

Semester 2

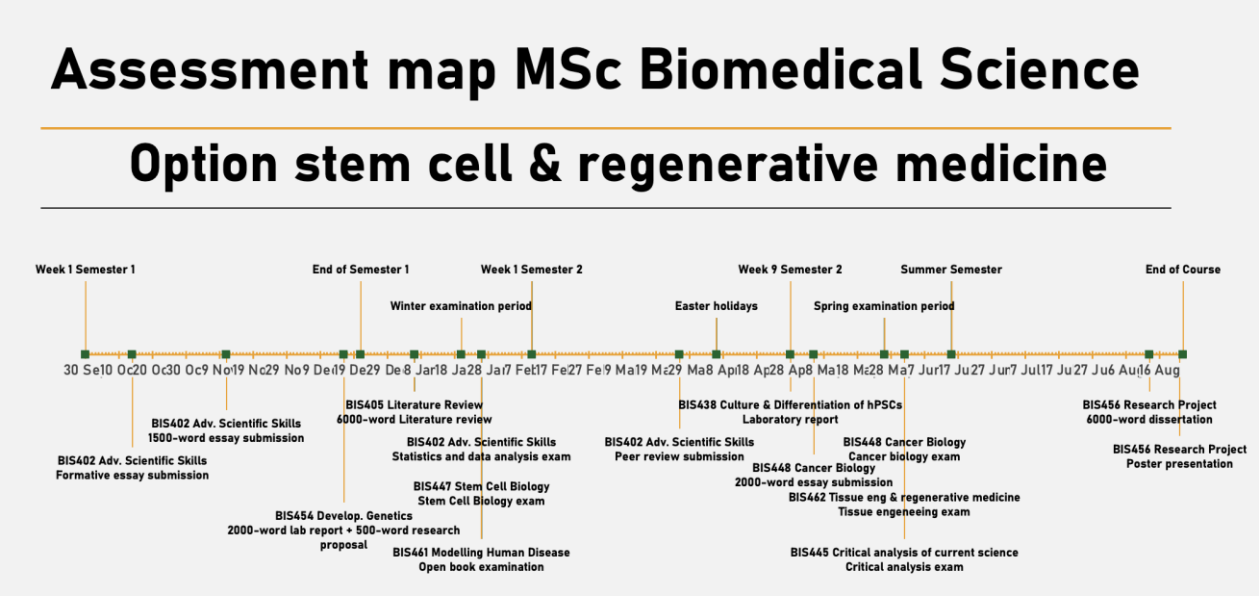
Module	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
BIS402 Advanced scientific skills	BIS402	BIS402			BIS402		BIS402	BIS402	BIS402	BIS402	BIS402	BIS402
BIS445 Critical analysis of current science			BIS445	BIS445	BIS445	BIS445	BIS445		BIS445	BIS445		
BIS456 Laboratory Research Project					BIS456	BIS456	BIS456	BIS456	BIS456	BIS456	BIS456	BIS456
Stem Cell and Regenerative Medicine Pathway												
BIS438 Culture and Differentiation of Human Pluripotent Stem Cells (P)	BIS438	BIS438	BIS438	BIS438								
BIS448 Cancer Biology	BIS448	BIS448	BIS448	BIS448	BIS448	BIS448						
BIS462 Principles of Regenerative Medicine and Tissue Engineering							BIS462	BIS462	BIS462	BIS462	BIS462	BIS462
Cell Biology and Drug Development Pathway												
BIS440 Genomic Approaches to Drug Discovery							BIS440	BIS440	BIS440	BIS440	BIS440	BIS440
BIS448 Cancer Biology	BIS448	BIS448	BIS448	BIS448	BIS448	BIS448						
BIS460 Sensory Neuroscience							BIS460	BIS460	BIS460	BIS460	BIS460	BIS460
BIS439 Small Molecule and Functional Genomic Screening (P)	BIS439	BIS439	BIS439	BIS439								

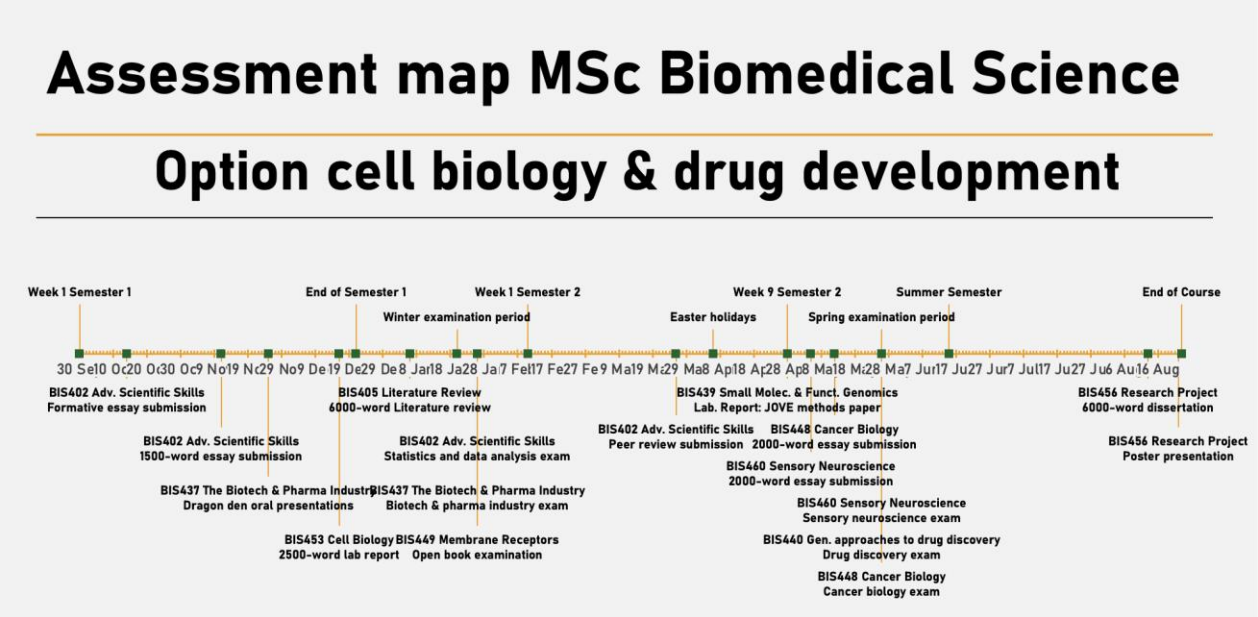
The Laboratory Research Project (BIS456) is part-time in the laboratory from week 5 and is full-time after the summer exams.

Assessment mapping and deadlines

Use the tables below to gain an overview of the format of assessment associated with each module and the timing of the assessment. The tables below contain the current proposed dates for coursework submissions and the period of examination (it does not indicate oral presentations for BIS445 spread throughout the year). These dates are subject to changes and will be confirmed at the start of the semester.

Using this information as well as the information related to the content of modules, choose your modules to ensure that you balance your workload.





Stem Cell and Regenerative Medicine pathway Deadlines

DATE	KEY DATES	MODULE
30/09/2024	Week 1 Semester 1	
14/10/2024	Formative essay submission	BIS402 Adv. Scientific Skills
11/11/2024	1500-word essay submission	BIS402 Adv. Scientific Skills
16/12/2024	2000-word lab report + 500-word research proposal	BIS454 Develop. Genetics
21/12/2024	End of Semester 1	
06/01/2025	6000-word Literature review	BIS405 Literature Review
20/01/2025	Winter examination period	
26/01/2025	Open book examination	BIS461 Modelling Human Disease
26/01/2025	Statistics and data analysis exam	BIS402 Adv. Scientific Skills
26/01/2025	Stem Cell Biology exam	BIS447 Stem Cell Biology
10/02/2025	Week 1 Semester 2	
26/03/2025	Peer review submission	BIS402 Adv. Scientific Skills
06/04/2025	Easter holidays	
28/04/2025	Laboratory report	BIS438 Culture & Differentiation of hPSCs
28/04/2025	Week 9 Semester 2	
05/05/2025	2000-word essay submission	BIS448 Cancer Biology
26/05/2025	Spring examination period	
01/06/2025	Tissue engeneeing exam	BIS462 Tissue eng & regenerative medicine
01/06/2025	Critical analysis exam	BIS445 Critical analysis of current science
01/06/2025	Cancer biology exam	BIS448 Cancer Biology
15/06/2025	Summer Semester	
13/08/2025	6000-word dissertation	BIS456 Research Project
22/08/2025	Poster presentation	BIS456 Research Project
23/08/2025	End of Course	

Cell Biology and Drug Development pathway Deadlines

DATE	KEY DATES	MODULE
30/09/2024	Week 1 Semester 1	
14/10/2024	Formative essay submission	BIS402 Adv. Scientific Skills
11/11/2024	1500-word essay submission	BIS402 Adv. Scientific Skills
25/11/2024	Dragon den oral presentations	BIS437 The Biotech & Pharma Industry
16/12/2024	2500-word lab report	BIS453 Cell Biology
21/12/2024	End of Semester 1	
06/01/2025	6000-word Literature review	BIS405 Literature Review
20/01/2025	Winter examination period	
26/01/2025	Open book examination	BIS449 Membrane Receptors
26/01/2025	Statistics and data analysis exam	BIS402 Adv. Scientific Skills
26/01/2025	Biotech & pharma industry exam	BIS437 The Biotech & Pharma Industry
10/02/2025	Week 1 Semester 2	
26/03/2025	Peer review submission	BIS402 Adv. Scientific Skills
06/04/2025	Easter holidays	
28/04/2025	Lab. Report: JOVE methods paper	BIS439 Small Molec. & Funct. Genomics
28/04/2025	Week 9 Semester 2	
12/05/2025	2000-word essay submission	BIS460 Sensory Neuroscience
05/05/2025	2000-word essay submission	BIS448 Cancer Biology
26/05/2025	Spring examination period	
26/05/2025	Drug discovery exam	BIS440 Gen. approaches to drug discovery
26/05/2025	Cancer biology exam	BIS448 Cancer Biology
26/05/2025	Sensory neuroscience exam	BIS460 Sensory Neuroscience
15/06/2025	Summer Semester	
13/08/2025	6000-word dissertation	BIS456 Research Project
22/08/2025	Poster presentation	BIS456 Research Project
23/08/2025	End of Course	

CHOOSING YOUR RESEARCH PROJECT

BIS456 is the module code for your research project. This module is coordinated by Dr. Anne-Gaelle Borycki and is worth 60 credits.

Your project is an individual project carried out in one of the laboratories in the School of Biosciences in the 2nd and 3rd semester (Mar-Aug). You will work part-time around your lecture and practical modules between March and June, and then join the lab full time after the summer examination period.

Below is a list of all projects offered as part of your MSc course. Each project listed belongs to one or two research themes, and we have grouped projects into 5 themes (Cancer Biology, Animal and Cellular Models of Human Disease, Stem Cell and Regenerative Medicine, Mechanisms of Drug Action, and Neurodevelopment and Neuroscience). The project description includes a title, the name of the supervisor and their website, a background to the project and the objectives as well as the techniques to be used and references.

To initiate the Research Project allocation process, please:

1) Read the projects and note the 3 themes that contain projects of interest to you.

2) Indicate your 3 preferred themes in the following Google form **by Friday September 13th**:

https://forms.gle/uHjcU6qbnVwQoRcs5

If you experience issues accessing the Google spreadsheet, email Anne-Gaelle Borycki (a.g.borycki@sheffield.ac.uk) your theme preference.

Once we have your choices, we will allocate you a project within one of your preferred themes. We will try as much as possible to allocate a project within your first or second theme. Note that project allocation will also take into consideration staff workload. Therefore, we cannot accept requests for specific projects.

Note that students registered on the Stem Cell and Regenerative Medicine pathway have priority on Stem Cell and Regenerative Medicine projects. Note also that you may choose any theme irrespective of your pathway choice.

If you have any questions, do not hesitate to contact me (a.g.borycki@sheffield.ac.uk).

LIST OF PROJECTS BY THEMES

PRIMARY THEME	SECONDARY THEME	Project #
cancer biology		6
cancer biology		7
cancer biology		16
cancer biology		26
cancer biology	cell and animal models of Human disease	28
cancer biology	cell and animal models of Human disease	30
cancer biology	cell and animal models of Human disease	31
cancer biology		33
cancer biology		34
cancer biology	cell and animal models of Human disease	38
cancer biology		43
cancer biology	cell and animal models of Human disease	45
cancer biology	cell and animal models of Human disease	46
cancer biology		56
cell and animal models of Human disease		10
cell and animal models of Human disease		11
cell and animal models of Human disease		13
cell and animal models of Human disease		15
cell and animal models of Human disease		17
cell and animal models of Human disease	cancer biology	27
cell and animal models of Human disease		32
cell and animal models of Human disease	cancer biology	35
cell and animal models of Human disease		37
cell and animal models of Human disease	stem cell & regenerative medicine	39
cell and animal models of Human disease	mechanisms of drug action	40
cell and animal models of Human disease		41
cell and animal models of Human disease	cancer biology	44
cell and animal models of Human disease	cancer biology	47
cell and animal models of Human disease		48
cell and animal models of Human disease		49
cell and animal models of Human disease	mechanisms of drug action	51
cell and animal models of Human disease		52
cell and animal models of Human disease		54
cell and animal models of Human disease		14
mechanisms of drug action	neurodevelopment & neuroscience	4
mechanisms of drug action	cell and animal models of Human disease	12
mechanisms of drug action	cancer biology	18
mechanisms of drug action	cancer biology	36
mechanisms of drug action	cell and animal models of Human disease	53
Neurodevelopment & neuroscience		2
Neurodevelopment & neuroscience		3
Neurodevelopment & neuroscience	cell and animal models of Human disease	5

Neurodevelopment & neuroscience	cell and animal models of Human disease	19
Neurodevelopment & neuroscience	cell and animal models of Human disease	20
Neurodevelopment & neuroscience	cell and animal models of Human disease	21
Neurodevelopment & neuroscience	cell and animal models of Human disease	22
Neurodevelopment & neuroscience		23
Neurodevelopment & neuroscience	cell and animal models of Human disease	29
Neurodevelopment & neuroscience		42
Neurodevelopment & neuroscience	cell and animal models of Human disease	50
stem cell & regenerative medicine		1
stem cell & regenerative medicine		8
stem cell & regenerative medicine		9
stem cell & regenerative medicine		24
stem cell & regenerative medicine	cell and animal models of Human disease	25
stem cell & regenerative medicine		55

University of Sheffield

School of Biosciences

MSc Projects List 2024/2025

Project 1

School of Biosciences Laboratory Research Project 2024/25

Identification of the cellular and molecular mechanisms of zebrafish regeneration

Stem Cell & Regenerative Medicine

Dr. Henry Roehl **h.roehl@sheffield.ac.uk**
Firth Court C30b **Tel: 0114 222 2351**
https://www.sheffield.ac.uk/biosciences/people/bms-staff/academic/henry-h-roehl

Background

The study of regenerative biology aims to elucidate the innate ability of organisms to replace tissues or organs after they have been removed or damaged. During vertebrate regeneration, tissue damage causes the immediate release of signals that initiate wound closure and inflammation. Following this, regenerative cells proliferate and migrate to the damaged area. These cells then grow to replace the missing organ or tissue. This process is very efficient in aquatic vertebrates such as salamanders, frogs and fish, and is not very successful in terrestrial vertebrates such as ourselves. The long-term goal of this project is to improve regenerative medicine approaches for patients.

Aims:

This project uses zebrafish as a model to identify the signals that recruit regenerative cells to the site of injury. Genetic and pharmacological inhibition of signalling pathways will be used to identify key regenerative signalling pathways.

Techniques:

The student will use cutting-edge techniques such as genetic and pharmacological inhibition of signalling pathways, light sheet microscopy and computational analysis.

References:

https://www.ncbi.nlm.nih.gov/pubmed/30275454
https://www.ncbi.nlm.nih.gov/pubmed/29938759

Project 2

School of Biosciences Laboratory Research Project 2024/25

Understanding hypothalamic development: gene expression analyses

Neurodevelopment & Neuroscience

Prof. Marysia Placzek **m.placzek@sheffield.ac.uk**
Firth Court D18a **Tel: 0114 222 2353**
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Background

The Placzek lab focuses on understanding how the hypothalamus develops in the embryonic chick. Over the years we have begun to identify how signals induce, then regionalise and direct growth of hypothalamic progenitor territories but many questions still remain, especially that of how early progenitor cells differentiate into distinct hypothalamic neurons and glia, including stem-like cells. Recently we have performed single cell RNA sequencing studies across early stages of hypothalamic development. This has yielded a data-rich resource, identifying new genes that mark hypothalamic progenitor domains. The key goal of this project is to perform expression profiling of a subset of these genes, to determine when and where they are expressed in the developing hypothalamus.

Aims:

(1) Select a subset of novel gene(s) potentially involved in hypothalamic induction, patterning, growth, neurogenesis or gliogenesis
(2) Perform in situ hybridisation studies with selected genes
(3) Incorporate into a 3-d map of the hypothalamus

Techniques:

Data mining of scRNA seq libraries, embryonic chick dissection, in situ hybridisation, cryostat sectioning and light microscopy, within the context of previous literature.

References:

Burbridge, S., Stewart, I. and Placzek M. (2016) Development of the neuroendocrine hypothalamus. Comprehensive Physiology DOI: 10.1002/cphy.c150023
Fu, SC, Towers, M, Placzek, M (2017) Fgf10+ progenitors give rise to the chick hypothalamus by rostral and caudal growth and differentiation. Development 144 3278-3288
Kim DW, Place E, Chinnaiya K, et al.(2022) Single-cell analysis of early chick hypothalamic development reveals that hypothalamic cells are induced from prethalamic-like progenitors. Cell Rep. 2022 Jan 18;38(3):110251. doi: 10.1016/j.celrep.2021.110251

Project 3

School of Biosciences Laboratory Research Project 2024/25

Investigating diabetes-induced anatomical changes in dorsal root ganglia

Neurodevelopment & Neuroscience

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Background

Diabetes is a serious health issue in developed and developing countries. About 415 million adults have diabetes, a number that is estimated to increase by 50% in 20 years. One of the most debilitating complications of diabetes is Diabetic Peripheral Neuropathy (DPN). DPN is characterised by a progressive distal to proximal degeneration of peripheral nerves which results in pain, loss of sensation and weakness. At least half of diabetics develop DPN. Neuropathy affecting small diameter fibers appear first leading to allodynia and hyperalgesia. Invasion of the dorsal root ganglia (DRG) by post- ganglionic sympathetic nerves (known as sympathetic sprouting, Ref 1) has been observed after damage to the sciatic nerve by injury. This invasion contributes to neuropathic pain. However, diabetes also is known to cause damage to the sciatic nerve and cause pain. It is not known if the effect of diabetes involves invasion of sympatric nerves.

Aims:

This project aims to examine for sympathetic invasion in DRG isolated from a mouse model of Type 2 Diabetes. The results of this project will help understand a cause of diabetes-induced pain and may suggest therapies based on blocking the activity of sympathetic nerves

Techniques:

Fixation of tissue and production of section histological sections
Immunolabelling for sympathetic fibres.
Imaging of labelled sections and using software for quantification of results.

References:

1) A role for nerve growth factor in sympathetic sprouting in rat dorsal root ganglia. Martyn G.JonesaJohn BMunsonbStephen W.N.Thompsona. Pain, Volume 79, Issue 1, 1 January 1999, Pages 21-29

Project 4

School of Biosciences Laboratory Research Project 2024/25

Modelling pain, Differentiation of a new neuronal cell line into a nociceptive phenotype for use in pain and pharmaceutical research.

Mechanisms of Drug Action **Neurodevelopment & Neuroscience**

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Background

Sensory neurons in dorsal root ganglia (DRG) detect and transmit pain signals to CNS. Changes in processing of pain signals in DRG neurons are thought to contribute to chronic pain. These changes are referred to as “peripheral sensitization”. It is important to understand the molecular events leading to peripheral sensitisation. However, this is hampered by the inability to obtained sufficient number of theses neurons from disassociated primary cultures for molecular and biochemical experiments. Furthermore, DRG cultures contain a variety of cell types including glia and fibroblasts besides neurons, making it difficult to interpret data from biochemical studies. We generated an immortal cell line from DRG which will lead to new set of experiments being carried out that would not have been possible with primary cultures. The generated cell line will replace the use of rodents to obtain primary cultures.

Aims:

The project aims to improve the differentiation protocol to produce sensory neurons of an adult phenotype similar to that is found in vivo. We will effect of sonic hedgehog, FGF1 and FGF12 on differentiation of MED17.11 cell line.

Techniques:

Tissue culture techniques.
Immunocytochemistry with imaging. Calcium imaging

References:

1) Ciara Doran, Jonathan Chetrit, Matthew C Holley, Mohammed A. Nassar. Mouse DRG Cell Line with Properties of Nociceptors. PLOS ONE
2) Mycobacterium tuberculosis Sulfolipid-1 Activates Nociceptive Neurons and Induces Cough.Ruhl CR, Pasko BL, Khan HS, Kindt LM, Stamm CE, Franco LH, Hsia CC, Zhou M, Davis CR, Qin T, Gautron L, Burton MD, Mejia GL, Naik DK, Dussor G, Price TJ, Shiloh MU. Cell. 2020 Apr 16;181(2):293-305.e11.

Project 5

Department of Biomedical Science Laboratory Research Project 2024/25

Activity-dependent plasticity of dendritic morphology in memory-encoding neurons

Neurodevelopment & Neuroscience **Cell & Animal Models of Human Disease** **Dr.**

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Background
How do neurons set up their synaptic inputs to achieve consistent levels and patterns of electrical activity despite extensive variation in morphology and electrical properties between even neurons of the same type? Perhaps variation on one physiological parameter serves to compensate for variation on another parameter.
This project tests this hypothesis using neurons called Kenyon cells (KCs) that store olfactory associative memories in the fruit fly Drosophila. Each KC receives inputs from a variable number of excitatory neurons. How many excitatory inputs should each KC have? Given a certain spiking threshold and excitability level, too many inputs and the KC will fire too often (making odour responses too broad, leading to failure to discriminate odours); too few and it won't fire enough (making odour responses too sparse, leading to failure to detect odours).

Aims:
We hypothesize that a KC's own electrical activity homeostatically affects how many inputs it receives, via how many dendrites the KC forms. We will decrease (or increase) the intrinsic excitability of single KCs and test if this increases (or decreases, respectively), the number of dendrites the KC forms.

Techniques:
Single-cell labelling by genetic techniques (MARCM); brain dissections; confocal microscopy; immunolabelling.

References:
Sparse coding in KCs: Lin, A. C., Bygrave, A. M., de Calignon, A., Lee, T., and Miesenböck, G. (2014).

Sparse, decorrelated odor coding in the mushroom body enhances learned odor discrimination. Nat Neurosci 17, 559–568. Anatomical and computational support for activity-dependent plasticity in KCs Abdelrahman, N.Y., Vasilaki, E., and Lin, A.C. (2021).

Compensatory variability in network parameters enhances memory performance in the Drosophila mushroom body. PNAS 118, e2102158118. https://doi.org/10.1073/pnas.2102158118. How to label single KCs Wu, J. S., and Luo, L. (2007).

A protocol for mosaic analysis with a repressible cell marker (MARCM) in Drosophila. Nat Protoc 1, 2583–2589.

Project 6

Department of Biomedical Science Laboratory Research Project 2024/25

Investigating the role of the extracellular matrix in supporting cancer cell growth under glucose starvation

Cancer Biology

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Background

Alteration in cell metabolism is of one of the hallmarks of cancer. Due to elevated cell growth and limited blood supply, the tumour microenvironment is often deprived of nutrients, including glucose and amino acid. Therefore, cancer cells adopt alternative strategies to obtain nutrients to support their growth under starvation. These include the scavenging of extracellular proteins, such as components of the extracellular matrix (ECM). The ECM is a complex network of secreted proteins that, besides providing support for tissues and organs, is involved in controlling a variety of cell function, including tumour formation and progression. Data from our lab demonstrate that the ECM can support the growth of invasive breast cancer cells under glucose starvation, through the rewiring of different metabolic pathways. Interestingly, the presence of ECM also results in the acquisition of drug resistance under glucose starvation.

Aims:

This project will characterise the mechanisms through which the ECM favours cancer cell growth and drug resistance under glucose starvation. Objectives: 1. Assessing the contribution of metabolic rewiring in supporting cell growth under glucose starvation in the presence of ECM, using a combination of 3D culture and metabolomics approaches 2. Elucidate the molecular mechanisms through which glucose starvation promotes drug resistance

Techniques:

Mammalian cell tissue culture is the base for the development of this project. Different types of ECM will be generated. Cell proliferation will be assessed in 2D and 3D environments using high throughput systems, while a mass-spectrometry based metabolomic approach will be used to investigate changes in cell metabolism.

References:

NAZEMI and Rainero, 2020. Cross-Talk Between the Tumor Microenvironment, Extracellular Matrix, and Cell Metabolism in Cancer. Front Oncol. 2020 Feb 26;10:239 Cox 2021. The matrix in cancer. Nature Reviews Cancer 21, pp.217-38

Nazemi M, Yanes B, Martinez ML, Walker HJ, Pham K, Collins MO, Bard F, Rainero E. The extracellular matrix supports breast cancer cell growth under amino acid starvation by promoting tyrosine catabolism. PLoS Biol. 2024 Jan 16;22(1):e3002406. doi: 10.1371/journal.pbio.3002406. eCollection 2024 Jan.

Project 7

Department of Biomedical Science Laboratory Research Project 2024/25

Mechanobiology of breast cancer

Cancer Biology

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Background

It has recently become clear that the mechanical properties of cells and of the tissue environment are crucial player in development and disease. The stiffness of the extracellular matrix can trigger differentiation of stem cells and is an important regulator of tumor progression, fibrosis, and cardiovascular disease, however the molecular mechanisms how stiffness is sensed by the cell and how it is translated into biochemical signalling is currently incompletely understood. We have identified a number of candidate proteins that translocate from the cytosol into the nucleus upon mechanical cues elucidating potentially mechanosensitive signalling. This master project will investigate in detail a subset of these protein candidates and aims to elucidate their contribution to regulation of cell proliferation and tumor progression.

Aims:

The project aims at identifying of novel molecular pathways how mechanical cues like cell density and extracellular matrix stiffness are translated into biochemical signalling. The contribution of these pathways to tumor progression in breast cancer will be investigated.

Techniques:

Spinning disc confocal microscopy, immunofluorescence analysis, cell culture, cell transfection, cDNA cloning and expression, western-blotting, proximity biotinylation, tumor sample microarray staining

References:

Mechanobiology of YAP and TAZ in physiology and disease.
Panciera T, Azzolin L, Cordenonsi M, Piccolo S.
Nat Rev Mol Cell Biol. 2017 Dec;18(12):758-770. doi: 10.1038/nrm.2017.87. Epub 2017 Sep 27.
Review.

Project 8

Department of Biomedical Science Laboratory Research Project 2024/25

Characterization of subpopulations of otic progenitors derived from human Embryonic Stem Cells (hESCs)

Stem Cell & Regenerative Medicine

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Background

The ability to define and purify otic progenitor cells effectively is key for the development of a stem cell based therapy for deafness. When hESCs are directed into otic differentiation, they generate at least two types of otic cells with different molecular profile and lineage potential: otic epithelial (OEPs) and otic neuroprogenitors (ONPs). ONPs have the ability to restore auditory function when transplanted into an animal model of deafness. However, a relatively subtle heterogeneity is apparent within the progenitor subpopulations. Such heterogeneity may be comparable to the diversity encountered in the differentiating otocyst. It would be important to gain a better understanding of the different molecular make-ups and lineage potential of these different cell types. To explore this, we have generated a reporter line that expresses GFP driven by an otic-specific enhancer and identified a signature of cell surface markers.

Aims:

This project will aim to characterise the heterogeneity and diversity of otic phenotypes obtained from hESCs.

Techniques:

The project will involve the culture of human embryonic stem cells and otic progenitors, reporter lines, flow cytometry, immunolabeling, qRT-PCR and fluorescent microscopy.

References:

Chen, W; et al. Restoration of auditory evoked responses by human ES cells-derived otic progenitors. Nature, 490:278-282, 2012

Project 9

Department of Biomedical Science Laboratory Research Project 2024/25

Defining the signalling pathways regulating the induction, differentiation and maintenance of human neuromesodermal progenitors

Stem Cell & Regenerative Medicine

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Background

During early embryogenesis the spinal cord, skeleton and body musculature are generated by a common precursor population known as neuromesodermal progenitors (NMPs). These are located at the posterior part of the embryo and they are marked by the co-expression of two transcription factors, TBXT and SOX2. NMPs have recently attracted considerable interest because of their capacity to give rise to cell populations which can be used for regenerative medicine and disease modelling applications. We have recently described the derivation of human NMPs from human pluripotent stem cells (hPSCs) following treatment with WNT and FGF signalling agonists. Under these conditions a large fraction of cells express both TBXT and SOX2 corresponding to NMPs after 3 days of differentiation. However, these cells cannot be maintained as NMPs for longer and lose TBXT expression after culture under the same conditions for a further 3-4 days.

Aims:

1) To determine the signalling pathways which regulate the emergence of TBXT+SOX2+ NMPs from human pluripotent stem cells
2) To determine the signalling pathways which regulate the maintenance of TBXT+SOX2+ NMPs for long periods in culture
3) To determine the signalling pathways which regulate the differentiation of TBXT+SOX2+ NMPs toward their downstream derivatives

Techniques:

hPSC culture and differentiation, immunocytochemistry, real time quantitative PCR

References:

1) Gouti et al. PLoS Biol. 2014 Aug 26;12(8):e1001937
2) Wymeersch et al Development. 2021 Feb 16;148(4):dev180612
3) Tzouanacou et al. Dev Cell. 2009 Sep;17(3):365-76.

Project 10

Department of Biomedical Science Laboratory Research Project 2024/25

Host senescence responses to the typhoid toxin of Salmonella Typhi

Cell & Animal Models of Human Disease

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Background

Typhoid fever is caused by Salmonella Typhi (~27 million infections, 200,000 deaths each year). In our recent Nature Communications article (doi:10.1038/s41467-019-12064-1), we discovered that typhoid toxin of S.Typhi induces a non-canonical DNA damage response in human cells - the RING phenotype, which accelerates an ageing-like process called senescence.

The senescent cells respond by secreting unidentified human proteins (secretome), which cause senescence in bystander cells that have increased susceptibility to Salmonella infection (henceforth txSASP phenotype). We have used proteomics to identify the secretome underlying the txSASP phenotype. Thus, we want an enthusiastic student to exploit our mass spectrometry data to help elucidate the mechanism by which candidate proteins in the secretome drive senescence and infection.

Aims:

1. Identify candidate txSASP proteins through bioinformatic analysis of senescent cell secretomes
2. Validate the txSASP proteins in the secretome of toxin-treated human cells
3. Establish the significance of the validated txSASP proteins in driving senescence and infection

Techniques:

Mammalian cell culture, bioinformatics, infection biology, RNAi, protein purification, immunofluorescence, fluorescence microscopy, image analysis and phenotyping, immunoblotting.

References:

1. Ibler AEM, ElGhazaly M, Naylor KL, Bulgakova NA, F. El-Khamisy S & Humphreys D (2019) Typhoid toxin exhausts the RPA response to DNA replication stress driving senescence and Salmonella infection. Nature Communications, 10(1). PMID: 31492859
2. Yang, Y. A., Chong, A. & Song, J. Why Is Eradicating Typhoid Fever So Challenging: Implications for Vaccine and Therapeutic Design. Vaccines-Basel 6, doi:ARTN 45 10.3390/vaccines6030045 (2018).
3. Song, J., Gao, X. & Galan, J. E. Structure and function of the Salmonella Typhi chimaeric A(2)B(5) typhoid toxin. Nature 499, 350-354, doi:10.1038/nature12377 (2013).

Project 11

Department of Biomedical Science Laboratory Research Project 2024/25

Immunosenescence: a novel mechanism by which pathogenic bacteria evade innate immunity.

Cell & Animal Models of Human Disease

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Background

Senescence is a hallmark of ageing. Senescent cells undergo permanent cell-cycle arrest and release a pro-inflammatory secretome - senescence-associated secretory phenotype (SASP) - that transmits senescence and recruits immune cells, which restrict pathological cells, e.g. cancer or virus-infected. Consequently, senescence is considered a powerful innate defence mechanism. Counter to this, we discovered that typhoid toxin of Salmonella Typhi hijacks senescence to drive infection (Ibler et al 2019). By inducing senescence, the toxin triggered cellular secretion of unidentified factors (txSASP) that transmit senescence to bystander cells, elicit DNA damage and drive Salmonella infection underlying typhoid fever. We seek an enthusiastic student to establish whether the toxin accelerates ageing in macrophage cells via immunosenescence, which we propose represents a novel immune evasion mechanism by bacterial pathogens.

Aims:

1. Establish protocols for immunosenescence using human macrophages and purified toxin Harvest intoxicated macrophages then:
2. Investigate immunosenescence in the intoxicated macrophages by immunoblotting and fluorescence microscopy
3. Identify genetic signatures of immunosenescence using Gene Chip Microarray and bioinformatic analysis

Techniques:

Mammalian cell culture, bioinformatics, Gene Chip Microarray, protein purification, immunofluorescence, fluorescence microscopy, image analysis and phenotyping, immunoblotting.

References:

1. Ibler AEM, ElGhazaly M, Naylor KL, Bulgakova NA, F. El-Khamisy S & Humphreys D (2019) Typhoid toxin exhausts the RPA response to DNA replication stress driving senescence and Salmonella infection. Nature Communications, 10(1). PMID: 31492859
2. Yang, Y. A., Chong, A. & Song, J. Why Is Eradicating Typhoid Fever So Challenging: Implications for Vaccine and Therapeutic Design. Vaccines-Basel 6, doi:ARTN 45 10.3390/vaccines6030045 (2018).
3. Song, J., Gao, X. & Galan, J. E. Structure and function of the Salmonella Typhi chimaeric A(2)B(5) typhoid toxin. Nature 499, 350-354, doi:10.1038/nature12377 (2013).

Project 12

Department of Biomedical Science Laboratory Research Project 2024/25

Development of JAK/STAT pathway-dependent cell lines resistant to JAK-inhibitors

Mechanisms of Drug Action

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Background

A number of cultured human cell lines are dependent on JAK/STAT pathway signalling for their growth. As such they can be used to test the activity of JAK inhibitors. However, in the clinic, patients can become resistant to JAK inhibitor therapy. This project seeks to develop cell lines which require JAK/STAT pathway activity, but which have been selected to be resistant to two clinically used JAKinibs. Development of such cells will allow the testing of other inhibitors in a 'resistant' background with the long term aim of identifying rescue therapies that could potentially be used in the clinic.

Aims:

Determine the Ki of methotrexate and ruxolitinib in naive BaF3 cells using cell counting, MTT/MTS assays and anti-pSTAT5 antibody assays. Develop cells resistant to the inhibitory effects of methotrexate and ruxolitinib. Repeat the Ki determination in both resistant cell lines and naïve cells for both drugs.

Techniques:

Cell culture, western blotting, MTT/MTS assays.

References:

Thomas S, Fisher KH, Snowden JA, Danson SJ, Brown S, Zeidler MP. Methotrexate Is a JAK/STAT Pathway Inhibitor. PLoS One. 2015 Jul 1;10(7):e0130078. doi: 10.1371/journal.pone.0130078. PMID: 26131691; PMCID: PMC4489434.

Meyer SC, Keller MD, Chiu S, Koppikar P, Guryanova OA, Rapaport F, Xu K, Manova K, Pankov D, O'Reilly RJ, Kleppe M, McKenney AS, Shih AH, Shank K, Ahn J, Papalexi E, Spitzer B, Socci N, Viale A, Mandon E, Ebel N, Andraos R, Rubert J, Dammassa E, Romanet V, Dölemeyer A, Zender M, Heinlein M, Rampal R, Weinberg RS, Hoffman R, Sellers WR, Hofmann F, Murakami M, Baffert F, Gaul C, Radimerski T, Levine RL. CHZ868, a Type II JAK2 Inhibitor, Reverses Type I JAK Inhibitor Persistence and Demonstrates Efficacy in Myeloproliferative Neoplasms. Cancer Cell. 2015 Jul 13;28(1):15-28. doi: 10.1016/j.ccell.2015.06.006. PMID: 26175413; PMCID: PMC4503933.

Project 13

Department of Biomedical Science Laboratory Research Project 2024/25

Investigating the role of the JAK/STAT pathway in the specification of Drosophila blood cell lineages

Cell & Animal Models of Human Disease

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Background

Until recently Drosophila blood was thought to be made up of just three cell types – macrophage-like plasmatocytes, platelet/thrombocyte-like crystal cells and specialist parasite engulfing cells called lamellocytes. Although the role of JAK/SAT signalling in the specification of lamellocytes has been well documented (Morin-Poulard 2013), it is not known what role the pathway plays in the specification or differentiation of other cell types. We have recently identified molecular reporters/markers that identify previously unknown subsets of plasmatocytes (Coates et al 2021). However the pathways that regulate the number and temporal dynamics of these subtypes remains unclear.

Aims:

1) Establish assays / approaches to manipulate the JAK/STAT pathway in vivo 2) Quantify / characterise the number and spatial distribution of cells labelled by subtype-specific markers in JAK/STAT-modified and control animals. 3) Examine the temporal distribution of subtypes at different developmental stages in JAK/STAT-modified and control animals. 4) Quantify the behaviour of the highlighted blood cell sub-lineages in JAK/STAT-modified and control animals.

Techniques:

Drosophila genetics, husbandry and embryo collections Collection, fixation and preparation of embryos, larvae and blood films for staining with antibodies and recombinant fluorescent proteins Use of spinning disc / confocal microscopes to capture data Data interpretation, preparation, quantification and statistical analysis

References:

Coates et al, 2021 Elife 10:e58686. doi: 10.7554/eLife.58686.

Morin-Poulard et al, 2013, JAKSTAT ; 2(3): e25700. doi: 10.4161/jkst.25700

Project 14

Department of Biomedical Science Laboratory Research Project 2024/25

Developing novel cell lines for modelling CEDNIK syndrome

Cell & Animal Models of Human Disease

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Background

CEDNIK (cerebral dysgenesis, neuropathy, ichthyosis and keratoderma) syndrome is a rare fatal disease caused by the loss of SNAP29. SNAP29 is member of a family of proteins required for membrane transport and specifically vesicle fusion. At present it is unclear why the loss of SNAP29 leads to CEDNIK syndrome. To elucidate the role of SNAP29 in this process we will use CRISPR/Cas9 based approaches to generate cell lines where the function of SNAP29 has been perturbed. In addition, we will also use this method to generate tagged version of SNAP29, which will allow the function of SNAP29 to assessed using live cell microscopy and proteomic based approaches.

Aims:

The aim of the project is to generate a panel of cell lines which will be useful for modelling CEDNIK disease. These cells will then be used to a) elucidate the molecular interactions which regulate SNAP29 function and b) monitor the intracellular localisation and trafficking of SNAP29.

Techniques:

The project will use cutting edge gene editing approaches combined with advanced microscopy and proteomic based methods. The project will also provide a strong grounding in cell culture and basic molecular biology techniques.

References:

Fuchs-Telem D., Stewart H., Rapaport D., Nousbeck J., Gat A., Gini M., Lugassy Y., Emmert S., Eckl K., Hennies H.C., Sarig O., Goldsher D., Meilik B., Ishida-Yamamoto A., Horowitz M., Sprecher E. CEDNIK syndrome results from loss-of-function mutations in SNAP29. British Journal of Dermatolgy 2011. 164, 610-616.

Gordon D.E., Bond L.M., Sahlender D.A. and Peden A.A. A targeted siRNA screen to identify SNAREs required for constitutive secretion in mammalian cells. 2010 Traffic. 11, 1191-1204. Wang H., La Russa M., Qi L.S. CRISPR/Cas9 in Genome Editing and Beyond.Annu Rev Biochem 2016. 85:227-64.

Project 15

Department of Biomedical Science Laboratory Research Project 2024/25

Elucidating the role of STX19 in secretory autophagy

Cell & Animal Models of Human Disease

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Background

STX19 is a poorly characterised Qa-SNARE which is targeted to the cell surface by palmitoylation. Depletion of STX19 using siRNA causes a block in constitutive secretion and its overexpression leads to a loss of LC3 positive autophagosomes from the cell. STX19 contains a LIR motif which may allow STX19 to bind and/or be recruited to LC3 positive autophagosomes. Using a range of approaches such as Y2H screening, proteomics and proximity based biotinylation assays several novel STX19 interacting proteins have been identified (SNAP29, ZWINT, Girdin and DST) which may regulate the function of STX19.

Aims:

The overarching aim of this project is to determine if STX19 has a role in secretory autophagy and whether its interacting partners also function on this pathway.

Techniques:

To determine if STX19 and its binding partners are functioning in secretory autophagy a range of cutting edge techniques will be used including protein pull downs, confocal microscopy and mitochondrial rerouting experiments.

References:

Cellular and molecular mechanism for secretory autophagy. 2017 Autophagy. DOI: 10.1080/15548627.2017.1307486.

S-acylation regulates the trafficking and stability of the unconventional Q-SNARE STX19. 2018 Journal of Cell Science. DOI: 10.1242/jcs.212498.

A targeted siRNA screen to identify SNAREs required for constitutive secretion in mammalian cells. 2010 Traffic. DOI: 10.1111/j.1600-0854.2010.01087.x.

Project 16

Department of Biomedical Science Laboratory Research Project 2024/25

Understanding cancer cell diversity through dynamics of intracellular messengers

Cancer Biology

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Background

Mammalian cells are constantly exposed to multiple extracellular signalling molecules and their behavioural decisions (e.g. to proliferate or differentiate) are defined by simultaneous outputs from numerous signalling pathways. Understanding how the dynamics of these pathways affect cell behaviour is crucial for normal and pathological tissue. High spatiotemporally-resolved reporters of extracellular and intracellular signalling molecules are invaluable but generation of multiple reporters is laborious and image analysis of their simultaneous activity is computationally demanding. To overcome this problem, you will apply a complementary approach: to use the dynamics of second messengers (STAT3 and calcium ions) as a single, integrated readout of multiple signalling pathways. Previous students in the lab have already developed a technique for long-term imaging of cultured cancer cells (Razlivanov et al. Biotechniques, 65(1); 37-39). This powerful technique allows us to address a range of important questions such as: a) how do individual cancer cells differ in terms of their signalling pathways? b) what is the optimal dynamics of activation of individual pathways? c) how do anticancer drugs inhibiting one pathway affect other pathways? In this project you will continue to address these fundamental problems.

Aims:

1. Describe the heterogeneity of cancer cells in culture using the dynamics of second messengers as a readout 2. Define the optimal dynamics of activation by extracellular signaling molecules (e.g. EGF, VEGF and ATP) 3. Define how treatment with epidermal growth factor antagonists affect the dynamics of other extracellular signaling pathways (e.g. FGF and ATP) 4. Optional: if you are a mathematics geek, you will have an opportunity to participate in development of novel approach that will use real cells in machine learning tasks to optimize drug treatment dynamics

Techniques:

Fluorescent imaging, cell culture techniques, molecular biology

References:

Nguen L.K., Kholodenko B.N., (2015) “Feedback regulation in cell signalling: Lessons for cancer therapeutics” Semin Cell Dev Biol. pii: S1084-9521(15)00185-6

Slack, M. D., Martinez, E. D., Wu, L. F. & Altschuler, S. J. (2008) “Characterizing heterogeneous cellular responses to perturbations” PNAS 105, 19306-19311 Noren, D. P. et al. Endothelial cells decode VEGF-mediated Ca2+ signaling patterns to produce distinct functional responses.Science signaling 9, ra20,
doi:10.1126/scisignal.aad3188 (2016).

Clapham, D. E. (2007). "Calcium signaling." Cell 131(6): 1047-1058. Santos S.D., et al. (2007). " Growth factor-induced MAPK network topology
shapes Erk response determining PC-12 cell fate." Nat Cell Biol 9(3): 324-30.

Project 17

Department of Biomedical Science Laboratory Research Project 2024/25

Imaging neuronal activity in zebrafish model of stroke

Cell & Animal Models of Human Disease

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Background

The goal of the project is to develop experimental paradigms to study how neuronal circuits differ in health and disease. For this purpose, we will use zebrafish larvae as an animal model. The main advantage of zebrafish is its transparency, well defined brain organisation and ease of pharmacological manipulations. Zebrafish has been successfully used as a model for a number of neurological diseases but to date, it was not used to understand the neural circuitry of these diseases. We will fill this gap by studying how processing of sensory and motor information is affected by stroke.

To achieve this goal, we will establish an experimental paradigm involving 2-photon imaging of neuronal activity in zebrafish larvae subjected to a range of stimuli evoking different forms of behaviour and study how neuronal activity changes in zebrafish model of stroke.

Aims:

1. To develop experimental paradigm of whole brain imaging of zebrafish larvae exposed to multisensory stimulation.
2. Define stereotypic subpopulations of neurons responsible for different forms of behaviours.
3. To define how the activity of these populations is affected in zebrafish model of ischemic brain damage.

Techniques:

1. Zebrafish handling
2. 2-photon calcium imaging of neuronal activity
3. In situ hybridisation
4. Image analysis

References:

1. Walcott and Peterson “Zebrafish model of cerebrovascular disease” J Cereb Blood Flow Metab 2014 Apr; 34(4): 571–577.
2. Kishimoto et al. “Neuronal regeneration in a zebrafish model of adult brain injury” Disease Models & Mechanisms 2012 5: 200-209; doi: 10.1242/dmm.007336
3. Yu and Yang “Zebrafish as an alternative model for hypoxic-ischemic brain damage” Int J Physiol Pathophysiol Pharmacol 2011;3(2):88-96

Project 18

Department of Biomedical Science Laboratory Research Project 2024/25

Targeting GBM Brain tumours with novel targeting therapeutics.

Mechanisms of Drug Action Cancer Biology

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Background

Approximately 3,200 adults are diagnosed with Glioblastoma multiforme (GBM) each year. Survival is ~12-18 months. GBM treatments have not improved in 20 years. Current treatments, such as chemotherapy are inefficient and non-specific and lead to recurrence within 15 months (Fernandes et al., 2017). Most therapies cause a multitude of secondary effects, due to the damage done to local tissue, so there is a need to have targeted and effective new medicines (Shen & Abate-Shen, 2010). Recent advances in targeted therapies, such as monoclonal antibodies, have had limited success. However, although effective in some cancers, they are ineffective in GBM, combined with the manufacturing and immunological side-effects, this makes them not ideal as a treatment.
Peptides and peptide drug conjugates (PDC) represent an emerging strategy that can reduce peripheral toxicity & adverse reactions, while enhancing drug uptake through tumour selectivity (Vrettos et al. 2018). Currently little work investigates their use in GBM. This project will use cell- penetrating peptides to deliver novel therapies to GBM cancer cells.

Aims:

Determine the interaction and efficacy of delivery with a cell surface receptor in cancer cells. Determine the subcellular locations of the peptide and receptor. Quantitate the activity of the peptide in cancer targeting with pulse chase experiments, using receptor ligand interactions.

Techniques:

The candidate will use cancer cell lines to characterize the peptide in cell assays. The project will use automated high throughput equipment including, high content microscopy and liquid handling robotics. Methods will include the drug inhibition of receptor internalisation. The project will use cell penetrating peptides, RNAi, immunological stains, small molecule inhibitors and biochemical assays.

References:

Fernandes C, Costa A, Osório L, et al. Current Standards of Care in Glioblastoma Therapy. In: De Vleeschouwer S, editor. Glioblastoma [Internet]. Brisbane (AU): Codon Publications; 2017 Sep 27. Chapter 11. Available from: https://www.ncbi.nlm.nih.gov/books/NBK469987/ doi: 10.15586/codon.glioblastoma.2017.ch11

Shen, M. M., & Abate-Shen, C. (2010). Molecular genetics of prostate cancer: new prospects for old challenges. Genes & development, 24(18), 1967-2000.

Vrettos, E. I., Mező, G., & Tzakos, A. G. (2018). On the design principles of peptide–drug conjugates for targeted drug delivery to the malignant tumuor site. Beilstein journal of organic chemistry, 14(1), 930-954.

Project 19

Department of Biomedical Science Laboratory Research Project 2024/25

Identification of TANK-binding kinase 1 (Tbk1) signalling pathways relevant to Motor Neurone Disease.

Neurodevelopment & Neuroscience **Cell & Animal Models of Human Disease**

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Background

Motor Neurone Disease or Amyotrophic lateral sclerosis (ALS) is a disorder that results in fatal paralysis within a few years of symptom onset. In recent years, large exome sequencing studies of ALS patients have independently identified loss of function mutations in the kinase, Tbk1. Repeat expansion in C9ORF72 is a major cause of ALS and results in the formation of aggregates that sequester RNA- binding proteins. Tbk1 phosphorylates optineurin and p62, cargo receptors for recruiting ubiquitinated proteins to the autophagosome for destruction. All three proteins are involved in autophagy and are relevant to the clearance of aggregates that result from C9ORF72 repeat expansion. Although mutations in Tbk1 are associated with ALS, it is not known how dysregulation of this kinase might contribute to development of disease.

Aims:

1) Use proteomic approaches to identify substrates and regulators of Tbk1 relevant to the formation and accumulation of protein aggregates characteristic of ALS.
2) To validate novel Tbk1 interactors/substrates using Co-IPs, kinase assays and Western blotting.

Techniques:

Cell culture and transfection, immunoprecipitation, western blotting, sample preparation for proteomic analysis, mass spectrometry, immunocytochemistry with confocal microscopy.

References:

Haploinsufficiency of TBK1 causes familial ALS and fronto-temporal dementia. Freischmidt A et al. Nat Neurosci. 2015 May;18(5):631-6.

Exome sequencing in amyotrophic lateral sclerosis identifies risk genes and pathways. Cirulli ET et al. Science. 2015 Mar 27;347(6229):1436-41.

The PINK1-PARKIN Mitochondrial Ubiquitylation Pathway Drives a Program of OPTN/NDP52 Recruitment and TBK1 Activation to Promote Mitophagy. Heo JM et al. Mol Cell. 2015 Sep 9. pii: S1097-2765(15)00662-0

Project 20

Department of Biomedical Science Laboratory Research Project 2024/25

Remodelling of the cell surface proteome by the palmitoyl-acyltransferase DHHC5

Neurodevelopment & Neuroscience **Cell & Animal Models of Human Disease**

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Background

Protein palmitoylation is the addition of a long-chain fatty acid to a cysteine residue via a thioester bond. This reversible lipid modification is most often used to facilitate the interaction of proteins with membranes, and dysfunction of the palmitoylation machinery has been implicated in several neurological disorders, including Huntington's disease and Schizophrenia. A family of 23 enzymes called palmitoyl-acyltransferases (PATs) mediate the palmitoylation of proteins in humans. DHHC5 is among the more unique members of the PAT family as it is localised to the plasma membrane, where it regulates the subcellular localisation of its substrates by palmitoylation. ZDHHC5 plays a vital role in a wide range of processes in different cell types, and we know that DHHC5 regulates the cell surface expression of specific proteins (-catenin, desmōglein-2 etc.) important for synaptic plasticity and cell adhesion. We have preliminary data that indicates that DHHC5 regulates the cell surface expression of a broader set of proteins. This project aims to determine how DHHC5 remodels the cell surface proteome using DHHC5 CRISPR knockout cells and state-of-the-art mass spectrometry-based proteomics.

Aims:

1) Determine changes in the cell surface proteome in DHHC5 CRISPR knockout cells using cell surface protein labelling, enrichment and analysis using quantitative mass spectrometry-based proteomics.
2) Validate changes in cell surface expression identified in aim 1 using western blotting and immunofluorescence microscopy

Techniques:

Cell culture and transfection, western blotting, sample preparation for proteomic analysis, mass spectrometry, data analysis/bioinformatics, immunofluorescence microscopy.

References:

Woodley KT, Collins MO. Regulation and function of the palmitoyl-acyltransferase ZDHHC5. FEBS J. 2021 Jan 8.

Woodley KT, Collins MO. S-acylated Golga7b stabilises DHHC5 at the plasma membrane to regulate cell adhesion. EMBO Rep. 2019 Oct 4;20(10):e47472.

Brigidi GS, Santyr B, Shimell J, Jovellar B & Bamji SX. (2015). Activity-regulated trafficking of the palmitoyl-acyl transferase DHHC5. Nature communications 6, 8200.

Project 21

Department of Biomedical Science Laboratory Research Project 2024/25

Remodelling of the lipid raft proteome by the palmitoyl-acyltransferase DHHC5

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Background

Protein palmitoylation is the addition of a long-chain fatty acid to a cysteine residue via a thioester bond. This reversible lipid modification is most often used to facilitate the interaction of proteins with membranes, and dysfunction of the palmitoylation machinery has been implicated in several neurological disorders, including Huntington's disease and Schizophrenia. A family of 23 enzymes called palmitoyl-acyltransferases (PATs) mediate the palmitoylation of proteins in humans. DHHC5 is among the more unique members of the PAT family as it is localised to the plasma membrane, where it regulates the subcellular localisation of its substrates by palmitoylation-meditated partitioning into lipid rafts. ZDHHC5 plays a vital role in a wide range of processes in different cell types, and we know that DHHC5 regulates the cell surface expression of specific proteins (-catenin, desmoglein-2 etc.) important for synaptic plasticity and cell adhesion. We have preliminary data that indicates that DHHC5 regulates the cell surface expression of a broader set of proteins and, in particular, lipid raft proteins. This project aims to determine how DHHC5 remodels the lipid raft proteome using DHHC5 CRISPR knockout cells, proximity-labelling of lipid raft proteins and state-of-the-art mass spectrometry-based proteomics.

Aims:

1) Determine changes in the lipid raft proteome in DHHC5 CRISPR knockout cells using APEX2 proximity-labelling, enrichment and analysis using quantitative mass spectrometry-based proteomics.
2) Validate changes in lipid raft protein expression identified in aim 1 using western blotting and immunofluorescence microscopy

Techniques:

Cell culture and transfection, western blotting, sample preparation for proteomic analysis, mass spectrometry, data analysis/bioinformatics, immunofluorescence microscopy.

References:

Woodley KT, Collins MO. Regulation and function of the palmitoyl-acyltransferase ZDHHC5. FEBS J. 2021 Jan 8.

Woodley KT, Collins MO. S-acylated Golga7b stabilises DHHC5 at the plasma membrane to regulate cell adhesion. EMBO Rep. 2019 Oct 4;20(10):e47472.

Brigidi GS, Santyr B, Shimell J, Jovellar B & Bamji SX. (2015). Activity-regulated trafficking of the palmitoyl-acyl transferase DHHC5. Nature communications 6, 8200.

Project 22

Department of Biomedical Science Laboratory Research Project 2024/25

Using behaviour to understand why synaptic depression might be better for learning than synaptic potentiation

Neurodevelopment & Neuroscience **Cell & Animal Models of Human Disease**

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Background

The fruit fly Drosophila can associate particular odours with rewards or punishments. These memories are stored in odour-encoding neurons called Kenyon cells (KCs), which synapse onto neurons called mushroom body output neurons (MBONs) that lead to approach or avoidance behaviour. Flies learn by weakening the incorrect action – e.g., when odour X is paired with punishment, the synapses from odour-X-responding KCs onto 'approach' MBONs are depressed. Why weaken the incorrect action rather than strengthen the correct action? We have developed a mathematical model of the circuit that predicts that synaptic depression is computationally superior to synaptic potentiation under certain conditions. These conditions make testable predictions about certain quantitative psychophysical features of fly learning behaviour. We will test these predictions to see if synaptic depression is 'optimal'.

Aims:

We will test these predictions by measuring how well flies can learn to avoid different concentrations of odours given different intensities of punishment. We predict that flies would learn better with stronger odours but that performance would reach a plateau at intermediate concentrations, and that this plateau should get higher with stronger punishment.

Techniques:

Behavioural experiments, computational simulations (if desired)

References:

Amin, H., and Lin, A.C. (2019). Neuronal mechanisms underlying innate and learned olfactory processing in Drosophila. Current Opinion in Insect Science 36, 9–17. https://doi.org/10.1016/j.cois.2019.06.003.

Project 23

Department of Biomedical Science Laboratory Research Project 2024/25

Using the connectome to analyse the relationship between dendritic morphology and number or placement of synaptic inputs

Neurodevelopment & Neuroscience **Cell & Animal Models of Human Disease**

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Background

How do neurons set up their synaptic inputs to achieve consistent levels and patterns of electrical activity despite extensive variation in morphology and electrical properties between even neurons of the same type? Perhaps variation on one physiological parameter serves to compensate for variation on another parameter.

This project tests this hypothesis using neurons called Kenyon cells (KCs) that store olfactory associative memories in the fruit fly Drosophila. For flies to distinguish many odors, it's important for all KCs to have similar activity levels, yet each KC receives variable amounts of excitatory input. Perhaps KCs compensate for variable amounts of excitation by altering their dendritic morphology: a KC receiving a lot of excitatory synapses might put them at the end of long, thin dendrites so the signals decay a lot before reaching the spike initiation zone.

Aims:

Use newly available connectome to automatically analyse the dendritic morphology of hundreds of real KCs and test for correlations among the following factors: dendritic morphology (e.g., length/thickness/branching of dendrites); number of excitatory synapses; relative placement of excitatory and inhibitory synapses.

Techniques:

Computational simulations, programming-based data analysis, data visualization, statistical analysis

References:

Scheffer, L.K., Xu, C.S., Januszewski, M., Lu, Z., Takemura, S.-Y., Hayworth, K.J., Huang, G.B., Shinomiya, K., Maitin-Shepard, J., Berg, S., et al. (2020). A Connectome and Analysis of the Adult Drosophila Central Brain. bioRxiv 12, 2020.04.07.030213.

Lin, A. C., Bygrave, A. M., de Calignon, A., Lee, T., and Miesenböck, G. (2014). Sparse, decorrelated odor coding in the mushroom body enhances learned odor discrimination. Nat Neurosci 17, 559–568.
Gruntman, E., and Turner, G. C. (2013). Integration of the olfactory code across dendritic claws of single mushroom body neurons. Nat Neurosci 16, 1821–1829.

Project 24

Department of Biomedical Science Laboratory Research Project 2024/25

Role of primary cilia in skeletal muscle regeneration

Stem Cell & Regenerative Medicine

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Background

The long-term regenerative capacity of adult stem cells relies on the ability of stem cell to self-renew in order to maintain a pool of stem cells. In the laboratory, we study the mechanisms that regulate the activity of satellite cells, which are adult skeletal muscle-specific stem cells responsible for the repair of muscles after injury and in diseases. We recently reported that self-renewing satellite cells have the singular ability to re-assemble a primary cilium following cell division, a characteristics not shared by satellite cells adopting a myogenic fate.

Given that the primary cilia is a cellular organelle acting as a signalling centre, we have hypothesised that primary cilia play an essential role in maintaining a pool of stem cells during repair by providing cues that control self-renewal cell fate decision.

Aims:

The aims of this project is to use ex vivo and in vivo genetic approaches in the mouse to decipher the role of primary cilia in skeletal muscle regeneration and in satellite cell self-renewal.

Techniques:

Techniques will involve muscle fibre cultures, immunofluorescence, tissue cryosectioning, imaging, image analysis, statistical analyses.

References:

Jaafar Marican H, Cruz Migoni S & Borycki AG (2016) Asymmetric Distribution of Primary Cilia Allocates Satellite Cells for Self-Renewal. Stem Cell Reports, 6(6), 788-805.

Project 25

Department of Biomedical Science Laboratory Research Project 2024/25

Patched 1 function in skeletal muscle stem cells and its implication in muscle wasting

Stem Cell & Regenerative Medicine **Cell & Animal Models of Human Disease**

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Background

Muscle wasting associated with muscular dystrophies, cancer, atrophy and aging remains a major cause of death. The potential role played by skeletal muscle stem cells (MuSCs) in the loss of muscle mass remains obscure and requires further investigations. In previous work, we demonstrated that Sonic hedgehog signalling is essential to maintain the self-renewing and proliferative capacity of MuSCs. Yet, we uncovered that conditional gain-of-function mutations in Patched 1, the receptor for Sonic hedgehog, in MuSCs caused loss of muscle mass and a phenotype reminiscent of premature aging of muscles (sarcopenia).

Aims:

The project aims to investigate further the relationship between MuSC homeostasis and control of muscle mass, and to determine the specific role of Patched 1 signalling in this process.

Techniques:

The project will make use of mouse genetics, tissue cryosectioning, immunofluorescence and histology, combined with microscopy and imaging to characterize the sarcopenic phenotype in Patched 1 mutant muscles.

References:

A switch in cilia-mediated Hedgehog signaling controls muscle stem cell quiescence and cell cycle progression
Sara Betania Cruz-Migoni, Kamalliawati Mohd Imran, Aysha Wahid, Oisharja Rahman, James Briscoe, Anne-Gaëlle Borycki
βιοΡχιω 2019.12.21.884601;΄δοι:¨https://doi.org/10.1101/2019.12.21.884601

Project 26

Department of Biomedical Science Laboratory Research Project 2024/25

Analysis of HIF as a DNA repair factor

Cancer Biology

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Background

HIF is a protein that normally protects cells against the consequences of a lack of oxygen, however, we found that HIF also protects DNA against damage, even when oxygen levels are normal. What was exciting is that HIF could also protect the DNA in our animal model the zebrafish, when certain DNA repair genes were inhibited. For instance, ATM deficient larvae were at least as resistant against DNA-damaging treatments as normal fish when HIF was active. This is exciting because drugs that activate the HIF pathway have become available, and they have recently been approved in the UK and Europe for the treatment of anaemia.

Aims:

We aim to both define the protective effect of HIF better and identify potential downstream factors.

Techniques:

We will try to identify potential downstream genes and use genome editing tools such as CRISPR/Cas9 to create fish models of choice to test if these genes are important for genoprtection. We will modulate HIF signalling chemically of genetically and challenge larvae with DNA damaging compounds We will use a variety of molecular biology methods and will measure outputs by analysis of GFP reporters, in situ hybridisation, qPCR.

References:

Kim HR, Santhakumar K, Markham E, Baldera D, Greenald D, Bryant HE, et al. Investigation of the role of VHL-HIF signaling in DNA repair and apoptosis in zebrafish. Oncotarget. 2020;11(13):1109-30.

Project 27

Department of Biomedical Science Laboratory Research Project 2024/25

Analysis of DNA repair genes in zebrafish

Cell & Animal Models of Human Disease Cancer Biology

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Background

DNA repair is a crucial process and defects in DNA repair are a crucial driver for ageing, neurodegeneration and cancer. This process is classically studied in cell culture but it is clear that such models have significant limitations. We have started to create zebrafish in vivo models using CRISPR/CAS9 technologies and genome editing. These models can you be used to understand in vivo consequences of defective DNA repair.

Aims:

We have created several zebrafish mutant models in crucial genes invoved in DNA repair, the student will contribute to the phenotypic analysis of these mutants, and creation of further ones using CRISPR/CAS9 technology.

Techniques:

Molecular biology, PCR, microinjection, fluorescence microscopy, zebrafish technologies, behavioural analysis.

References:

Mammalian RNase H2 removes ribonucleotides from DNA to maintaingenome integrity. Hiller B, Achleitner M, Glage S, Naumann R, Behrendt R, Roers A.J Exp Med. 2012 Jul 30;209(8):1419-26

Zaksauskaite R, Thomas RC, van Eeden F, El-Khamisy SF. Tdp1 protects from topoisomerase 1-mediated chromosomal breaks in adult zebrafish but is dispensable during larval development. Sci Adv. 2021 Jan 29;7(5)

Project 28

Department of Biomedical Science Laboratory Research Project 2024/25

Dissecting the cellular and molecular mechanisms underlying mesenchymal-to epithelial transitions

Cancer Biology

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Background

During development many cells migrate over large distances to form tissues and organs. To do this they undergo an epithelial-to mesenchymal transition (EMT), migrate, and then undergo a mesenchymal-to epithelial transition (MET) at distant sites. While many upstream signals driving EMTs are known, MET remains poorly understood: neither the signals required for its induction, nor how it occurs at the cell and molecular level. The selected student will work on a project targeted towards identifying the molecular mechanisms underlying MET during midgut development in Drosophila melanogaster embryos. We have recently carried out transcriptomic approaches to identified genes differently regulated during midgut-MET. The project will involve validation of expression of these genes using fluorescence in situ hybridisation in wild type embryos, and in mutants – to understand how and when these genes become active.

Aims:

The lab is currently carrying out a high-throughput expression screen for genes involved in MET in the Drosophila embryo. The aim of this project will be for the student to investigate a number of candidate genes, to understand when and how these genes become active in the embryonic midgut.

Techniques:

This project will involve collating data from online databases; Drosophila genetics and husbandry; collecting, fixing and performing FISH on embryos; microscopy and image analysis.

References:

Campbell K, Whissell G, Franch-Marro X, Batlle E, Casanova J: Specific GATA factors act as conserved inducers of an endodermal-EMT. Dev Cell 2011, 21:1051-1061. 2.

Campbell K, Casanova J: A role for E-cadherin in ensuring cohesive migration of a heterogeneous population of non-epithelial cells. Nat Commun 2015, 6:7998. 3. Pitsidianaki I, Morgan J, Adams J, Campbell K: Mesenchymal-to-epithelial transitions require tissue specific interactions with distinct laminins. Journal Cell Biology 2021, 220 (8): e202010154.

Project 29

Department of Biomedical Science Laboratory Research Project 2024/25

How does rapamycin affect the Drosophila sleep cycle?

Neurodevelopment & Neuroscience **Cell & Animal Models of Human Disease**

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Background

A drug called Rapamycin was first used as an immune system suppressor in organ transplant recipients, and later discovered to extend the yeast and worm lifespans. In 2009, it was found to lengthen the mice lives by up to 14 per cent, even though the drug was started at 600 days old, the human equivalent of being about 60. But what kind of changes does rapamysin have in animal behaviour and life style? how does it affect an animal's sleep cycle/circadian rhythm?

Aims:
To investigate how adding rapamysin in the food affects the Drosophila sleep cycle and life span.

Techniques:
Using Drosophila activity monitoring system to measure and compare the sleep cycles and lifespan of control wild-type flies to those of test flies, which consume rapamysin-added food.

References:
Bitto et al. Transient rapamycin treatment can increase lifespan and healthspan in middle-aged mice. eLife 2016;5:e16351

Project 30

Department of Biomedical Science Laboratory Research Project 2024/25

Investigating the role of proteases in controlling extracellular matrix remodelling in breast cancer

Cancer Biology **Cell & Animal Models of Human Disease**

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Background

The extracellular matrix (ECM) is a complex network of secreted proteins, such as collagens and laminins, which is involved in controlling several cell functions, including proliferation and migration. It is well established that there is extensive ECM remodelling associated with breast cancer progression, leading to the formation of a collagen-rich microenvironment which promotes tumour progression. This is often associated with an increase production of proteases, which are able to degrade ECM components to foster cancer cell invasion. We have recently identified ECM endocytosis as a key mechanism upregulated in cancer cells, promoting both cell invasion and cell proliferation under nutrient deprived conditions, however the contribution of ECM degradation by proteases to this process is unknown. Our preliminary data suggest that cathepsins, and not matrix metalloproteases (MMPs) are involved in this process.

Aims:

This project will characterise the contribution of proteases to ECM internalisation, focusing cathepsins.
Objectives:

1. Assessing the ECM internalisation in the presence of cathepsin inhibitors.
2. Investigating the role of the cathepsins in breast cancer cell growth, migration and invasion

Techniques:

2D and 3D breast cancer cell culture, cell proliferation, migration and invasion assays, immunofluorescence and confocal microscopy, live-cell imaging

References:

Pengfei Lu, Valerie M. Weaver and Zena Werb. The extracellular matrix: A dynamic niche in cancer progression. J Cell Biol. 2012 Feb 20; 196(4): 395–406.

Osuala KO, Ji K, Mattingly RR, Sloane BF. Breast Cancer: Proteolysis and Migration. Adv Exp Med Biol. 2019;1152:401-411.

Mona Nazemi, Bian Yanes, Montserrat Llanses Martinez, Heather Walker, Frederic Bard, Elena Rainero. The extracellular matrix supports cancer cell growth under amino acid starvation by promoting tyrosine catabolism. bioRxiv 2021.06.09.447520; doi: https://doi.org/10.1101/2021.06.09.447520

Project 31

Department of Biomedical Science Laboratory Research Project 2024/25

Macropinocytosis as a regulator of cancer cell growth

Cancer Biology **Cell & Animal Models of Human Disease**

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Background

Cancer cells use macropinocytosis to take up nutrients to allow them to grow and survive (Commisso et al., 2013), Macropinocytosis is an endocytic pathway that allows cells to take up large volumes of extracellular material (King and Smythe, 2020). Actin-driven ruffles form on the cell surface which fuse to form macropinosomes and the material trapped within the macropinosome is targeted to lysosomes where it is broken down and used by the cell (Yoshida et al., 2018). In order to identify novel regulators of macropinocytosis we have carried out a genome wide siRNA screen of the kinome and have identified a number of novel and specific regulators of micropinocytosis. Understanding how these signalling molecules regulates macropinocytosis raises the exciting possibility of identifying novel therapeutic targets that could be developed for cancer treatment.

Aims:

The aim of the project is to select a small number of the most potent ‘hits’ from the siRNA screen and to determine their mechanism of action in macropinocytosis. We are particularly interested to determine whether interfering with the function of these candidate proteins will affect cancer cell growth.

Techniques:

Cell culture and transfection; CRISPr knock-out and knock-in experiment rescue experiments; high resolution microscopy, and quantitative mass spectrometry

References:

King JS, Smythe E. (2020) Water loss regulates cell and vesicle volume. Science 367: 246-247.

Commisso et al. (2013) Macropinocytosis of protein is an amino acid supply route in Ras-transformed cells Nature 497: 633-637

Yoshida et al. (2018) Macropinocytosis, mTORC1 and cellular growth control. Cell Mol Life Sci 75: 1227-1239

Project 32

Department of Biomedical Science Laboratory Research Project 2024/25

Investigating how Hyaluronic Acid shapes the embryonic heart.

Cell & Animal Models of Human Disease

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Background

The heart is a highly complex organ that arises from a linear heart tube in the early embryo. This heart tube undergoes complex morphogenesis including looping of the tube and regional ballooning of the cardiac chambers, which are important to establish the correct shape of the heart. The developing heart comprises an inner and outer tube, made up of myocardium and endocardium, between which is sandwiched a layer of extracellular matrix (ECM) rich in the glycosaminoglycan Hyaluronic Acid (HA). The cardiac ECM is dynamic during heart development, undergoing regionalized expansion and reduction during different stages of morphogenesis. HA- synthesising enzymes, HA-degrading enzymes, and HA-binding proteins have all been broadly implicated in heart development, yet how they help sculpt the heart is poorly understood. In this project you will use live fluorescent imaging of transgenic embryonic zebrafish hearts harbouring single or combinatorial mutations in various HA pathway genes to understand how HA dynamics fine tune heart morphogenesis.

Aims:

Using a combination of genetic zebrafish models, live fluorescent imaging, and 3D morphometric image analysis you will: Investigate the role of specific HA degrading or HA synthesizing enzymes in zebrafish heart morphogenesis. Use combinatorial genetic models to define the role of HA-binding proteins in HA homeostasis and heart morphogenesis.

Techniques:

Zebrafish embryology Molecular biology (for example genotyping, or plasmid generation) Immunohistochemistry Live fluorescent imaging (for example lightsheet or confocal) 3D image analysis R (for multiparametric data analysis)

References:

Derrick CJ, Sánchez-Posada J, Hussein F, Tessadori F, Pollitt EJG, Savage AM, Wilkinson RN, Chico TJ, van Eeden FJ, Bakkers J, Noël ES. Asymmetric Hapln1a drives regionalized cardiac ECM expansion and promotes heart morphogenesis in zebrafish development. Cardiovasc Res. 2022 Jan 7;118(1):226-240

Sánchez-Posada J and Noël ES. morphoHeart: a novel quantitative tool to perform integrated 3D morphometric analyses of heart and ECM morphology during embryonic development. bioRxiv 2024 https://doi.org/10.1101/2024.02.19.580991

Derrick CJ and Noël ES. The ECM as a driver of heart development and repair. Development 2021 Mar 5;148(5):dev191320.

Project 33

Department of Biomedical Science Laboratory Research Project 2024/25

New strategies for cancer drug development: protein-protein interaction inhibitors to inhibit the critical cancer target Fen1

Cancer Biology

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Background

Tumour suppressor genes are implicated in cancer suppression - via control of genomic stability. However, they may also be implicated in tumorigenesis & tumour progression, as subversion of the DNA damage response (DDR) plays an important role in generating genomic instability, a cancer hallmark. Genomic instability is also the evolutionary mechanism by which drug resistance emerges in cancer. We can conceive ways in which disruption of DDR in drug resistant cancer cells would restore sensitivity to first line treatment, opening up the opportunity of reducing or eliminating evolution of drug resistance in cancer. Fen1 is a non-essential component of the DNA replication machinery, which confers cisplatin resistance in non-small cell lung carcinoma. The aim is to investigate the role of a prototypic protein-protein interaction inhibitor drug which is predicted to disrupt Fen1's normal repertoire of cellular interacting proteins.

Aims:

The Fen1 gene is a non-essential component of the DNA replication machinery and the DDR, which confers cisplatin resistance in non-small cell lung carcinoma. The aim is to investigate the role of a prototypic protein-protein interaction inhibitor drug which is predicted to disrupt Fen1's normal repertoire of cellular interacting proteins.

Techniques:

This project will involve the use of the Flp-recombinase system to engineer cell lines that inducibly express peptidomimetic drugs, the use of state of the art immunoprecipitation using GFP-Nanotrap technology, coupled with proteomics to identify the effects of drug treatment on multi-protein complex composition.

References:

1 Exell, J. et al. Cellularly active N-hydroxyurea FEN1 inhibitors block substrate entry to the active site. Nat. Chem. Biol. Advanced online publication, doi:10.1038/nchembio.2148 (2016).
2 Scott, D. E., Bayly, A. R., Abell, C. & Skidmore, J. Small molecules, big targets: drug discovery faces the protein-protein interaction challenge. Nat Rev Drug Discov 15, 533-550, doi:10.1038/nrd.2016.29 (2016).
3 Rothbauer, U. et al. A versatile nanotrap for biochemical and functional studies with fluorescent fusion proteins. Mol Cell Proteomics 7, 282-289, doi:10.1074/mcp.M700342- MCP200 (2008).

Project 34

Department of Biomedical Science Laboratory Research Project 2024/25

Investigating a novel gene of unknown function involved in transmitting replication stress signals to the mitotic apparatus

Cancer Biology

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Background

Healthy aging is frequently affected by the onset of cancers. One in 3 of us will be affected over our lifetime, & 50% will have significantly reduced lifespan as a consequence. Cell cycle checkpoint genes are often mutated in cancers, while paradoxically, in appropriate circumstances, they may be attractive targets for rational drug design. Our laboratory was one of the first labs to identify the significance of the Chk1 gene product in checkpoint control. Colorectal cancer remains a significant challenge, often due to late presentation of symptoms & Chk1 inhibitors are currently in clinical trials for a subset of colorectal cancers. Recently we have undertaken a genome-wide siRNA screen in colorectal cancer cells to identify previously unknown checkpoint pathway genes in order to identify novel potential therapeutic targets. As a result we have identified a range of genes whose roles in checkpoint regulation are completely unknown.

Aims:

This project will focus on a novel gene's (MiCatS1) cellular function, in checkpoint control. MiCatS1-deficient cells do not respond to cellular signalling pathways indicating replication stress. This project will generate an understanding of how MiCatS1 controls genome integrity.

Techniques:

These will include a range of molecular cell biology techniques, tissue culture, confocal fluorescence microscopy, protein expression and functional characterisation approaches including proteomics, quantitative PCR, siRNA-mediated knockdown of gene expression, and immunoblotting.

References:

(1) The Biology of Cancer, 2nd ed., by Robert Weinberg, Garland Press, Ch 16, pp 815-816
(2) Feijoo, C., Hall-Jackson, C., Wu, R., Jenkins, D., Gilbert, D., and Smythe, C (2001). Activation of mammalian Chk1 during DNA replication arrest: a role for Chk1 in the intra-S phase checkpoint monitoring replication origin firing. The Journal of Cell Biology, 154(5), 913–924.
(3) Bowen, E., (2015). PhD Thesis. "A genomic screen for the identification of novel components of the S phase checkpoint", University of Sheffield.

Project 35

Department of Biomedical Science Laboratory Research Project 2024/25

Understanding the role of DRAM in infection and autophagy-related disease

Cell & Animal Models of Human Disease Cancer Biology

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Background

Lysosomal digestion is important in many diseases. The capture and degradation of cytoplasmic components by autophagy allows poorly vascularised tumour cells to survive starvation and neurons to remove the protein aggregates associated with neurodegeneration. Lysosomes also enable immune cells to kill engulfed microbes within phagosomes and use autophagy to capture and kill intracellular pathogens. DRAM (Damage Regulated Autophagy Regulator) is a conserved lysosomal protein implicated in several diseases. In cancer, DRAM1 mediates regulation of autophagy and cell death by the tumour suppressor p53, and is decreased in many primary tumours (Crichton et al. Cell 2006). DRAM upregulation also aids the autophagic clearance of intracellular bacteria during mycobacterial infection (van der Vaart et al. Cell Host Microbe 2014). However, how DRAM regulates lysosomal activity and autophagy remains unclear.

Aims:

The main aims of this project is to develop a new experimental model to study DRAM function by generating CRISPR mutants in our amoebae model system. You will then test how this affects both autophagy and the ability to capture and kill bacteria within the phagosome.

Techniques:

The primary model organism used will be Dictyostelium amoebae, which are widely to model macrophages. It will involve tissue culture, molecular biology (CRISPR,GFP expression, fluorescent microscopy and functional assays of host-pathogen interaction, phagosome maturation and survival.

References:

1. Criollo A, Dessen P, Kroemer G. Cell Cycle. 2009 Aug;8(15):2319-20. DRAM: A phylogenetically ancient regulator of autophagy.
2. O'Prey J, Skommer J, et al. Cell Cycle. 2009 Jul 15;8(14):2260-5.Analysis of DRAM-related proteins reveals evolutionarily conserved and divergent roles in the control of autophagy
3. Crighton D, Wilkinson S, O'Prey J et al. Cell. 2006 Jul 14;126(1):121-34. DRAM, a p53-induced modulator of autophagy, is critical for apoptosis.

Project 36

Department of Biomedical Science Laboratory Research Project 2024/25

Targeting Cancer with novel targeting therapeutics

Mechanisms of Drug Action Cancer Biology

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Background

Cancer is a growing health issue, with an ageing population. Current treatments, such as chemotherapy or hormonal therapy, are inefficient and non-specific (Shen & Abate-Shen, 2010). Most therapies cause a multitude of secondary effects, due to the damage done to local tissue, so there is a need to have targeted and effective new medicines (Frame & Maitland, 2019). Recent advances in targeted therapies, such as monoclonal antibodies, have used combinations of a biologic and a small molecule conjugated to deliver cargoes to the cancer. However, although effective, there are problems with manufacturing and immunological side-effects. Some wards are half full of patients with adverse reactions.

Peptides and peptide drug conjugates (PDC) represent an emerging strategy that can reduce peripheral toxicity & adverse reactions, while enhancing drug uptake through tumour selectivity (Vrettos et al. 2018). This project will use cell-penetrating peptides to deliver novel therapies to cancer cells.

Aims:

Determine the interaction and efficacy of delivery with a cell surface receptor in cancer cells. Determine the subcellular locations of the peptide and receptor. Quantitate the activity of the peptide in cancer targeting with pulse chase experiments, using receptor ligand interactions.

Techniques:

The candidate will use cancer cell lines to characterize the peptide in cell assays. The project will use automated high throughput equipment including, high content microscopy and liquid handling robotics. Methods will include the drug inhibition of receptor internalisation. The project will use cell penetrating peptides, RNAi, immunological stains, small molecule inhibitors and biochemical assays.

References:

Frame, F.M., and Maitland N.J. (2019). Epigenetic Control of Gene Expression in the Normal and Malignant Human Prostate: A Rapid Response Which Promotes Therapeutic Resistance. Int J Mol Sci. May; 20(10): 2437.

Shen, M. M., & Abate-Shen, C. (2010). Molecular genetics of prostate cancer: new prospects for old challenges. Genes & development, 24(18), 1967-2000.

Vrettos, E. I., Mező, G., & Tzakos, A. G. (2018). On the design principles of peptide–drug conjugates for targeted drug delivery to the malignant tumor site. Beilstein journal of organic chemistry, 14(1), 930-954.

Project 37

Department of Biomedical Science Laboratory Research Project 2024/25

Modelling human bone disorders in zebrafish

Cell & Animal Models of Human Disease

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Background

Whole genome sequencing studies have led to a vast amount of new candidate genes for human diseases. One such gene is NBAS which when mutated results in acute liver failure and skeletal abnormalities (SOPH syndrome, Short stature; Optic atrophy; Pelger-Huet anomaly). Patients with NBAS mutations are subjected to a lifetime of recurrent fractures, episodes of acute liver failure and immune deficiency. Recently we have developed a zebrafish model which carries mutations in NBAS and displays skeletal malformations similar to the human condition. Analysis done in our lab as well as others suggests that NBAS may play a role in the secretion of collagen. This project builds upon our work focusing on the zebrafish model to unravel the molecular causes of this disease and to develop a platform for high-throughput drug screens to identify drugs that may be used in the clinic.

Aims:

1) To characterise the collagen secretion defect in nbas mutant zebrafish
2) To screen for drugs that affect the nbas phenotype in zebrafish

Techniques:

Cutting-edge techniques such as lightsheet and AIRY scan microscopy and robot-based drug library screening.

References:

Maksimova N, et al. Neuroblastoma amplified sequence gene is associated with a novel short stature syndrome characterised by optic nerve atrophy and Pelger-Huët anomaly. J Med Genet. 2010 Aug;47(8):538-48. doi: 10.1136/jmg.2009.074815

Balasubramanian M, et al. Compound heterozygous variants in NBAS as a cause of atypical osteogenesis imperfecta. Bone. 2017 Jan;94:65-74. doi: 10.1016/j.bone.2016.10.023.

Project 38

Department of Biomedical Science Laboratory Research Project 2024/25

Regulation of Epidermal Growth Factor Receptor (EGFR) trafficking and degradation by Syndecan-4

Cancer Biology **Cell & Animal Models of Human Disease**

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Background

Syndecan-4 is a co-receptor that cooperates with growth factor receptors and integrins to regulate cell adhesion and migration. The relationships specifically regulate wound healing by controlling fibroblast recruitment to wounds, with the result that syn4-knockout mice have healing defects. Mass spectrometry revealed that syn4 regulates the surface expression of several receptors including EGFR, which regulates membrane protrusion signals during migration and may mediate several of the syn4 effects. The mass spectrometry also indicated that syn4 regulates surface expression of receptors by triggering caveolar endocytosis. The questions are whether syn-4 affects endocytosis or degradation of EGFR or both, and whether it affects EGFR signalling. Finally, despite the correlation between syn4 and EGFR roles, the way they cooperate during functional processes such as migration is not understood.

Aims:

The aim is to resolve whether and how syndecan-4 affects the endocytosis, surface availability, and function of EGFR. You will test whether engagement of syn-4 triggers active endocytosis of EGFR, subsequent degradation of EGFR, and the effect of syn-4 on EGFR activation by EGF.

Techniques:

You will use cell culture assays and membrane fractionation to test protein expression and distribution. Cell surface labeling and antibody uptake to follow endocytosis live and phospho western blotting to assess EGFR activity. You will use lysosomal and proteosomal inhibition to test the effect on and role of EGFR degradation in the syn4 response.

References:

Williamson RC, Cowell CAM, Reville T, Roper J, Rendall TCS, Bass MD*. (2015) Coronin-1C protein and caveolin protein provide constitutive and inducible mechanisms of Rac1 protein trafficking. J Biol Chem. 290(25) 15437-49.

Bass MD*, Williamson RC, Nunan RD, Humphries JD, Byron A, Morgan MR, Martin P, Humphries MJ. (2011) A syndecan-4 hair trigger initiates wound healing through caveolin- and RhoG-regulated integrin endocytosis. Dev Cell. 21: 681-93.

Sigismund S, Argenzio E, Tosoni D, Cavallaro E, Polo S, Di Fiore PP. (2008) Clathrin-Mediated Internalization Is Essential for Sustained EGFR Signaling but Dispensable for Degradation Dev Cell. 15: 209

Project 39

Department of Biomedical Science Laboratory Research Project 2024/25

The effect of therapeutic ultrasound on matrix deposition and organization during skin healing and scarring

Cell & Animal Models of Human Disease **Stem Cell & Regenerative Medicine**

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Background

Chronic ulcers are non-healing wounds that affect 200,000 UK patients annually and frequently result in amputation. During healthy healing, fibroblasts migrate to the wound bed, differentiate into myofibroblasts and apply force to contract the wound. Deposition and reorganisation of extracellular matrix, particularly collagen, make a crucial contribution to rapid healing, but also result in scarring. We recently published that delayed healing could be accelerated using ultrasound to activate fibroblasts, but do not know whether differentiation into myofibroblasts or extracellular matrix is affected. The reorganisation and alignment of collagen fibres makes as big a contribution to scarring as the molecular composition, so the big questions are how ultrasound affects matrix deposition, cross-linking and organisation in 3D.

Aims:

The aim is to examine the effect of ultrasound on deposition and organisation of extracellular matrix. You will determine whether ultrasound treatments affect the composition or synthesis rate of matrices generated by fibroblasts, the level of crosslinking and the 3D structure of resultant matrix.

Techniques:

You will use fibroblasts to generate 3D matrices that resemble skin dermis, analyse the molecular composition by western blot or qPCR and the organisation by confocal microscopy. You will test the effect of ultrasound on your readouts.

References:

Pounder, Harrison (2008) Low intensity pulsed ultrasound for fracture healing: a review of the clinical evidence and the associated biological mechanism of action. Ultrasonics 48:330-8.

Roper, Williamson, Bally, Cowell, Brooks, Stephens, Harrison, Bass. (2015) Ultrasonic stimulation of mouse skin reverses the healing delays in diabetes and aging by activation of Rac1. J Invest Dermatol. 135: 2842

Ehrlich & Hunt. (2012) Collagen organization critical role in wound repair. Advances in Wound Care 1:3

Project 40

Department of Biomedical Science Laboratory Research Project 2024/25

The Role of EGFR in Matrix-Directed Migration

Cell & Animal Models of Human Disease Mechanisms of Drug Action

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Background

Cell migration is guided by the fibrous extracellular matrix that provides “tracks” along which cells migrate to allow efficient translocation over great distances. The transmembrane receptor Syndecan- 4 is known to cooperate with matrix receptors to guide migration by regulation of the small signalling molecule, Rac1, which stimulates membrane protrusion to both initiate and migration and establish polarity and direction. Despite its importance, the mechanism of matrix-directed Rac1 regulation remains unresolved. We know some of the molecules involved but not how they connect. Our recent observation that Syn4 triggers trafficking of EGFR could provide the link. EGFR and integrins are known to interact in matrix-dependent migration and EGFR binds the Rac1 activator, Vav2, which we know to be important. Therefore, we hypothesise that Syn4-dependent EGFR trafficking is responsible for Rac1 activation and migration.

Aims:

The aim is to resolve whether EGFR mediates syndecan-4-dependent Rac1 activation and regulation of fibroblast migration.

Techniques:

You will use cell culture assays to test the effect of EGFR inhibitors and siRNA knockdown on Rac1 activation by effector pull down and activation of downstream signals by western blot. You will test the effect of EGFR inhibition on migration through 3D matrix and using scratch assays.

References:

Bass MD, Roach KA, Morgan MR, Mostafavi-Pour Z, Schoen T, Muramatsu T, Mayer U, Ballestrem C, Spatz J, Humphries MJ. (2007) Syndecan-4-dependent Rac1 regulation determines directional migration in response to the extracellular matrix. J Cell Biol. 177:527.

Bass MD*, Williamson RC, Nunan RD, Humphries JD, Byron A, Morgan MR, Martin P, Humphries MJ. (2011) A syndecan-4 hair trigger initiates wound healing through caveolin- and RhoG-regulated integrin endocytosis. Dev Cell. 21: 681-93.

Marcoux N, Vuori K (2003) EGF receptor mediates adhesion-dependent activation of the Rac GTPase: a role for phosphatidylinositol 3-kinase and Vav2. Oncogene. 22: 6100-6.

Project 41

Department of Biomedical Science Laboratory Research Project 2024/25

mRNA expression analyses of genes involved in chicken wing development

Cell & Animal Models of Human Disease

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Background

The longstanding view of how proliferative outgrowth terminates following the patterning phase of limb development involves the breakdown of reciprocal extrinsic signalling between the distal mesenchyme and the overlying epithelium (e-m signalling). However, by grafting distal mesenchyme cells from late stage chick wing buds to the epithelial environment of younger wing buds, our lab has shown that this mechanism is not required. We have performed RNA sequencing analyses that reveals that distal mesenchyme cells complete proliferative outgrowth by an intrinsic cell cycle timer in the presence of e- m signalling. In this process, e-m signalling is required permissively to allow the intrinsic cell cycle timer to run its course. In this study we isolated many genes that could be involved in controlling the intrinsic cell cycle timer, which need to be analysed.

Aims:

The aim of this project is to characterise the mRNA expression patterns of some of the genes we have isolated in our RNA-sequencing experiments. Following the characterisation of the expression of these genes, the student will analyse the literature to produce a model of how they could control the intrinsic cell cycle timer.

Techniques:

RNA in situ hybridisation, chicken embryo dissection and literature analyses.

References:

An Intrinsic Cell Cycle Timer Terminates Limb Bud Outgrowth. Elife 2018 Sep 3;7:e37429. doi: 10.7554/eLife.37429

Project 42

Department of Biomedical Science Laboratory Research Project 2024/25

Assessing the integration of sensory inputs in the zebrafish brain with electrophysiology

Neurodevelopment & Neuroscience

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Background

The integration of sensory information is vital for survival, communication and navigation. However we know very little about the different circuits or locations where this occurs in the brain. We know quite a bit about the sensory receptors themselves and the individual sensory systems but not much about how one sensory input can influence the perception of others. This project aims to show how sensory integration occurs at the single neuron level in the zebrafish brain.

Aims:

The aim is to assess the degree of sensory integration and interaction in different areas of the zebrafish brain using whole cell or cell attached electrophysiological recording.

Techniques:

Young zebrafish (less than 5 days post fertilisation) will be immobilised in agarose and viewed under a microscope. Glass electrodes will be inserted into the brain and individual neurons will be recorded with a glass patch pipette in response to different combinations of sensory stimuli.

References:

Olt et al (2016) In Vivo Physiological Recording From the Lateral Line of Juvenile Zebrafish. J Physiol 594: 5427-38

Boulanger-Weill & Sumbre (2019) Functional Integration of Newborn Neurons in the Zebrafish Optic Tectum. Front Cell Dev Biol. 7: 5

Project 43

Department of Biomedical Science Laboratory Research Project 2024/25

Understanding the role of AP-1 transcription factors in cancer cell motility

Cancer Biology

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Background

During cancer metastasis cells acquire the capacity to break away from their tissue of origin and invade distant organs. Oncogenic mutations in cancer cells promote cell invasion and motility by altering actin cytoskeleton organisation. Our previous work has showed how activation of RAS oncogenes induces rapid cytoskeletal rearrangements promoting cell ruffling and motility. RNA Sequencing has revealed a RAS-dependent transcriptional signature associated with this invasive phenotype. The project will investigate how the AP-1 transcription factor complex controls this transcriptional response and hence, cell motility and invasion. This will enhance our understanding of the molecular mechanisms that underpin cancer invasion and metastasis.

Aims:

1. Characterise the temporal expression patterns of different AP-1 subunits following HRAS activation

2. Determine how AP-1 transcription factors affect cytoskeletal organisation & cell motility

3. Identify AP-1 target genes that regulate cell motility.

Techniques:

Cell culture, western blotting and immuno-fluorescence microscopy will be used to characterise expression patterns. siRNA knockdown of AP-1 subunits will be then used to assess their effect on cytoskeletal organisation (immuno-fluorescence) and cell motility (timelapse microscopy). Bioinformatic analysis will identify AP-1 target genes.

References:

Ganguli, S., Wyatt, T., Nyga, A., Lawson, R.H., Meyer, T., Baum, B. and Matthews, H.K. (2023). Oncogenic Ras deregulates cell-substrate interactions during mitotic rounding and respreading to alter cell division orientation. Current Biology 33, 2728–2741 https://doi.org/10.1016/j.cub.2023.05.061

Casalino L, Talotta F, Cimmino A, Verde P. The Fra-1/AP-1 Oncoprotein: From the "Undruggable" Transcription Factor to Therapeutic Targeting. Cancers (Basel). 2022 Mar 14;14(6):1480. doi: 10.3390/cancers14061480.

Project 44

Department of Biomedical Science Laboratory Research Project 2024/25

Does SUMOylation regulate communication between mitochondria and endoplasmic reticulum?

Cell & Animal Models of Human Disease Cancer Biology

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Background:

SUMOylation is a post-translational modification involving the covalent attachment of small ubiquitin-like modifier (SUMO) proteins to target proteins, regulating their function, localization and stability. Dysregulated SUMOylation is implicated in ageing and age-related diseases, contributing to pathological features. However, the fundamental mechanisms underlying the role of SUMOylation under these conditions remain largely elusive (Chen et al., 2021). Emerging evidence indicates that SUMOylation can modify specific bridging proteins such as MFN2 and ACSL4 (Hendriks et al., 2018; Xu et al., 2024), which are involved in forming mitochondria-associated membranes (MAMs). This leads to the hypothesis that SUMOylation is a critical regulatory mechanism for MAM formation. Successful completion of this project would uncover a novel SUMO-dependent mechanism underlying inter- organellar communication and pave the way for developing a new tractable therapeutic strategy against ageing and age-related diseases linked to MAMs.

Aims:

The aims of this project are to i) detect MAMs in cultured human cells and ii) investigate the potential role of SUMOylation in regulating MAM formation.

Techniques:

The project will use a range of molecular cell biology techniques including cell cultures, SUMOylation inhibition, cDNA transfection and RNA interference, immunoblotting, and imaging and luciferase assays.

References:

Chen, X., Zhang, Y., Wang, Q., Qin, Y., Yang, X., Xing, Z., Shen, Y., Wu, H., and Qi, Y. (2021). The function of SUMOylation and its crucial roles in the development of neurological diseases. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 35, e21510.

Hendriks, I.A., Lyon, D., Su, D., Skotte, N.H., Daniel, J.A., Jensen, L.J., and Nielsen, M.L. (2018). Site-specific characterization of endogenous SUMOylation across species and organs. Nature communications 9, 2456.

Xu, X., Mao, Y., Feng, Z., Dai, F., Gu, T., and Zheng, J. (2024). SENP1 inhibits ferroptosis and promotes head and neck squamous cell carcinoma by regulating ACSL4 protein stability via SUMO1. Oncology reports 51.

Project 45

Department of Biomedical Science Laboratory Research Project 2024/25

Is BiP SUMOylation important for cell survival following endoplasmic reticulum stress?

Cancer Biology **Cell & Animal Models of Human Disease**

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Background:

Binding immunoglobulin Protein (BiP) is an essential heat shock protein 70 family chaperone, known as the master regulator for Unfolded Protein Response (UPR) elicited by endoplasmic reticulum (ER) stress, for cell survival. BiP is implicated in the development and metastasis of cancer and the pathogenesis of neurodegenerative diseases (Casas, 2017). Intriguingly recent mass spectrometry- based studies reveal BiP as a potential target protein for SUMO conjugation (termed as SUMOylation) (Hendriks et al., 2018). Most recently we have validated BiP as a *bona fide* SUMO target and identified the site for SUMO conjugation (unpublished data from Guo lab). We, and others, have previously demonstrated that increased protein SUMOylation is a cytoprotective response against extreme cell stress (Guo et al., 2013). Therefore we hypothesize that BiP SUMOylation is important for cell survival following ER stress. The findings from this project may lead to establishing a new tractable approach for treating age-related diseases.

Aim:

The aim of this project is to define the role for BiP SUMOylation in ER stress-induced cell death in human cells.

Techniques:

The project will use a range of molecular cell biology techniques including cell cultures, cDNA transfection and RNA interference, assays, ER stress/UPR induction by pharmaceutical inducers tunicamycin and/or thapsigargin, and biochemical examinations of cell viability (*e.g.*, cytochrome c release, caspase activation, and LDH assays).

References:

Casas, C. (2017). GRP78 at the Centre of the Stage in Cancer and Neuroprotection. Frontiers in neuroscience *11*, 177.

Guo, C., Hildick, K.L., Luo, J., Dearden, L., Wilkinson, K.A., and Henley, J.M. (2013). SENP3-mediated deSUMOylation of dynamin-related protein 1 promotes cell death following ischaemia. The EMBO journal *32*, 1514-1528.

Hendriks, I.A., Lyon, D., Su, D., Skotte, N.H., Daniel, J.A., Jensen, L.J., and Nielsen, M.L. (2018). Site-specific characterization of endogenous SUMOylation across species and organs. Nature communications *9*, 2456.

Project 46

Department of Biomedical Science Laboratory Research Project 2024/25

Investigating the Roles of SUMO Protease SENP6 in Regulating SUMOylation and Cell Survival following Heat Shock

Cancer Biology **Cell & Animal Models of Human Disease**

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Background:

Hyperthermia is an important therapy in the treatment of cancer (Crezee et al., 2021). Hyperthermia is known to induce heat shock response (HSR), a vital fundamental mechanism that allows cells to survive sudden increases in environmental temperature that have pronounced damaging effects on important cellular structures and function. It is generally accepted that dramatically increased global SUMO conjugation (also termed as SUMOylation), as part of HSR, antagonizes cell death following heat shock (HS) (Golebiowski et al., 2009). Moreover, HS inactivates 5 of 6 SUMO-specific proteases (SENPs) (Pinto et al., 2012), the enzymes capable of removing SUMOs from SUMOlyated proteins in a process called deSUMOylation, suggesting that HS-induced SUMOylation is attributable to decreased SENP-mediated deSUMOylation. Interestingly, however, it remains unclear if SENP6, whose activity does not appear to be affected by HS, has roles in regulating SUMOylation levels and cell death following HS. The findings from this project may lead to uncover a new tractable approach for bettering hyperthermia-based cancer treatment.

Aims:

The aim of this project is to determine the roles for SENP6 in HS-induced SUMOylation and cell death in human cervical cancer cells.

Techniques:

The proposed work will be conducted using a combination of techniques including cell cultures, DNA & siRNA transfections for changing SENP6 levels, and HSR induction using water-baths at different temperatures, SDS-PAGE & western blotting for monitoring expression levels of SENP6, SUMO-1- ylation or SUMO-2/3-lyation, and biochemical and imaging examinations of cell viability (e.g., cytochrome c release, caspase activation, and LDH assays).

References:

Crezee, J., Franken, N.A.P., and Oei, A.L. (2021). Hyperthermia-Based Anti-Cancer Treatments. Cancers *13*.

Golebiowski, F., Matic, I., Tatham, M.H., Cole, C., Yin, Y., Nakamura, A., Cox, J., Barton, G.J., Mann, M., and Hay, R.T. (2009). System-wide changes to SUMO modifications in response to heat shock. Sci Signal *2*, ra24.

Pinto, M.P., Carvalho, A.F., Grou, C.P., Rodríguez-Borges, J.E., Sá-Miranda, C., and Azevedo, J.E. (2012). Heat shock induces a massive but differential inactivation of SUMO-specific proteases. Biochimica et biophysica acta *1823*, 1958-1966.

Project 47

Department of Biomedical Science Laboratory Research Project 2024/25

Does glucose deprivation induce remodelling of mitochondria-associated membranes?

Cell & Animal Models of Human Disease Cancer Biology

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Background:

Glucose is a critical nutrient for mammalian cells, serving as the primary energy source through cellular respiration. Its deprivation leads to energy stress, endoplasmic reticulum (ER) stress, and apoptosis (Graham et al., 2012). Conversely, high glucose levels disrupt the integrity of mitochondria-associated ER membranes (MAMs), decreasing ER-mitochondria communication, inducing mitochondrial fission, and impairing mitochondrial respiration (Rieusset, 2018). This highlights the importance of MAMs in glucose sensing and regulating cellular bioenergetics, which is crucial for maintaining glucose homeostasis. We hypothesize that glucose deprivation enhances MAM formation in mammalian cells. Successful investigation of this hypothesis would improve our understanding of ageing-related conditions, including neurodegeneration, muscle aging, insulin resistance and metabolic dysfunction. Furthermore, it could pave the way for developing novel therapeutic strategies against ageing and age- related diseases linked to MAM dysfunction (Brewer et al., 2016).

Aim:

The aims of this project are to i) detect MAMs in cultured human cells and ii) determine the effect of glucose deprivation on MAM formation.

Techniques:

The project will use a range of cell biology techniques including cell cultures, cDNA transfection, immunoblotting & co-immunoprecipitation, and imaging and luciferase assays.

References:

Brewer, R.A., Gibbs, V.K., and Smith, D.L., Jr. (2016). Targeting glucose metabolism for healthy aging. Nutrition and healthy aging 4, 31-46.

Graham, N.A., Tahmasian, M., Kohli, B., Komisopoulou, E., Zhu, M., Vivanco, I., Teitell, M.A., Wu, H., Ribas, A., Lo, R.S., et al. (2012). Glucose deprivation activates a metabolic and signaling amplification loop leading to cell death. Molecular systems biology 8, 589.

Rieusset, J. (2018). The role of endoplasmic reticulum-mitochondria contact sites in the control of glucose homeostasis: an update. Cell death & disease 9, 388.

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Department of Biomedical Science Laboratory Research Project 2024/25

Does SUMOylation regulate FKBP8-mediated formation of mitochondria-associated membranes?

Cell & Animal Models of Human Disease

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Background:

FKBP8 (also known as FKBP38) is a versatile protein involved in protein folding and trafficking, autophagy regulation, apoptosis modulation, immunoregulation, inhibition of viral infections, and potentially neural functions (Bhujabal et al., 2017; Shirane and Nakayama, 2003). These diverse roles are crucial for maintaining cellular homeostasis and responding to cellular stress. Emerging evidence indicates that FKBP8 plays a significant role in the formation of mitochondria-associated membranes (MAMs), which are contact sites between the mitochondria and the endoplasmic reticulum (ER) (Kwak et al., 2020). Our unpublished findings indicate that FKBP8 undergoes SUMO conjugation, leading to the hypothesis that SUMOylation regulates FKBP8-mediated MAM formation. This project would uncover a novel SUMO-dependent mechanism underlying inter-organellar communication and enhance our understanding of diseases associated with FKBP8, including Hepatitis C, Pineal Region Germinoma and Spina Bifida.

Aim:

The aims of this project are to i) detect MAMs in cultured human cells and ii) investigate the potential role of FKBP8 SUMOylation in regulating MAM formation.

Techniques:

The project will use a range of molecular cell biology techniques including cell cultures, cDNA transfection and RNA interference, immunoblotting, and imaging and luciferase assays.

References:

Bhujabal, Z., Birgisdottir, A.B., Sjottem, E., Brenne, H.B., Overvatn, A., Habisov, S., Kirkin, V., Lamark, T., and Johansen, T. (2017). FKBP8 recruits LC3A to mediate Parkin-independent mitophagy. EMBO reports 18, 947-961.

Kwak, C., Shin, S., Park, J.S., Jung, M., Nhung, T.T.M., Kang, M.G., Lee, C., Kwon, T.H., Park, S.K., Mun, J.Y., et al. (2020). Contact-ID, a tool for profiling organelle contact sites, reveals regulatory proteins of mitochondrial-associated membrane formation. Proceedings of the National Academy of Sciences of the United States of America 117, 12109-12120.

Shirane, M., and Nakayama, K.I. (2003). Inherent calcineurin inhibitor FKBP38 targets Bcl-2 to mitochondria and inhibits apoptosis. Nature cell biology 5, 28-37.

Project 49

Department of Biomedical Science Laboratory Research Project 2024/25

Investigation into a novel therapeutic for the treatment of allergic diseases

Cell & Animal Models of Human Disease

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Background

Every 10 seconds someone in the UK has a potentially life threatening asthma attack; the underlying cause of most attacks are allergic reactions instigated by activation of mast cells to pollutants and innocuous substances. It is through their secretion of inflammatory mediators that mast cells exert their devastating effects: eg. secreted histamine causes constriction of smooth muscle to restrict breathing, secreted cytokines like TNF-alpha recruit other immune cells into the area causing inflammation and long term restructuring and damage. Research in the Seward lab is focussed on understanding the molecular mechanisms controlling mediator secretion from mast cells. SNARE proteins and their regulators have been highlighted as suitable candidates due to their critical role in regulating the final vesicle fusion step essential to mediator secretion.

Aims:

This project will investigate the role of synaptotagmins (Syts) in regulating mast cell responses and evaluate the effectiveness of a newly described peptide inhibitor of Syt-2, SP9 to inhibit mediator secretion.

Techniques:

Tissue culture, fluorescent microscopy, immunofluorescence, western blotting, transient transfection, siRNA knockdown, flow cytometry, mediator secretion assays

References:

Melicoff, E., et al. (2009). Synaptotagmin-2 Controls Regulated Exocytosis but Not Other Secretory Responses of Mast Cells. J. Biol. Chem., 284(29), 19445-19451.

Wajdner, H. E., et al. (2017). Orai and TRPC channel characterization in FcεRI-mediated calcium signaling and mediator secretion in human mast cells. Physiological Reports, 5(5), e13166. Lai, Y., et al. (2022). Inhibition of calcium-triggered secretion by hydrocarbon-stapled peptides. Nature, 603(7903), 949-956.

Project 50

Department of Biomedical Science Laboratory Research Project 2024/25

Misregulated dense core vesicle exocytosis in amyotrophic lateral sclerosis and frontotemporal dementia

Neurodevelopment & Neuroscience **Cell & Animal Models of Human Disease**

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Background

Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are devastating adult-onset neurodegenerative diseases characterised by the loss of motor neurons and atrophy of the frontal and temporal regions of the brain. A GGGGCC hexanucleotide repeat expansion in the first intron of the C9orf72 gene is the most common cause of familial ALS and FTD. Haploinsufficiency, RNA toxicity and dipeptide protein toxicity are all thought to contribute mechanistically towards C9orf72-related ALS/FTD. Because synaptic impairment precedes axon degeneration and progresses through a dying-back mechanism, it is likely that synaptopathy is not just a secondary manifestation to neuronal loss but rather plays an active role in pathogenesis. Recent data from DeVos lab (Sheffield) indicates disrupted dense core vesicle (DCV) function caused by C9orf72 haploinsufficiency may contribute to the functional deficits of synapses.

Aims:

The project aims to examine the impact of C9orf72 haploinsufficiency on DCV biogenesis and exocytosis using adrenal chromaffin cells isolated from knockout mice. Chromaffin cells are enriched in DCVs whose trafficking and fusion can easily be quantified using electrophysiology and imaging, making them a useful model system for mechanistic studies into the underlying cellular defects underpinning ALS/FTD.

Techniques:

Primary tissue culture, electrophysiology, fluorescent microscopy and live cell imaging, transient transfection

References:

1. Balendra R., and Isaacs A.M. (2018). C9orf72-mediated ALS and FTD: multiple pathways to disease. Nat Rev Neurol. 14, 544-558.
2. Bauer C.S., Cohen R.N., Sironi F., Livesey M.R., Gillingwater T.H., Highley J.R., Fillingham D.J., Coldicott I., Smith E.F., Gibson Y.B. et al. (2022). An interaction between synapsin and C9orf72 regulates excitatory synapses and is impaired in ALS/FTD. Acta Neuropathol. 144, 437-464.
3. de Diego A.M.G., Ortega-Cruz D., and García A.G. (2020). Disruption of Exocytosis in Sympathoadrenal Chromaffin Cells from Mouse Models of Neurodegenerative Diseases. Int J Mol Sci. 21, 1946..

Project 51

Department of Biomedical Science Laboratory Research Project 2024/25

Investigating the role of candidate ubiquitin enzymes in mechanoflammation

Cell & Animal Models of Human Disease **Mechanisms of Drug Action**

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Background

The key features of a number of musculoskeletal diseases including osteoarthritis are chronic inflammation, pain, tissue damage and loss of function. Osteoarthritis is a very common disease for which the only treatments are pain relief and joint replacement. Mechanical injury is a key factor in initiating these diseases. The cellular and molecular mechanisms of this tissue destruction are poorly understood. Our group key aim is to identify therapeutic targets for osteoarthritis and related musculoskeletal diseases through understanding how mechanical tissue injury causes inflammation (mechanoflammation) and tissue damage. Identifying the cellular mechanisms involved in injury responses is crucial for understanding disease mechanisms and for designing specifically tailored therapeutic regimens for musculoskeletal diseases.

Our studies showed mechanical injury to articular cartilage activates within seconds intracellular signalling pathways that are characteristic of the inflammatory response. Similar pattern of intracellular signalling was observed in diverse mammalian tissues, including synovium, skin, and mesentery tissues of mice as well as in zebrafish larvae, suggesting that the response is not unique to cartilage and may be generic to all connective tissues. Recent evidence from our studies showed a great significance of the ubiquitin system as an upstream regulator of these cellular events. The ubiquitin system comprises a group of enzymes that facilitate post-translational modifications of proteins by ubiquitination which is emerging as a critical regulator of inflammatory signalling pathways.

Ubiquitination is a reversible and dynamic reaction which is tightly controlled by the opposing actions of ubiquitin ligases and deubiquitinating enzymes (DUBs). We propose that a group of candidate ubiquitin enzymes are critical regulators of chronic inflammation, underpinning tissue damage upon injury. Targeting these enzymes is emerging as a significant new drug discovery opportunity for modulating tissue damage and identifying new therapeutic target for musculoskeletal diseases including osteoarthritis.

Aims:

1- to target candidate ubiquitin enzymes in zebrafish embryos using CRISPR/Cas9 approaches
2- to investigate the role of these enzymes in injury induced inflammatory signalling

Techniques:

We combine the use of Zebrafish model systems for high-throughput screening and a number of cutting-edge technologies including advanced transgenesis, advanced live imaging techniques and CRISPR/Cas9 gene editing methodologies as well as cellular and biochemical advanced techniques.

References:

Kaokhum N, Pinto-Fernández A, Wilkinson M, Kessler BM, Ismail HM*. The Mechano-Ubiquitinome of Articular Cartilage: Differential Ubiquitination and Activation of a Group of ER-Associated DUBs and ER Stress Regulators. Mol Cell Proteomics. 2022 Dec;21(12):100419. doi: 10.1016/j.mcpro.2022.100419. Epub 2022 Sep 28. PMID: 36182100; PMCID: PMC9708921.

Khatoum N, Pinto-Fernandez A, Wilkinson M, Kessler B, Ismail HM*. Ubiquitylome analysis of articular cartilage post mechanical injury reveals a differential ubiquitination pattern of a subset of DUBs and proteins linked to the ERAD cellular response. bioRxiv 2022.01.19.476879; doi: https://doi.org/10.1101/2022.01.19.476879

Project 52

Department of Biomedical Science Laboratory Research Project 2024/25

Unravelling the role of *P4HB* in skeletal development using zebrafish as a disease model

Cell & Animal Models of Human Disease

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Background

Osteogenesis Imperfecta (OI) is the commonest form of heritable bone fragility and all genes so far implicated in OI are involved in production, processing or secretion of type 1 collagen. Cole Carpenter syndrome-1 (CCS1) is a syndromal form of OI caused by a recurrent variant in P4HB, which encodes for protein disulphide isomerase (PDI). P4HB is a ubiquitously expressed and evolutionarily conserved multifunctional protein. It has protein disulphide isomerase activity and acts as a chaperone to ensure correct protein folding with the correct cysteine bridges. It is also a component of multi-subunit enzyme complexes. It provides the beta subunit of the alpha2, beta2 tetrameric prolyl-4-hydroxylase enzyme (where the prolyl-4-hydroxylase catalytic site is provided by the alpha subunit partner). The proline hydroxylation enzyme activity modifies collagen. Collagen proline hydroxylation is required for the structural integrity of collagen fibrils and for recognition by collagen binding proteins such as integrins. In this project, we plan to analyse in-depth the skeletal abnormalities of mutant specific p4hb zebrafish and how this compares to the human disease. Biomarkers generated in this study will provide the basis for a drug screening assay to identify potential therapeutics.

Aims

Evaluate skeletal manifestations in a *p4hb* disease-specific mutant and phenotype *p4hb* mutant zebrafish model in comparison to human phenotype.

1. Generate a disease-specific fish model for *p4hb*
2. Evaluate skeletal manifestations and phenotype of *p4hb* mutant zebrafish model

1. A zebrafish model of CCS1 will be made by engineering the equivalent Tyr-Cys substitution in the endogenous zebrafish p4hb.
2. We will use staining methods to study the cartilage and bone defect in more detail (Alcian Blue for cartilage/ Alizarin Red and Calcein for bone) at different stages of development on larval/adult skeleton. Defects will be quantified using morphometrics to determine any differences in chondrocyte number and cell shape and in the overall bone structures. We will analyse expression of p4hb gene throughout development in both wild-type and mutant fish. Previous studies have shown ubiquitous expression in early stages of embryogenesis, we will look at later stages to determine if there is any specific expression in the developing skeletal structures.

1. Marini JC, Forlino A, Bächinger HP, Bishop NJ, Byers PH, Paepe A, Fassier F, Fratzl-Zelman N, Kozloff KM, Krakow D, Montpetit K, Semler O. Osteogenesis imperfecta. Nat Rev Dis Primers. 2017 Aug 18;3:17052. doi: 10.1038/nrdp.2017.52. PMID: 28820180
2. Ricard-Blum S. The collagen family. Cold Spring Harb Perspect Biol. 2011 Jan 1;3(1):a004978. doi: 10.1101/cshperspect.a004978. PMID: 21421911; PMCID: PMC3003457.
3. Rauch F, Fahiminiya S, Majewski J, Carrot-Zhang J, Boudko S, Glorieux F, Mort JS, Bächinger HP, Moffatt P. Cole-Carpenter syndrome is caused by a heterozygous missense mutation in P4HB. Am J Hum Genet. 2015 Mar 5;96(3):425-31. doi: 10.1016/j.ajhg.2014.12.027. Epub 2015 Feb 12. PMID: 25683117
4. Balasubramanian M, Pollitt RC, Chandler KE, Mughal MZ, Parker MJ, Dalton A, Arundel P, Offiah AC, Bishop NJ. CRTAP mutation in a patient with Cole-Carpenter syndrome. Am J Med Genet A. 2015 Mar;167A(3):587-91. doi: 10.1002/ajmg.a.36916. Epub 2015 Jan 21. PMID: 25604815.
5. Balasubramanian M, Padidela R, Pollitt RC, Bishop NJ, Mughal MZ, Offiah AC, Wagner BE, McCaughey J, Stephens DJ. P4HB recurrent missense mutation causing Cole-Carpenter syndrome. J Med Genet. 2018 Mar;55(3):158-165. doi: 10.1136/jmedgenet-2017-104899. Epub 2017 Dec 20. PMID: 29263160.

Project 53

Department of Biomedical Science Laboratory Research Project 2024/25

An in vivo chemical screen for antagonists of an adhesion GPCR signalling pathway in zebrafish

Mechanisms of Drug Action Cell & Animal Models of Human Disease

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Background

Adhesion GPCRs have great potential as therapeutic targets for drug discovery. The adhesion GPCR Adgrg6 has a conserved role in myelination of the peripheral nervous system. In humans, mutations in ADGRG6 are known to be causative for a myelination disorder, congenital contracture syndrome 9, and may also underlie some forms of idiopathic scoliosis. In zebrafish, adgrg6 mutations lead to defects both in peripheral myelination and in formation of the semicircular canals of the inner ear. The project will involve identification of compounds through an unbiased screen of zebrafish embryos, or via treatment of embryos with selected candidate compounds. Hit compounds will be validated using dose- response assays together with expression of mRNA or protein markers. A structural similarity network will be used to identify related compounds that may have similar activities.

Aims:

To identify novel antagonists for the adhesion GPCR Adgrg6, an important regulator of myelination in the vertebrate peripheral nervous system.

Techniques:

Collection and staging of zebrafish embryos and recognition of different stages of ear development; use of wild-type and mutant lines; chemical screens; in situ hybridisation; quantitative and statistical analysis of imaging data. Students will also gain insight into chemoinformatics and the approaches that underpin drug discovery programmes.

References:

Diamantopoulou E, Baxendale S, de la Vega de León A, Asad A, ... and Whitfield TT (2019). Identification of compounds that rescue otic and myelination defects in the zebrafish adgrg6 (gpr126) mutant. eLife 8: e44889

Geng F-S, Abbas L, Baxendale S, ... and Whitfield TT (2013). Semicircular canal formation in the zebrafish inner ear requires the function of gpr126 (lauscher), an adhesion class G protein-coupled receptor gene. Development 140: 4362-74.

Stoveken HM, ... and Tall GG (2016). Dihydromunduletone Is a Small-Molecule Selective Adhesion G Protein-Coupled Receptor Antagonist. Mol. Pharmacol. 90: 214–224.

Project 54

Department of Biomedical Science Laboratory Research Project 2024/25

Epithelial dynamics in the developing zebrafish inner ear

Cell & Animal Models of Human Disease

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Background

The vertebrate inner ear is the organ of hearing and balance. The ear develops from the otic vesicle, a simple monolayer of epithelium, which gives rise to a complex series of interlinked tubes and chambers during embryonic development. Morphogenesis of the developing ear involves dynamic changes in cell behaviour (for example, changes in cell shape, motility, division and death) to generate shape and form in the developing organ. This project will involve an analysis of epithelial morphogenesis in the zebrafish ear, using live fluorescence imaging, in both wild-type and a selected mutant background.
The student will formulate hypotheses for gene function and test these using one or more available mutant and transgenic lines.

Aims:

To identify and understand the molecular and cellular requirements for generation of the semicircular canal system of the developing zebrafish inner ear.

Techniques:

The project will be a mix of wet and dry work. Collection and staging of zebrafish embryos; use of wild-type, transgenic and mutant lines; fluorescence imaging. Computational work will involve quantitative and statistical analysis of imaging data, making use of software such as Fiji, arivis and Origami.

References:

Alsina, B. and Whitfield, T. T. (2017). Sculpting the labyrinth: Morphogenesis of the developing inner ear. Semin. Cell Dev. Biol. 65, 47–59.

Baldera, D., Baxendale, S., Hateren, N. J. Van, Marzo, M., Glendenning, E., Geng, F.-S., Yokoya, K., Knight, R. D. and Whitfield, T. T. (2023). Enhancer trap lines with GFP driven by smad6b and frizzled1 regulatory sequences for the study of epithelial morphogenesis in the developing zebrafish inner ear. J. Anat. 243, 78–89.

Mendonca, T., Jones, A. A., Pozo, J. M., Baxendale, S., Whitfield, T. T. and Frangi, A. F. (2021). Origami: Single-cell 3D shape dynamics oriented along the apico-basal axis of folding epithelia from fluorescence microscopy data. PLoS Comput. Biol. 17, e1009063.

Project 55

Department of Biomedical Science Laboratory Research Project 2024/25

The mechanisms of genetic changes in human pluripotent stem cells

Stem Cell & Regenerative Medicine

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Background

Human pluripotent stem cells (hPSCs) represent powerful tools for modelling early embryogenesis and provide a source of differentiated cells for use in regenerative medicine applications. The assurance of genetic stability is essential for uses of hPSCs in basic research and regenerative medicine. Nonetheless, hPSCs are known to acquire genetic changes upon prolonged culture. The commonly observed genetic changes in hPSCs are non-random and involve gains of whole or parts of chromosomes 1, 12, 17, 20 and X, indicating that genes within these regions confer selective advantage to mutant cells. Variant hPSC with recurrent aneuploidies often show signs of neoplastic progression, including reduced apoptosis, growth-factor independence and higher cloning efficiency. With hPSCs derivatives entering the clinical trials, a possibility that genetic changes may confer malignant properties to hPSCs or their differentiated progeny is a major cause of regulatory concern.

Aims:

The goal of this project is to investigate the molecular mechanisms that underlie the maintenance of the integrity of the hPSC genome and how disruption of these mechanisms can lead to undesired genetic changes.

Techniques:

Human pluripotent stem cell culture, time-lapse imaging and image analysis, immunocytochemistry.

References:

1. Price CJ, Stavish D, Gokhale PJ, Stevenson BA, Sargeant S, Lacey J, Rodriguez TA, Barbaric I (2021) Genetically variant human pluripotent stem cells selectively eliminate wild-type counterparts through YAP-mediated cell competition. Developmental Cell 56:2455-70.

2. Halliwell J, Barbaric I, Andrews PW (2020) Acquired genetic changes in human pluripotent stem cells: origins and consequences. Nature Reviews Molecular

Project 56

Department of Biomedical Science Laboratory Research Project 2024/25

Characterising membrane identity changes during cell division

Cancer Biology

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Background

Cell division is arguably the single most traumatic event in the live cycle of a cell and mishaps are often the root cause of cancer. Among the many changes that occur is the complete rearrangement of all membrane compartments: The nuclear envelope has to be broken up and rebuild; the Golgi apparatus is dissolved and reformed; endosomes and mitochondria have to be distributed and the plasma membrane is massively restructured during cleavage furrow formation and abscission. Key towards controlling this dramatic rearrangement are changes to the identity of the membranes, imparted by the Phosphoinositides, a small family of phospholipids. At the heart of this project is to better understand and characterise the complex changes to the identity of membranes during cytokinesis. This will be done using microscopy and novel fluorescent probes that have been developed by the lab. The work will be part of an international collaboration with the potential of real impact on the field.

Aims:

Comprehensive characterisation and quantification of the phosphoinositide identity of membranes during different stages of cell division. You will produce a detailed timeline of how the identity of membranes change during cell division. This will be a crucial resource to the field with strong implications to cancer cell biology.

Techniques:

- High resolution confocal microscopy
- Tissue culture and synchronisation of cells
- Staining of cells using custom made fluorescent probes developed by the lab
- Image analysis and quantification

References:

Maib H, Adarska P, Hunton R, Vines JH, Strutt D, Bottanelli F, Murray DH. Recombinant biosensors for multiplex and super-resolution imaging of phosphoinositides. J Cell Biol. 2024 Jun 3; doi: 10.1083/jcb.202310095. PMID: 38578646; PMCID: PMC10996583.

Carlton JG, Jones H, Eggert US. Membrane and organelle dynamics during cell division. Nat Rev Mol Cell Biol. 2020 Mar;21(3):151-166. doi: 10.1038/s41580-019-0208-1. PMID: 32034394.

Cauvin C, Echard A. Phosphoinositides: Lipids with informative heads and mastermind functions in cell division. Biochim Biophys Acta. 2015 Jun;1851(6):832-43. doi: 10.1016/j.bbalip.2014.10.013. PMID: 25449648.

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Department of Biomedical Science Laboratory Research Project 2024/25