# BSDB Newsletter


## British Society for Developmental Biology

The Company of Biologists

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# Communication Officer's Report: Ben Steventon

2021 has been a tough year on all of us, and in writing this many of the same thoughts are coming to me as they did a year ago. However, there are some very important differences to this newsletter, and they do provide examples to the robustness of our field and of our society. In many conversations I have had with our membership over the last few years, it has been increasingly apparent that many of us feel that the field of Developmental Biology is under threat. It is certainly the case that it is sometimes difficult to put a pin in exactly what Developmental Biology is, and its importance to human society relative to other scientific disciplines. However, it is critical to be clear on what we all set out to do each time we look at cells and embryos under a microscope, or indeed, a T-SNE plot. We are here to ask a set of questions, and these questions have not changed. Not in a long time, in fact. It is perhaps rare for a scientific field to bring in so many other disciplines in search of answers to questions that remain constant over such dramatic alterations in approach and methodology.

I'll leave it to Jane Oppenheimer to point out how the deep curiosity of an embryologist endures over successive waves of incorporation of Anatomy, Cell biology, Molecular Biology and Genetics. Here, she comments on how there has been a progression in the study of embryos from considering them in terms of whole embryos by the likes of Aristotle, Fabricius, Harvey, Malpighi and Wolff, then in terms of their separate layers by Wolff, Pander, von Baer, His, Haeckel and Spemann, then in relation to their cells (Roux, Driesch, Spemann, Harrison and others) and their components (O.Hertwig, Boveri, E.B. Wilson and Conklin). We are now well into a phase of examining the complex relations of molecules within cells and between them, but as Oppenheimer nicely points out:

*"The integrative powers of the embryo, at all of its levels, are however so pervasive that they never permit themselves to be overlooked by those who avail themselves the privilege of looking at the embryo at all. The result has been that when each of the practices just enumerated became fashionable, the previous one was never outmoded; and when, at each stage of its development, embryology has added a new dimension to its studies, it has never wholly discarded the old ones."*
(Oppenheimer, 1967 pg. 9)

In this sense, one could see Developmental Biology as a field that succeeds through continually recruiting other disciplines. The fascination that is given from watching an embryo development, and to consider the full complexity of what is going on inside an organism as it is forming its own self, is something almost unmatched in the natural world. As such we bring people into the field, and once there, they remain as captivated as we are. It is therefore hard to imagine such a force feeling threatened in the academic landscape, but we often do. One recent and painful example is how hard model organism databases have had to fight for their continued funding. Facing such threats, it is now more important than ever to arm ourselves with the fascination of our subject, and to point out just how much of the sciences has benefitted from bringing their steel to our blade. It is only through application to a problem that new scientific approached become honed, and we have the best problem going. So, lets own it.

Where can you find evidence of our robustness in this newsletter? For the first, I would point you in the direction of our last round of Gurdon Summer Students. The reports are yet again, fabulous. Even more credit should be given to this year's students to have been able to take on such interesting projects during a pandemic, especially having endured many months of online teaching. It is a relief to see this next round of developmental biologists were able to get into labs over the summer and tell us about their work. We are also very happy to have contributions from Crick Summer Students included in this issue, as this makes for even better reading.
For the second, I'd like to point to the life of our much loved, Lewis Wolpert. It was a great

loss to our community to see him go. His life's work has demonstrated the importance of continually asking sharp questions, and thereby recruiting the right skills to tackle them. If you were able to have discussed his work with him, he would have been the first to have even handedly pointed to where his skills lay as a developmental biologist. "I'm terrible at doing experiments", he would say. "But very good at getting others to do them for me". We at the BSDB are very happy introduced a new medal to his honour this year, and trust that this will send our message loud and clear: Recruit, Recruit, Recruit.

Ben Steventon

*The BSDB gratefully acknowledges the continuing financial support of The Company of Biologists Ltd (CoB).*

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# Chair's Report: Paul Martin

It has been a crazy year but the BSDB has coped well and has attempted to be a solid rock in stormy seas. Our REMO driven BSDB Spring meeting was a big success and allowed us to taste some fabulous science plus also to socialise at a virtual meeting in ways that almost resembled the real thing. This was testament to our organisers: Tanya Whitfield, Clare Baker, Sally Lowell (BSDB), and Stefan Hoppler, Aziz Aboobaker, Marieke Charalambous, (GenSoc), alongside the folk at HG3 who we have a longstanding relationship with. It has been good to have reliable friends like them in covid times.

We (our treasurer Cynthia Andoniadou) have given out lots of "travel" grants for students and post-docs in our community, in order for them to attend other "online" meetings. Of course, these will not be a perfect substitute for the impromptu interactions that occur at real meetings but they do enable some networking, and they are a decent stopgap, and will guide us towards blended meetings which will mean not everyone has to travel to all meetings in the future which will be better for climate and mental health. Indeed, one such blended meeting is currently being planned for Oxford in 2023. Sally Lowell will be in charge, with local organisers, and the plan is for the bulk of attendees to be listening to talks for real in Oxford, but with spokes extending to hubs in at least three other European cities where their local organisers will take charge of focussed half day sessions. This is a pioneering experimental approach, so please help us make it work.

A week after our BSDB Spring meeting we held a very competitive round of voting for four new members of our BSDB committee (Kyra Campbell, Jeremy Green, Anestis Tsakiridis, and Helen Weavers), and we now have a full complement of committee members with a nice geographical spread who, between them, work across all of our favourite model organisms and cover most tissues and lineages that our community work on. And we have a new student rep, Lara Busby, and new post-doc rep Anahí Binagui-Casas, who we are pleased to welcome to the committee.

Since I last wrote for this newsletter, we have suffered the sad loss of one of the Godfathers of our community, Lewis Wolpert. But from this sadness good things have arisen. We had been talking for a while about having a new BSDB medal to celebrate the best of UK developmental biology teaching and writing including popularisation/outreach for our field, and we decided to name this new medal after Lewis. Our inaugural awardee is Jamie Davies from Edinburgh. For this BSDB medal we will arrange for the awardee to give a series of three or four talks at Institutes where developmental biology is taught (or should be). We are in the process of selecting these venues, so please let me know if your Institute fancies a bit of Jamie action!

Related to this, our next BSDB autumn meeting will be a celebration of Lewis' life and works, organised by several of his ex-students and colleagues (Jim Smith, Jonathan Slack, Claudio Stern, Cheryll Tickle, and Neil Vargesson), and held at the Crick. More details to follow.

We have several other BSDB projects on the go. One of these is a trailer/mini movie about UK developmental biology that we'd like to function as something we could take into schools but which might also serve as a bait to tempt funders and production companies to consider a more ambitious foray into the movies for our developmental biology community. Again, we will update you as these plans unfold.

Hopefully we will all get to meet each other a lot more in the coming year. I'm looking forward to interacting more with many of you f2f, particularly at our next BSDB Spring meeting. In the meantime, those of you, like me, who are currently embroiled in teaching a new influx of undergraduates… keep showing them pictures and movies of beautiful embryos. Remember, our every day "data" is wondrous and makes it easy to inspire the next generation of developmental biologists!

# Secretary's Report: Tristan Rodriguez

It has been a great pleasure to be selected to take over as Secretary of the BSDB. I would like to start by extending a big thank you to my predecessor Megan Davey for all her hard work over the past years. I would also like to thank her, our treasurer Cynthia Andoniadou and our meetings secretary Sally Lowell for their patience in helping me to learn the ropes over the last few months.

My role as Secretary has been made ever so much easier by all the efforts of Megan to update our membership database and facilitate its transfer to Hg3 conferences, who will manage our membership going forward. Many of you will already be familiar with Hg3 as they have managed the Annual Spring Meetings for many years now and they already manage the membership database for our sister society, the BSCB. Given how important communicating with our members is, we hope that by transferring the handling of our membership to a known professional company will help deal with any queries efficiently on a day-to-day basis.

This year has been a big year of change for our committee. In addition to Megan, Berenika Plusa and Alistair McGregor also finished their terms as elected committee members and our graduate representative Jessica Foryth also stepped down, that together with Charlotte Sophie Louise Bailey stepping down last year as our Postdoc representative means there will be a lot of new faces on the committee. All our departing members have made important contributions to the BSDB and I would like to thank them on behalf of everyone in our community. I will also extend a warm welcome to our incoming elected committee members, Helen Weavers, Kyra Campbell, Anestis Tsakiridis and Jeremy Green, and to Lara Busby and Anahí Binagui-Casas, who will serve as our graduate and post-doctoral representatives, respectively.

These have been a difficult couple of years for our everyone, I am sure, but the membership numbers remain buoyant and we now stand at 1016 paying members. The nominations for the 2021 Waddington, Cheryll Tickle and Beddington Medals were absolutely outstanding this year, so electing a winner proved close to impossible. But there is no doubt that Dame Ottoline Leyser, Dr. Tatjana Sauka-Spengler and Dr. Kristina Stapornwongkul respectively were all extremely worthy winners of each one of these awards. I very much look forward to seeing your nominations for 2022 and very much encourage you all to submit nominations that help reflect the strength and diversity of our community.

I wish you all a good end to 2021 and look forward to seeing you at the 2022 BSDB Spring meeting in Warwick.

Tristan Rodriguez

# Meetings Officer's Report: Sally Lowell

These are interesting times to be a Society Meetings Officer (or indeed to be a human being of any type). We were disappointed this year to spend another spring away from the charms of Warwick University campus but delighted to come together online at our joint meeting with GenSoc. Big thanks to organisers Tanya Whitfield & Clare Baker (BSDB), and Stefan Hopper, Aziz Aboobaker & Marika Charalambous (GenSoc) for much hard work in adapting their excellent programme to an online format. Special thanks to postgrad reps Jessica Forsyth (BSDB) and Emily Baker (GenSoc) for creating superb online workshops and social events, including a virtual pub quiz that the BSDB committee managed not to win despite being extremely clever and knowledgeable, and also having three times as many team members as everyone else. The conference party went with its usual swing, even though it was just me dancing on my own in my kitchen. Another highlight was our online art exhibition, organised by Tanya Whitfield, featuring a wide variety of beautiful art works, both from professional artists and our talented BSDB/GenSoc members. We hope to repeat our online art exhibition in future years, so please do consider submitting your own science images, sci-art, or any-other-sort-of-art. There will probably be prizes, and there will definitely be glory.

Next Spring, we make our magnificent return to Warwick on April 3-6, arm in arm with our friends from BSCB. We hope to be able to deliver a hybrid format for people who prefer to link arms with us virtually. The organisers, Jens Januschke & Raman Das (BSDB), and Tom Nightingale, Alison Twelvetrees & Susana Godinho (BSCB) have put together a great line up of speakers (see poster), while our student and postdoc reps, Lara Busby (BSDB), Anahí Binagui-Casas (BSDB), Rowan Taylor (BSCB) and Alex Fellows (BSCB) will be bringing you our always-popular careers workshop and social events. Want to be a speaker yourself? We'll be choosing plenty of short talks from abstracts, hopefully with options for both in-person and remote speakers. Abstract submission now open, registration open soon. Check out the website for more details: https://tinyurl.com/BSCBDB2022. It is going to be great.

The meeting formerly known as the 2020 joint Autumn meeting of the BSDB and the International Society for Differentiation will finally take place 4-8 September 2022 in Valetta, Malta and will be completely fabulous. Note that BSDB members INCLUDING PIs can apply for BSDB Conference Grants to attend this meeting. We hope to retain our original programme and will keep you posted on our plans as they develop. Please also keep an eye out for a very special meeting in London on 13-14 September 2022 to celebrate the life of Lewis Wolpert. More details here: https://tinyurl.com/Wolpert22.

In 2023 we will host the quadrennial gathering of the European Congress for Developmental Biology, which will replace our usual Spring Meeting and take place somewhere lovely that is not Warwick. We are busy hatching plans for that and will let you know more in our next newsletter and at our AGM.

Have you got an idea for a BSDB Autumn meeting that you'd like to organise? If so, then please do get in touch with me at meetings@bsdb.org. We have vacancies from 2024 onwards so you have plenty of time to put your meeting together, and you'll get plenty of support from the BSDB to help you.

I look forward VERY much to seeing many of you in Warwick next year!

# Treasurer's Report: Cynthia Adoniadou

The Society remains vibrant with 1016 verified members at the end of July 2021, meaning a steady maintenance of our membership, despite the challenging environment for securing funding for recruitment of students and postdocs to labs in the UK and worldwide. Despite a roller coaster year for global financial markets, the financial situation of the Society remains in good shape and this has allowed us to continue our activities to promote developmental biology, described below.

**Report on the financial year 2020-21**

As shown in the accompanying provisional accounts for the Society for the period August 2020 - July 2021, this last year has seen us invest in activities to bring our community together after a tough year, to which end our Society's expenditure exceeded our income by £8,672.

In the previous financial year, we incurred an underspend due to the pandemic restrictions, which led to postponing our 2020 Spring Meeting, the suspension of Gurdon Summer Studentships and the lack of travel awards throughout the year. To offset this underspend, we applied for proportionately lower funding from the Company of Biologists (CoB). The yearly sum received from the CoB is essential for the running of the Society. The block grant helps us to support the running costs of meetings in spring and autumn, as well as Gurdon CoB Summer Studentships. This was reduced from £35,000 to £21,360. The Society awarded 60 registration grants to allow student and postdoc members to attend the virtual BSDB/Genetics Society Joint Spring meeting 2021 (£5,000). In order to bring as many members of the community together as possible, the Society made a decision to incur the significant costs of an excellent virtual platform, whilst setting very low registration cost for both full and student members (£150 and £25 respectively). The total cost for this highly successful meeting to the BSDB was £43,971. This amount would have been much greater if it wasn't for the great efforts of our conference organisers, who strive to raise income via sponsorship and keep costs under control. Gurdon Summer Studentships resumed for the summer of 2021 and we were pleased to award a record 16 studentships, funded at a competitive rate to ensure we attract the best students (£24,000 total). This year's projects spanned both wet lab and hybrid wet/dry lab research, and for the first time, outreach activities.

We also receive a grant to spend on CoB/BSDB travel awards to help towards the costs of our members' attendance/travel to overseas meetings. This grant was reduced from £37,500 to £19,465; in line with the previous years underspend. To facilitate worldwide conference participation of students and postdoc members, the Society opened up the CoB/BSDB Travel Grants for virtual meeting registrations. In total, 6 CoB/BSDB travel awards were made in 2020-21 (£1,091), reflecting the low demand during the pandemic, with awards granted to all eligible applicants.

**Future Plans**

The Society investments have substantially increased in value during the reporting period. It must be noted that the figures reported are unrealised valuations of our holdings, therefore subject to future fluctuations. However, our overall solid financial health means that we will continue to invest in new activities to promote developmental biology in the UK, and can do this without any significant threat to the core business of the Society.

# New BSDB Committee Members

**Kyra Campbell**

I have long been fascinated by the question of how cells assemble into functional tissues at both the subcellular and intercellular levels. After studying Natural Sciences at the University of Cambridge and being fired up by my final year course in Developmental Biology, I stayed on to do my PhD with Helen Skaer. In close collaboration with Elisabeth Knust, I explored how cell polarity is established and maintained as cells undergo the extensive remodelling that underlies tissue morphogenesis. For my postdoctoral training, I moved to the lab of Jordi Casanova in Barcelona, and focused on developing a novel model system in *Drosophila melanogaster* for studying the mechanisms underlying cell plasticity during development, and in collaboration with Eduard Batlle's lab at the IRB, in tumourigenesis. I enjoyed my postdoc in Barcelona tremendously, not only for the science and the people, but also in growing my family – I had both my daughter and son there.

We moved to Sheffield in 2017, when I activated a Sir Henry Dale Fellowship that I was awarded by the Wellcome Trust/ The Royal Society, and started my research group in the University of Sheffield. My lab is focused on identifying the molecular mechanisms underlying epithelial cell plasticity during development and disease. We study how this fundamental property is orchestrated during morphogenesis of the *Drosophila* midgut, and also in our recently developed *Drosophila* models for metastatic cancer.

**Jeremy Green**

Jeremy Green was the co-discoverer with Jim Smith of the dose-threshold action of growth factor morphogens, experimentally validating the concept of Positional Information and laying the groundwork for directed-differentiation of stem cells. After a Fellowship at UC Berkeley with John Gerhart and Ray Keller, he became Assistant Professor at Harvard Medical School's Dana Farber Institute. He returned to the UK and started his group at King's College London in 2005. The Green lab investigates morphogen action, self-organising Reaction-Diffusion Turing patterning and physical morphogenesis in mammalian (mouse) and amphibian development using advanced microscopy, image analysis and computational modelling.


**Anestis Tsakiridis**

I graduated with BSc(Hons) in Biochemistry from the University of Edinburgh in 2001 and, after an MSc in Life Sciences, I did a PhD (2002-2006) in the lab of Lesley Forrester in the same University, focusing on gene trap mutagenesis in mouse embryonic stem cells. This was followed by a postdoc first with Josh Brickman and then Val Wilson in the Institute for Stem Cell Research/MRC Centre for Regenerative Medicine in Edinburgh studying cell fate decisions in the early mouse embryo through the use of pluripotent stem cell-based models. In 2016 I was awarded a Vice-Chancellor's Fellowship from the University of Sheffield, and subsequently, a BBSRC New Investigator grant to start my own research group at the Centre for Stem Cell Biology (CSBC) in the Biomedical Sciences Department (BMS) where I am currently a lecturer. My group's research aims to understand how human embryonic cells adopt different fates as they transit from pluripotency to lineage commitment, and exploit this knowledge for regenerative medicine and disease modelling applications with a particular focus on the anteroposterior regionalisation of neural crest and spinal cord cells.

**Helen Weavers**

Helen Weavers started her own research group in the Faculty of Life Sciences at the University of Bristol in 2018 funded by a Wellcome Trust and Royal Society Sir Henry Dale Fellowship. Helen was first introduced to the wonderful world of developmental biology as a Wellcome Trust PhD student studying *Drosophila* renal development in Prof Helen Skaer's lab at the University of Cambridge; for this work Helen was awarded the BSDB Beddington Medal in 2013. Helen then moved to Bristol to undertake MRC-funded post-doctoral research on tissue repair and inflammation with Profs Paul Martin and Will Wood, for which Helen received the BSDB's 2017 Dennis Summerbell Lecture Prize. Research in Helen's own lab now integrates studies in *Drosophila* and human genetic epidemiology to explore the molecular and cellular mechanisms that drive stress resistance during tissue development, inflammation and wound repair. For further information see the lab website https://www.tissueresilience.com/

**Lara Busby**

Lara is currently a PhD student with Ben Steventon at the University of Cambridge. She is studying the interplay between time and cell fate decisions during avian posterior body development, exploiting the chicken embryo as a system for experimental embryology. During development, cells must coordinate their behaviours and fate decisions with the overall progression of developmental time, to allow for normal morphogenesis. Lara hopes to gain insight into the mechanisms which allow cells to "tell the time", by performing heterochronic grafting experiments (moving cells between embryos of different ages).

On the committee, Lara is a representative for student members who are pursuing a master's or PhD, and aims to present the needs and preferences of BSDB postgraduate members to the rest of the committee. She will be organising events for postgraduate members at the annual BSDB meeting, including opportunities to network and meet other early career researchers, as well as a careers workshop. Please feel free to get in touch with Lara if you have any questions or ideas, at **students@bsdb.org**.

**Anahí Binagui-Casas**

I am excited to join the committee and represent the BSDB postdoc community. My aim is to help ensure our needs are met by the society and propose ways by which BSDB researchers could be supported during their postdoctoral posts. I also hope to use my experience as member of the equality, diversity, and inclusion (EDI) advisory group at my university to advocate for EDI values within BSDB.

I am a postdoctoral researcher working with Val Wilson in the Institute for Stem Cell Research at the University of Edinburgh. It fascinates me how cells undergo fate commitment, and I am currently interested in understanding the ontogeny of mesoderm progenitors that will produce different tissues during mammalian embryo development.

My background as a developmental biologist started early on during my undergraduate studies, which let me to pursue a masters in developmental genetics and genomics while embedding myself in bench life. Firstly, in the lab of Florenci Serras at the University of Barcelona, I studied early signals that trigger epithelial regeneration after damage in *Drosophila* larval imaginal discs. Secondly, in the lab of Sjaak Philipsen and Thamar van Dijk, at the Erasmus MC in Rotterdam, I looked at transcription factor interactions that regulate the gamma to beta haemoglobin switching during mammalian embryonic erythropoiesis. For my PhD studies, I moved

to Alexander Medvinsky's Lab at University of Edinburgh, where I looked at the role of endothelial-specific pathways in the emergence of haematopoietic stem cells during mouse embryonic development.

Looking forward to meeting you in any of the BSDB meetings! If you have any suggestions or questions, do not hesitate to contact me by email at **postdocs@bsdb.org**.

# Beddington Medal 2021: Kristina Stapornwongkul

We are very pleased to announce the 2021 Beddington Award winner as Kristina Stapornwongkul from the Crick Institute. She completed her PhD with Jean-Paul Vincent as part of the Wellcome Trust funded Developmental and Stem Cell Biology programme. Her work on understanding the mechanisms of diffusion-based signalling gradient formation represents a hugely innovative and original piece of research that will form a bench-mark study in terms of its application of synthetic biology approaches to understand fundamental questions of developmental biology in vivo. During her PhD, Kristina has won multiple prizes and awards, that include best talk prize at the "From Cells to Embryo" meeting in Paris, a selected talk at the "Physics of Living matter meeting" in Marseille, and a poster prize at the "From Gene Circuits to Tissue Architecture" meeting at EMBL Heidelberg. Her works represents the cutting edge of modern developmental biology in its application of inter-disciplinary approaches.

The central question of her PhD was to ask how effective simple diffusion can be in establishing a morphogen gradient in vivo. She started by asking how effectively GFP can diffuse from a localised stripe source within the wing disk epithelium of drosophila, when fine-tuning the tethering of this protein to extracellular binding partners. This was achieved through the use of surface associated anti-GFP nanobodies to modulate the ligand gradient by trapping the ligand and limiting its leakage. In a bold next step, Kristina then asked whether this engineered gradient would be able to substitute for the endogenous morphogen: Decapentaplegic (Dpp). To engineer the Dpp signalling pathway to be responsive to extracellular GFP, anti-GFP nanobodies were added to Dpp receptors. Strikingly, this was sufficient for GFP to activate phosphorylation of the downstream effectors of the signalling pathway. Such a finding opens up potential for many similar synthetic biology approaches to interrogate the mechanisms of intra-cellular signalling during development. For Kristina, it meant that she could go on to prove the sufficiency of an engineered GFP gradient to appropriately pattern the wing disk in the background of a Dpp loss of function mutant. This response was further improved through the targeted expression of synthetic glypicans, where a low-affinity GFP nanobody was fused to the glycosylphosphatidylinositol (GPI)-anchored components of the ECM. Therefore, the study provided direct proof of the importance of additional ECM binding partners in the modulation of gradient diffusion. Pairing these studies with models developed by the Salbreaux lab, the team were able to better understand how signal receptor, and non-receptor binding interact to tune gradient formation. Therefore, not only does her work provide direct evidence for the sufficiency of morphogen gradients in pattern formation in vivo, it also points the direction towards engineering approaches to generate pattern formation while leaving endogenous signals intact.

The independence and drive of Kristina in the development of her PhD project is clear from reading her nomination letter of JP Vincent:

"It had been shown previously that an inert protein like GFP does not form a gradient on its own in vivo (and this had actually been used as an argument that diffusion could not account for gradient formation). But Kristina argued that this was not a fair test and that gradient formation had to be assessed in the presence of extracellular binders. She realised that recent development (in the availability of anti-GFP nanobodies & in genome engineering expertise) would allow her to design such a test". JP Vincent

"To create a GFP gradient in vivo is on its own a remarkable achievement but Kristina had the boldness of thinking (and demonstrating) that it could suffice to provide positional information. This came as a shock to me and many developmental biologists and opens the way to further rigorous studies of signalling dynamics in living tissues. Kristina can take most of the credit for the design and execution of her work and for linking up with the physicists, who helped with modelling". JP Vincent

I'll leave you with some comments of Kristina herself on her PhD experience:

"First of all, I should say that I have been really lucky with my PhD. I feel that I've grown a lot as a scientist in the last years and this is

of course all thanks to JP, the Vincent lab and the entire scientific community I was interacting with. I'm incredible grateful to all these people not only for teaching and supporting me but also for making my PhD real fun (especially when the science didn't work). JP has been a great boss and I think it's quite impossible not to like him and his laid-back Californian surfer-style. It's also impressive how travelling with him always ends in chaos  (there are too many stories). I am also so thankful to the Vincent lab, a great mix of extremely bright and helpful people". Kristina Stapornwongkul

Congratulations Kristina on such great achievements during your PhD! The BSDB wish you all the best in the continuation of your scientific career, and are very happy to award you the 2021 Beddington award.

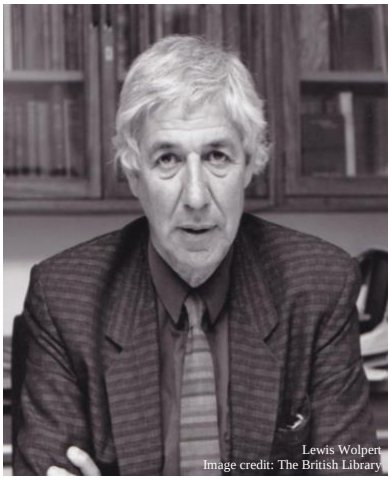
# Wolpert Medal 2021: Jamie Davies

**Wolpert Medallist 2021: Jamie Davies (University of Edinburgh)**

"You'll all be aware that one of the great thinkers and inspirers (and frankly, heroes) in our field of Developmental Biology, Lewis Wolpert, sadly passed away earlier this year. For several years our BSDB committee has been considering an award that might honour important aspects for our community beyond simply bench lab achievements, in particular teaching and communication. What better than to initiate such an award this year and name it after Lewis who was himself such a fantastic communicator of Developmental Biology ideas. As the inaugural recipient of this medal our committee unanimously agreed that Jamie Davies from Edinburgh exemplifies all that we would like to reward with this new medal. All of us on the committee have experienced interviewing undergrads from Edinburgh (perhaps applying for PhD studentships), who raved about Jamie's inspirational lectures. He has also written several very popular books in and around our field. And, he even has Lewis lineage history – he did his post-doc with David Garrod who had worked in Lewis' lab in the early pre-chick limb days on Dictyostelium motility."

Paul Martin (BSDB Chair)

## The Wolpert Medal



Lewis Wolpert
Image credit: The British Library

Following this year's sad passing of one of the greats of Developmental Biology, Lewis Wolpert, the BSDB committee has decided to launch a new annual medal in his honour. Lewis was well known for his ability to distill our subject's most engaging and fundamental problems into concise and well-grounded core concepts of Biology. This led to vastly important contributions to research in our field, but also to the communication of its problems to a broader audience. Through teaching, popular science writing and acting as a spokesperson for Science as a whole, Lewis inspired many of us into the deeper study of Developmental Biology. Therefore, annual 'Wolpert medal' will be presented to an individual who has made extraordinary contributions to the teaching and communication of Developmental Biology.

We have been incredibly fortunate to have the Scientist and Artist, Steph Nowotarski design the Lewis medal. In designing the Wolpert medal, we wanted to include various different model organisms that Lewis worked with through his career, including Hydra, chick limbs and sea urchins. At the centre is an image of Hydra as they represent the concepts of continual regeneration and renewal, and it is our best teachers and communicators that bring about the constant regeneration and renewal of our field.

To learn more of Lewis' life and work, please read this Obituary by Jim Smith. Read more of Lewis' impact on the BSDB here, and watch the inaugural Lewis Wolpert Memorial Lecture, hosted by UCL.

**You can watch Lewis's 2015 Waddington medal lecture here**


Wolpert Medal Design
Image credit: Stephanie Nowotarski

**Some memories of Lewis from the BSDB Committee.**

"Lewis was one of my heroes. He was my PhD supervisor, Julian Lewis' post-doc mentor, so I considered myself a grandson. He examined my PhD and was completely distracted throughout the viva because he'd just received a Fed Ex parcel with a juicy MS or antiquarian book that he wanted to look at....but when he did look at me and raise his glasses and ask me a question I felt like I was in the presence of royalty. He was a superstar in our field and a fantastically inspirational thinker and speaker for all of us in the Dev Biol community." – *Paul Martin*

"Lewis Wolpert had beautiful ideas and expressed them with elegant words. He inspired so many of us to become developmental biologists." – *Sally Lowell*

"I had the pleasure of meeting Lewis in the setting of my PhD Program annual symposium - he was a friend of the Portuguese scientific community. Having chosen a PhD on gastrulation, I amused myself by baffling others with his famous quote. I also read with great interest his scholarly book on depression, endlessly inquisitive and thought-provoking. He will forever remain in the consciousness of so many he had no idea about…" – *Rita Sousa-Nunes*

"I remember being inspired by Lewis Wolpert's beautiful textbook on developmental biology as an undergraduate student. This has led to a lifelong passion for development, for which I am exceptionally thankful." – *Raman Das*

"I was very fortunate to share lab meetings during my PhD with him as part of Claudio Stern's Monday evening lab meetings. Whenever it was his turn to present, it was often to re-iterate how little evidence there was for the role of morphogen gradients in development, and that it was extremely important to continually look for new explanations for pattern formation that go beyond his concept of positional information. In particular, he emphasised the importance of considering how individual cells receive and transmit information, stressing the importance of getting down to the details in order to gain the big picture. That he continually criticised the theory he was most famous for is a lesson to us all.

Lewis was also known as a great communicator of science, acting as a conduit between the general public and scientists in many different ways. His public lectures were always driven with honesty and a great degree of wit. He was clearly on the side of leaving ethical decisions for the general public to make, with the role of the scientist to provide the necessary information for people to form their own opinions on even complex issues. As he would say, while he trusted his science colleagues on a great deal of issues relating to biology- he would not allow a single one of them to even choose the colour of his tie.

In my first months as a PhD student I approached him at a wine and cheese session and asked him what he would study if he were to do his PhD again now. He looked down at his wine glass and began to explain how, when on holiday, he often drinks wine on a terrace here or there. Often he would be astounded when a fly would land on the rim of his glass- never failing to land precisely without a single slip. "If I were to start a PhD now, I would ask how flies manage to do that", he said." – *Ben Steventon*

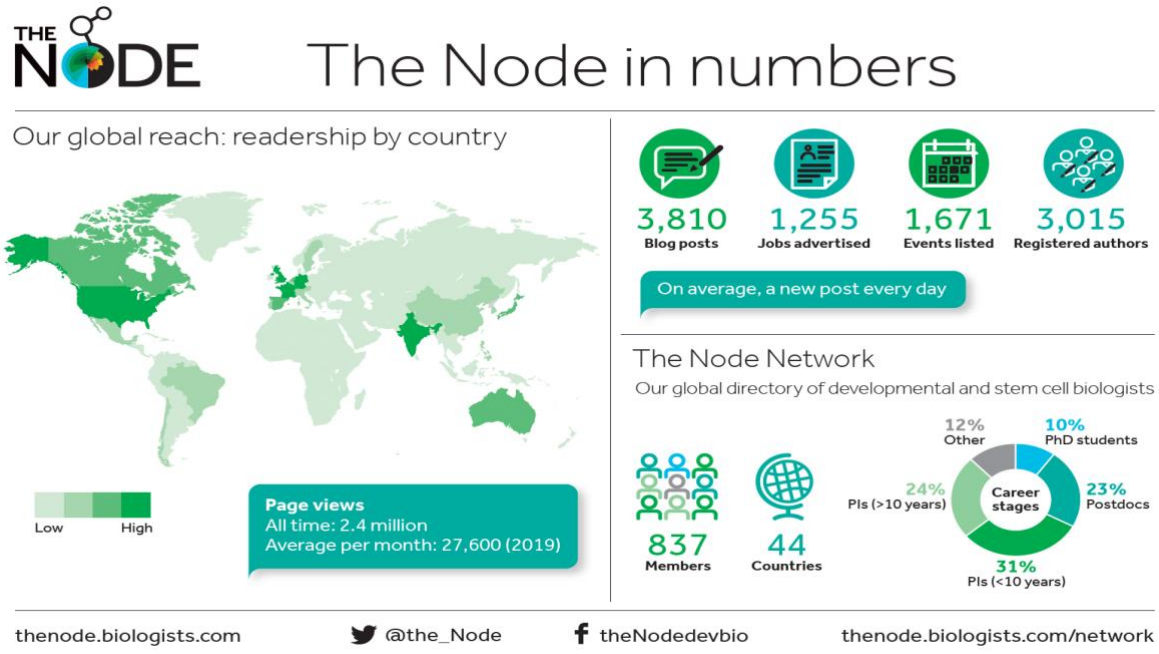
"Principles of Development" by Lewis Wolpert and colleagues was my introduction to developmental biology as a student and continues to be a corner stone of my teaching. I only had the chance to experience Lewis Wolpert in person once when he gave his Waddington medal lecture at the BSDB spring meeting in 2015 which left me very moved". – *Jens Januschke*

# Building communities at The Company of Biologists

The Company of Biologists was founded by George Parker Bidder III in 1925, initially acquiring the publication now known as Journal of Experimental Biology. Since then, our portfolio has grown to include Journal of Cell Science, Development, Disease Models & Mechanisms, and Biology Open. As a not-for-profit publishing organisation, we use our funds to support biologists and inspire the biological community. With our 100th birthday fast approaching, we reflect on recent initiatives at the Company and explain what they can offer developmental and stem cell biologists.

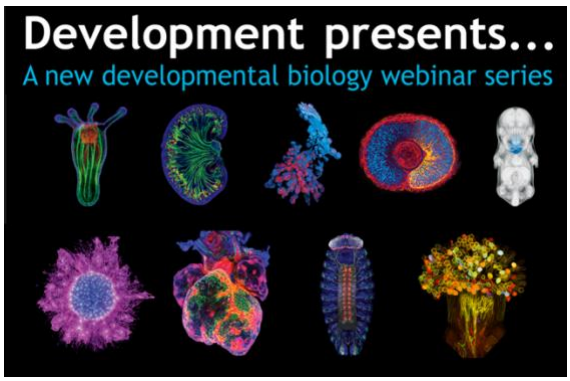
The Company of Biologists has been busy, with many exciting new initiatives aimed at supporting the biological research community. The Node (thenode.biologists.com) remains a highly successful platform for developmental and stem cell biologists to connect online, and in January 2020 we added another string to its bow with the launch of the Node Network. The Node Network is an inclusive directory of researchers with a focus on diversifying the developmental biology landscape. Here, users can share details about their research, including their area of expertise, the model organism they use, their institute and their career stage. There is also the option to share details about gender, race/ethnicity, LGBTQ+ identity and disability status. Anyone can join the network and it's free to sign up. We would particularly encourage conference organisers looking for speakers, editors looking for reviewers, or students looking to invite academics to their departments, to begin their search for candidates there. With almost 1,000 users, the Node Network is a great place to make new connections and discover inspiring members of the community.

Online communities like the Node have become particularly important in the context of the pandemic, which has had a profound impact on the scientific community. One of its most noticeable effects was the immediate cessation of in-person conferences. In response to this, the team at Development launched 'Development presents...'. This online webinar series premiered in October 2020 and is open to all, with a different Development Editor taking the role of host each month. The Editors select speakers that have recently published exciting papers or uploaded interesting preprints, with a focus on




*The Node is a well-established online community with a global reach. In January 2020 we launched the Node Network, which already has nearly 1,000 members from 44 different countries.*

providing a platform for early-career researchers (ECRs). The talks are hosted on Remo, an interactive platform that allows attendees to join virtual tables and chat with other participants. With registration open for future webinars, we hope to continue building on the vibrant community we have created.




*The Development presents… webinars have provided an online platform for the community to share their research and to network.*

Meetings are now on the rise again, with many adopting inclusive and ever more innovative hybrid formats. Our programme for 2022 is evolving, and one event of particular relevance for BSDB members will be Development's 'From Stem Cells to Human Development' meeting. Organised by James Briscoe, Prisca Liberali, Samantha Morris and Wei Xie, the meeting will be held at the historic Wotton House, Surrey. The meeting has been running every two years since 2014 and, after a virtual event in 2020, it is back for September 2022. More details, as well as links to our other Meetings and Workshops, can be found at biologists.com/meetings.

The Company of Biologists is particularly passionate about supporting ECRs. As well as showcasing their work through Development presents…, Development has been encouraging ECRs to act as reviewers, curating a database of ECRs that can be directly approached in future. We also have a blossoming preLights community, a group of ECRs who write about preprints that have caught their eye. The hope is to highlight preprints of interest to different fields in an increasingly crowded medium, whilst also providing ECRs with science writing experience. Our 'preLighters' come from around the world, ranging from PhD students to early PIs. More information, including details on how to become a preLighter, can be found at prelights.biologists.com. Finally, as part of their new 'Transitions in Development' interview series, Development has been talking to scientists who have established their own research group within the last five years. The aim is to demonstrate the diversity of experiences that come with achieving independence, providing ECRs with insight into the many possible routes.

As well as widening access to science via our online communities, The Company of Biologists is committed to Open Access. We already have two fully Open Access journals (Biology Open, and Disease Models & Mechanisms) while our remaining, hybrid publications (Development, Journal of Cell Science and Journal of Experimental Biology) have Transformative Journal status. This means that they will increase the proportion of their content that is published Open Access while still supporting our authors, whose publishing needs vary. Watch this space to hear more about our progress with this project. We also have a Read & Publish initiative, which is available worldwide. When institutions sign a Read & Publish agreement, they pay an annual fee that allows their researchers unlimited access to our hybrid journals as well as the ability for corresponding authors to publish their articles Open Access without having to pay an article processing charge (APC). We are thrilled to have recently signed a new Read and Publish agreement with Jisc, which will run from January 2022 to December 2024. You can check if your institution is participating at biologists.com/library-hub/read-publish/participating-institutions/.

Looking ahead to our 100th birthday in 2025, The Company of Biologists hopes to continue building on the online communities it has already formed, as well as addressing the challenges of the future. Some of these challenges are well-established. Others have developed more recently, as a result of global events such as the ongoing pandemic. In a shifting research landscape, one constant is that The Company of Biologists exists to support you and your research community. Find out more and keep in touch with all the opportunities we offer by going to our website

(biologists.com) or following us on Twitter (@Co_Biologists).

**Drunk D*rosophila*: Feeding larvae with ethanol to study developmental effects in adult learning and memory**

This summer I had the fantastic opportunity, thanks to the BSDB Gurdon/Company of Biologists Summer Studentship, to work in Dr Vincent Croset's lab at Durham University. The project I undertook aimed to investigate whether methamphetamine or ethanol consumption at the larval stage of *Drosophila melanogaster* resulted in developmental defects and a subsequent change in the learning and memory ability in adult flies.

In *Drosophila*, clusters of dopaminergic neurons specifically influence memory, motivation, sleep and locomotion (Aso et al., 2014). Because these neurons develop sequentially, methamphetamine or ethanol consumption at the larval stage may have a different impact on each population (Alves dos Santos and Smidt, 2011). Identifying these differences may help understand the links between drug or alcohol consumption and the impairment of specific brain functions. Initially, the focus of the project was whether feeding larvae with methamphetamines had an effect on the learning and memory of adult flies. Unfortunately, due to unforeseen circumstances, the drugs did not arrive in time but thankfully we were able to adapt the project to investigate whether feeding larvae with ethanol had an effect on the learning and memory phenotypes in the adults.

To begin with, I focused on method optimisation to find the best way to feed larvae with ethanol. This was important to determine whether the larvae would feed during a set amount of exposure time so we could be confident that any results in subsequent learning and memory tests were due to the consumption of ethanol. Multiple feeding assays were therefore conducted on different media, using blue dye as an indicator of feeding. The most successful trial was determined by the proportion of larvae with blue guts when observed under the microscope (Figure 1). Both liquid (sucrose solution) and solid (fly food) media were assayed across a range of time frames (15 minutes, 30 minutes, 1 hour, 2 hours). The most successful of the trials was using standard fly food for 1 hour (Figure 1).

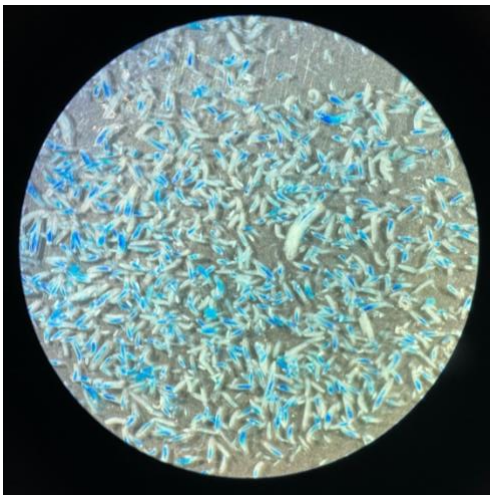


**Figure 1.** Larvae viewed under the microscope following 1 hour on blue fly food.

Next, I determined whether the control flies could learn to associate an odour, either 4-methylcyclohexanol (MCH) or 3-octanol (OCT), with a sucrose reward in a T-maze assay (Figure 2). In *Drosophila,* individual clusters of dopaminergic neurons innervate the Mushroom Body, the fly's main memory centre, conveying reinforcing information about a variety of rewards (Cognigni, Felsenberg and Waddell, 2018). For example, different groups of dopaminergic neurons encode the short-term reinforcing sweetness or the long-term reinforcing nutritional value of sugars (Yamagata et al., 2015). Therefore, assaying learning and memory in adult flies will help determine any developmental defects of ethanol feeding at developmental stages where the brain is particually vulnerable. This proved to be successful in both immediate memory and long-term memory, 24 hours after learning, for the control flies indicating that the concentration of the odours and set up of the T-maze was adequate.

Whilst these preliminary learning and memory assays were being conducted, I collected eggs from wildtype flies to transfer them into food either supplemented with water as a control or different concentrations of ethanol (5%, 7%, 10% and 14%) to then use the adults

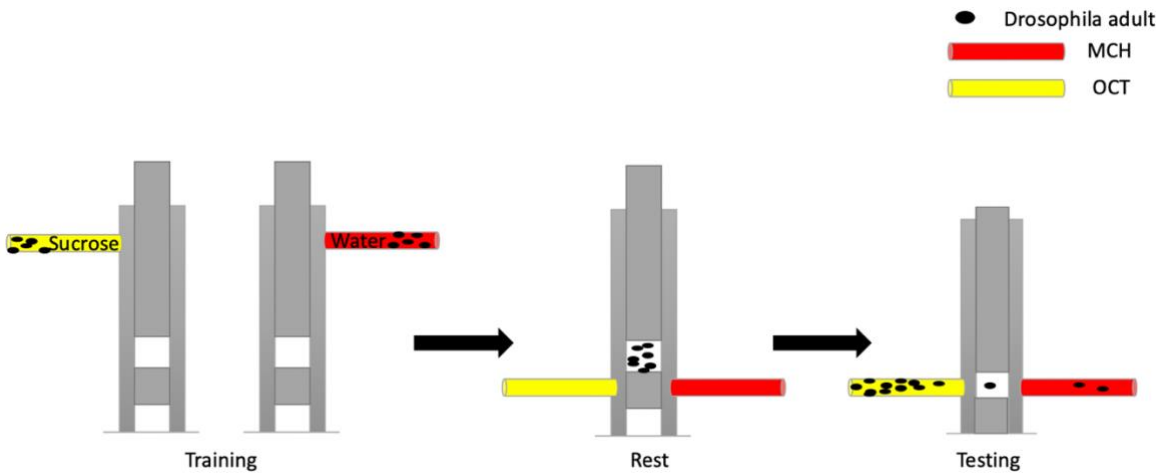



**Figure 2.** Diagram illustrating T-maze set up and protocol for appetitive learning.

in the learning and memory experiments. The control flies hatched within the standard time frame of 10 days when incubated at 25°C. However, the eggs on the food supplemented with ethanol at every concentration had a much greater developmental time frame, with the majority of flies still at the larval or pupa stage even after three weeks of incubation, resulting in very limited numbers of flies. Unfortunately, due to the unexpected apparent defects in development and increased developmental time I was unable to perform any learning and memory assays on the ethanol treated flies. However, this is something I hope to resume work on over the coming term in my free time.

I am extremely grateful for the opportunity that this studentship has given me and I would urge any other students to apply. I also wish to thank Vincent for all his help and support in undertaking this project, including directing me to appropriate papers to widen my understanding in this field. Although due to the nature of research, not everything goes to plan, it has still taught me valuable skills in experimental planning, new laboratory techniques and vastly increased my confidence working in research. It has confirmed my hope to apply for a career in research and to explore opportunities to study for a PhD.

***Abigail Stretch***

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## Dynamics of mesenchymal cell migration and proliferation in the chicken embryo

The research interest of the Headon group, and to which I had the opportunity to contribute, focus primarily on the behaviour of embryonic cells leading to the development of epithelial-mesenchymal organs. Within many of these organs, such as the skin and the intestine, are ordered substructures, such as feathers or villi, that develop in a periodically patterned matter in the embryo. Reciprocal interactions between the epithelium and the underlying mesenchyme are known to be the major driver of their emergence and pattern. My project focused on cell movement and aggregation in these tissues, mapping the orientation of cell divisions and measuring displacement of the resulting daughter cells, with the aim of understanding how specific cell distributions are attained in these tissues as they grow.

Through my project I worked on chicken embryos, an excellent model due to their ease of manipulation and the availability of appropriate transgenic reporter lines. Using membrane GFP transgenic embryos, I was able to track cell divisions in mesenchyme and epithelium from different embryonic stages, identifying mitotic events and orientations based on cell and nuclear shape. I learned how to use Fiji software, adopted to detect dividing cells and the orientation they take after separating. In addition, to better track the direction of individual daughter cells, their speed, and the plane over which they move after division, I utilised TAT-Cre driven random labelling of the Chameleon transgenic chicken line, followed by time lapse confocal imaging and tracking of cells using FIJI software.

I then decided to culture embryonic tissues to assess the roles of different signalling molecules in the development of the repetitive structures in the gut, utilising signalling inhibitors to block the activity of specific pathways. I found that culturing tissues gave me a deeper understanding the developmental phases that follow one another until the complete formation of the embryo.

During these months I had the chance to study animals, my central attraction, from a developmental point of view. Since one of my greatest aspirations for the future is to understand the cellular basis behind animals' variation, and the processes through which animal species differ so much in form even while utilising extremely similar developmental pathways, working with the Headon group gave me the opportunity to work on some of these fundamental processes and to understand that this career path is one that really interests me. Participating in this project constituted a major opportunity for my personal growth: I worked with an inspiring group of scientists in a world-renowned institute, and with their guidance I had the chance of taking ownership on a specific part of the project, actively and personally contributing to the team's research objective.

Through this internship I learned how to use specific experimental tools and ways of thinking about how organs are constructed. I had the opportunity to learn advanced techniques, as confocal microscopy, organ culture and manipulation, and microdissection, which I can apply in future research projects, especially since I would like to continue towards doing a PhD as a next step after graduation.

I would like to thank Dr. Denis Headon for welcoming me in a highly supportive and active environment, and Dr. Jon Riddell for helping and following me through the steps of the project with endless patience, giving me a great learning opportunity through which I applied research tools which will undoubtedly be useful for my future career path. A huge thanks also to the BSDB for making this research experience possible through the award of a Gurdon Studentship.

***Arianna Berbeglia***

**Host Lab and Project Background**

My summer project at the Sanson lab was the result of unfinished business. Having conducted my undergraduate final year lab project with them, I wanted to chase up some of the unexpected findings I'd happened across. The lab, headed by my PI Dr Bénédicte Sanson, uses a *Drosophila* model to investigate how physical forces and cell biomechanics affect morphology during development. My previous work involved analysis of *Drosophila* embryos during germband extension (GBE), a profound morphological change that occurs early in development. During GBE, several distinct cell behaviours contribute to elongation of the embryo, one of which is oriented cell divisions (OCD)[1]. Ectoderm cells preferably orient themselves to divide parallel with the embryos ventral midline (VML). One of the mutants we used for our investigation exhibited cell divisions that not only didn't display OCD, but some were completely out of the plane of the tissue. The literature suggested that this may be related to the apical area of the cells, in which a very small surface area may cause the cell to divide out of plane due to inability of the cell spindle to align appropriately in a vastly confined space[2]. Our results agreed; the affected mutants had cells with constricted apical surfaces, far smaller than the wild type (WT). For this project we produced the same

mutants, plus two additional mutants which we believed would exhibit some apically constricted cells, with the hope this would result in out of plane divisions to analyse.

All Things Practical

I was very fortunate to get to attend the lab in person. I was even more fortunate to have an amazing supervisor, Dr Jenny Evans, who helped me learn practical skills and plan the investigations we chose. My first day felt like a baptism by fire, and I was convinced that I'd never be able to pick up an embryo with forceps without squishing it. Many were lost. Fortunately, by the end of my eight-week project I could wrangle those embryos any which way I liked, and we had acquired some great movies. Said movies were taken on a confocal microscope with a spinning disk. We used transgenes for the fluorescent tagging of the embryos, with membrane protein fluorescing under a green laser, and myosin under red. The most difficult part was timing the movies correctly, to capture an adequate amount of cell divisions without photobleaching the embryo. The fly crosses we chose produced *Drosophila* embryos with mutations in the early anterior posterior patterning genes, which normally divide the embryo up into progressively narrower segments[3]. Our three chosen mutants were null for the gap genes Knirps and Hunchback ($KniHb^{-/-}$), Kruppel ($Kr^{-/-}$) and the pair-rule gene Evenskipped ($Eve^{-/-}$). To verify that the embryos were the mutants we though they were, Jenny and I conducted HCR (hybridisation chain reaction) experiments. This allowed us to visualise where specific RNAs were being expressed in the embryo. We didn't do this until after starting the imaging and analysis; one of the most valuable things I learned about lab work is that experiments mostly do not occur in the order that

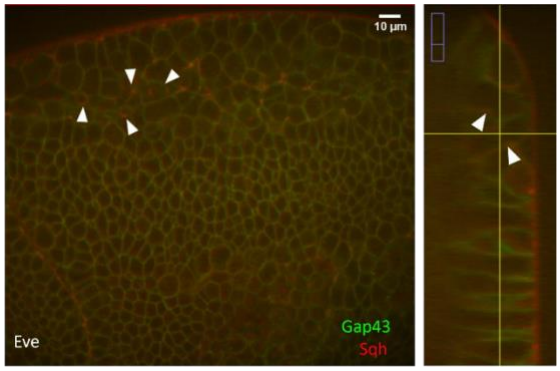


**Figure 1.** Confocal image of an $Eve^{-/-}$ embryo during GBE. Note the large, round cells around the midline (top) are actively dividing. Arrows mark out of plane divisions. Corresponding z plane to the right.

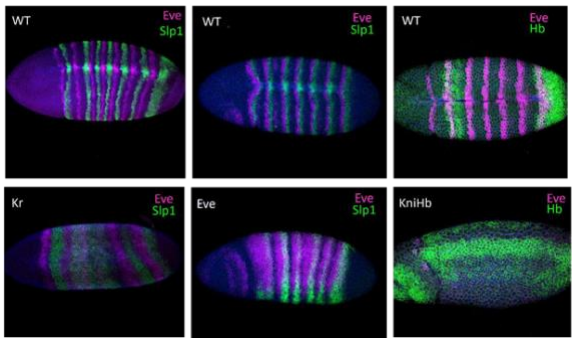


**Figure 2.** HCR experiment results; WT embryos (top) compared with KniHb (right), Eve (middle) and Kr (left) mutants. Note that the lab did not stock a Kr probe, so both probe sets used for the Kr experiment were for downstream genes.

seems most logical. For each mutant we chose two RNA probe sets; one for the gene/s we had supposedly knocked out, and one for a gene downstream which would have altered expression as a result. This is because in some cases it appears that the null protein is still being expressed, so to check that it is in fact a non-functioning protein (resulting in a null embryo) we check the downstream elements. For the $Kr^{-/-}$ and $Eve^{-/-}$ HCR I was forced to be independent as Jenny had to isolate, during which we were stuck communicating on Microsoft teams. I was fully prepared for my HCR to go wrong, but we were both really thrilled when it worked AND showed that we had produced the correct mutants. For the confocal movies, once they were captured I used the software Fiji[4] and Icy[5] to manually track and measure the midline ectoderm cells of the embryo over multiple parameters, as well as assessing the out of plane divisions.

Results, and More Questions

Our results were perplexing. This was the next most valuable thing I learned about lab work, you don't always get what you want (or in our case, what you expect). The KniHb$^{-/-}$ embryos, which had plenty of out of plane divisions in the movies taken before summer, seemed to have less than half that amount this time around. Furthermore, there was no difference between the apical area of the out of plane divisions and 'normal', planar divisions in KniHb$^{-/-}$. Fortunately, the other mutants had a staggering amount of out of

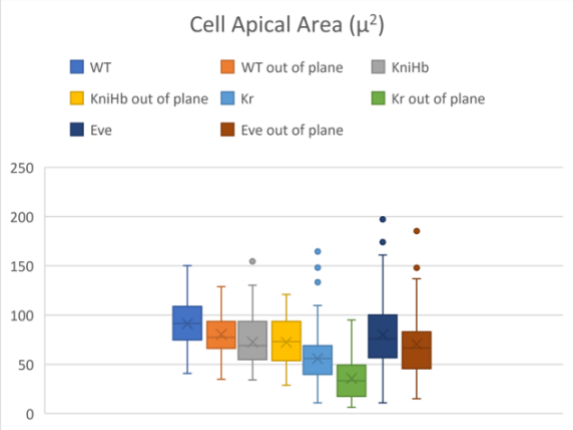


**Figure 3.** Box and whisker plots of cell apical areas. Note how the distributions show a smaller mean for almost all the out of plane divisions compared to the in plane divisions in flies of the same genotype.

plane divisions. We compared the WT flies and the mutants based on apical area at the end of interphase and found that in Kr$^{-/-}$ they were significantly smaller in both normal and out of plane divisions, with a similar finding to a lesser extend in Eve$^{-/-}$. I was keen to do some analysis of the cells in three dimensions, measuring their depth and angle to the surface at interphase and division for those dividing out of plane. We imaged to a depth of 40 microns but found that it was difficult to find the boundaries of the cell depth as the resolution seemed to decrease with depth. The next phases of this project will involve imaging the embryos with a more powerful microscope to overcome these limitations and allow us to collect more accurate data regarding cell depth.

I am extremely grateful to the BSDB for offering the Gurdon studentship which gave me the opportunity to conduct this project.

During my lab stay I learned a variety of practical skills, but more importantly how to design and troubleshoot experiments, all while working with some extremely skilled and knowledgeable colleagues; time just *flies* when you're having fun! Hopefully, this won't be my last time working with the Sanson lab, and in the near future I will be starting another image analysis based project which I feel far more prepared for thanks to this experience.

***Bridget Ryan***

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## Investigation into the role of DRAM2 on the autophagic activity of retinal pigment epithelium and retinal organoids

Being a student within STEM over the previous year and a half has been difficult, being unable to fully access labs and experience the degree to the full extent it was intended. The Biomedical department within Newcastle University has done very well in enabling some in person lab experience where other Universities and courses struggled due to the pandemic. In light of the situation, I believed I needed to acquire more experience, in the form of a summer placement. As well as wanting to practice more lab work, I wanted to experience working in a medical research setting to get an insight into the lifestyle and to understand how the natural progression of research unfolds.

The Retinal Stem Cell Research group led by Prof. Majlinda Lako in the Institute of Genetic Medicine at Newcastle University facilitated the exposure to medical research I had been awaiting. Their core research is to better understand inherited and age-related retinal disease to fight against blindness. Their research aims to determine which mechanisms within the retina and surrounding cells, retinal pigment epithelium (RPE), cause

disruptions to visions, then subsequently find ways to repair the damage by exploiting developmental, genetic, and cellular information. The lab use induced pluripotent stem cells (IPSC) which are derived from adult somatic cells that have been genetically reprogrammed to an embryonic stem cell-like state. These IPSC are able to differentiate into any type of adult cell and will grow indefinitely. This allows the lab to produce patient specific retinal organoids that are then being analysed, manipulated and have been used for successful corneal epithelial stem cell transplantation in 33 patients to date.

I worked alongside my supervisor, Rozaliya Tsikandelova, on her project investigating the role of DRAM2 on the autophagic activity of RPE and retinal organoids. This research is important as the autophagic pathway is a key homeostatic process where impaired cellular components are sequestered into autophagosomes that then go on to be degraded by lysosomes. Disruption to this system creates an accumulation of waste materials within the cell which has the potential to cause damage and cell death. This process is especially important within retinal cells as they are exposed to constant light, high oxygen utilization and high levels of lipid peroxidation. The effects of an impaired autophagic pathway has been linked to inflammatory, neurodegenerative, and age-related diseases including age related macular dystrophy (AMD). AMD is the commonest causes of blindness in the developed world and affects one in three people by age 75. There are two forms of AMD: 'dry' AMD and 'wet' AMD. Currently there is no treatment that exists for dry AMD so there is a huge un-met need to investigate into therapies for this disease.

The biallelic autophagy regulator DRAM2 results in the development of retinal dystrophy with early macular cone photoreceptor involvement. Patients with absent/ dysfunctional DRAM2 present loss of function of central vision during the third decade of life.

DRAM2 encodes a protein that's localizes in lysosomal membranes. DRAM2 initiates the conversion of endogenous LC3-I (microtubule-associated protein light chain 3) to the general autophagosome marker protein, LC3-II (LC3-1/phosphatidylethanolamine conjugate) during autophagosome formation. DRAM2 can interact with BECN1 complex to initiate phagophore formation in human macrophages. Furthermore, DRAM2 was shown to bind LAMP1 and LAMP2 which may be indicative of a role in autophagosome maturation.

Alongside experiencing experimental lab work I was tasked with the analysis and quantification of cellular structures from the control and CRISPR-Cas9 corrected IPSC lines collected from two patient's retinal organoids at day 220 of differentiation. The cellular structures I analysed included: autophagosomes, autolysosomes, lysosomes, mitochondria, and cristae. There were 4 groups; Group 1 (control) and Group 2 (CRISPR-Cas9 corrected) came from patient 1 who had no functioning DRAM2. Group 3 (control) and Group 4 (CRISPR-Cas9 corrected) came from patient 2 who had partial function of the DRAM2 gene. The data I produced was used to determine how the DRAM2 mutations effected the morphology and quantities of organelles involved in the autophagy pathway in the inner segment and cell body of the RPE cells. This was achieved by comparing the controlled cell lines to the patient specific corrected cell lines. I started my project using a software called Microscopy Image Browser which allows sensitive and accurate analysis of Transition electron microscopy (TEM) images. I analysed a total of 84 TEM images with varying magnification spanning the 4 groups producing large amounts of data. I began by determining what effects partial and full DRAM2 dysfunction had on mitochondrial morphology by assessing the form factor ((perimeter)/($4\pi$*surface area)) which indicates the complexity and branching aspect of mitochondria, and the aspect ratio ((major axis)/(minor axis)) which provides data on the circularity of mitochondria. Collecting data points from over 6,500 mitochondria. This was then used to produce comparable graphs with statistical analysis to determine the effects of DRAM2 on the morphology of mitochondria and how this could affect the autophagic activity of RPE cells and retinal organoids. After this analysis I began analysing the other organelles I was assigned. A highlight of the summer project was presenting my graphed data to Prof. Lako and discussing the results obtained with my supervisor. The most

exciting part of this whole project for me was knowing that this contribution will be presented within an upcoming research paper.

I am very lucky and thankful to have been awarded the BSDB Gurdon Studentship as it facilitated the insight into medical research that allowed me to truly gain so many new skills and experiences. I know that I am going to miss being a part of the friendly, intellectual environment within Prof. Lako's multidisciplinary team which made my summer so great. I highly recommend to any undergraduate student debating a summer research project to take the opportunity, as it will surprise you how much fun it is and the intensity of knowledge you will absorb.

***Daniel Singleton***

**My Summer Studentship Studying A Causative Gene of Primary Ciliary Dyskinesia**

To describe my summer studentship in words would only be reductive, as it surpassed all my expectations and beyond– and this is still an understatement. I pursued this research experience so strongly because I sought the confirmation that conducting research was what I wanted to pursue in life. However, I walk away from Professor Jarman's laboratory not only with an answer to my question, but having been enriched on so many different levels: I learned new laboratory techniques; I acquired greater knowledge in the field of research of my project; I was intellectually challenged and stimulated with questions that are yet to be answered; I strengthened skills and qualities I already possessed, such as the ability to communicate effectively or work independently which are so important in science. Moreover, this opportunity allowed me to witness first-hand what working in a research laboratory entails. It is a job that does not cease when you step foot outside the laboratory doors, but one that embraces your everyday life. It is a job that requires determination and hard work, but most of all, persistence and patience. A researcher spruces passion from every pore. It is a job that seeks curious minds and driven characters; researchers do not resign when faced with obstacles since it is exactly this, the consciousness of 'not knowing' and seeking understanding that is at the core of what motivates them. This I learned.

Alongside Professor Jarman and his group, I dedicated two months to a research project on Primary Ciliary Dyskinesia (PCD). PCD is an inherited disease characterized by the failure of cilia (hair-like projections) to 'beat.' As a result, babies and children have recurring lung infections, situs inversus, and are infertile if they survive long enough. More than forty genes cause PCD when mutated; they either encode the components of the dynein motor protein complexes that form the axoneme, or encode the proteins which build these complexes during cell

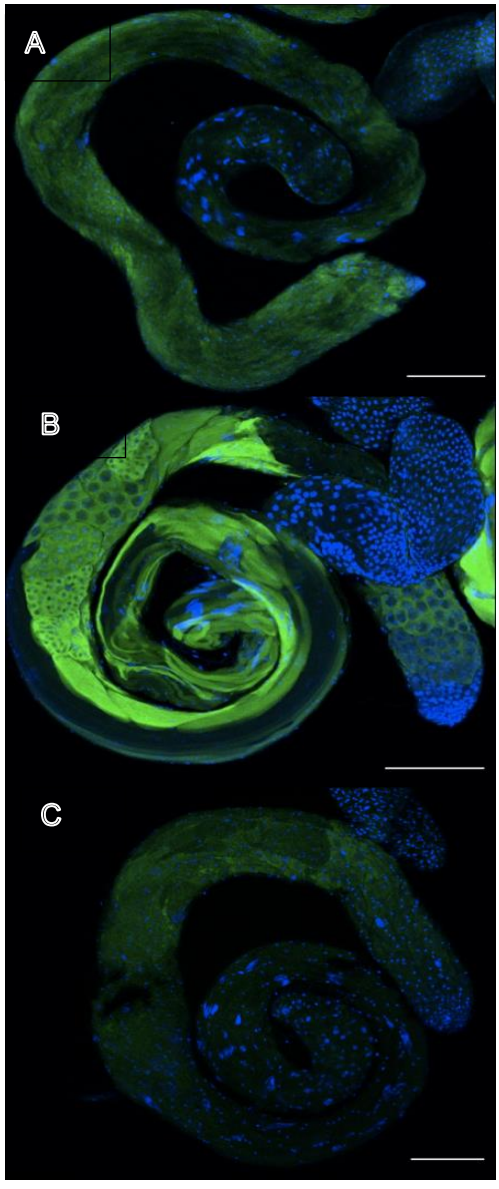


**Figure 1. Ttc12 Knockout *Drosophila* testes stained for an Inner Dynein Arm.** (A) The negative control (OreR) shows uniform staining. (B) The inner dynein arm in the wildtype testes fluoresces strongest in the

spermatocytes and the sperm bundles. This is the positive control. (C) The inner dynein arm does not fluoresce strongly in the spermatocytes and the sperm bundles in the Ttc12 knockout flies. The nuclei are stained in blue (DAPI) and the scale is 100microns.

differentiation. One of these genes is TTC12, a recently discovered PCD causative gene whose function is yet to be determined. Hence, the aim of my summer studentship was to investigate the function of Ttc12, the equivalent gene in *Drosophila melanogaster* (fruit fly).

The fruit fly is a compelling model organism for genetic analysis of PCD because it only has two ciliated cell types: the sensory neurons and sperm. Indeed, this experimental model has been successful in determining the function of other PCD causative genes. My project consisted of dissecting fly testes and conducting immunostainings. First, we identified a suitable staining technique for *Drosophila* testes. Then, we used the confocal microscope to observe whether the localization of different axonemal components was affected by the gene knockout. For example, we stained specific inner and outer dynein arms since their mislocalisation or loss (Fig.1), resulting in a disorganised axoneme, is a phenotype of PCD. Unfortunately, when we checked the fly stocks with PCR, we realized that they were not all correct. This revelation put a stall on the research project as new fly stocks had to be generated. While we began doing that, we decided to identify other molecular markers (different axonemal components) that could be tested for in the Ttc12 knockout flies. We were successful in this.

Although we were unable to test our hypothesis that Ttc12 is a co-chaperone during motile protein assembly in motile cilia to the extent that we hoped, we did not let the fly stocks stall further research. With more time, I am certain the team would have advanced in understanding. In the future, if the hypothesis is validated, chaperone therapy could be a potential treatment for patients with PCD. Helping in the pursuit of cures that will ameliorate patient lives is one of the main reasons researching these diseases is so fundamental and motivating. I am delighted to have contributed even only a little.

I want to thank Professor Jarman, Petra zur Lage and the entire research team. It is thanks to you that I walk into my final year of University with greater confidence, knowledge, and experience that will lead me to greater success in my research project and future studies. Thank you BSDB for funding my studentship, I am extremely grateful.

***Eleonora Scalia***

## Establishment of an *in vitro* system to test the Domestication Syndrome hypothesis

Traits associated with animal domestication, such as reduced fight or flight responses, smaller brains, shorter skulls, and floppy ears are associated with tissues derived from neural crest cells (NCCs) (Larson and Fuller 2014). This has led to formulation of the “NCC domestication syndrome hypothesis” in which genetic changes in NCCs are proposed to underpin domestication (Wilkins et al. 2014). NCCs are difficult to obtain from embryos but can be derived in vitro from induced pluripotent stem cells (iPSCs) (Prescott et al. 2015). The Burdon group has produced iPSCs from domesticated pigs and wild boar (both *Sus scrofa*), as well as another wild porcine species, the red river hog (*Potamochoerus porcus*). Preliminary experiments in the Roslin labs using the protocol outlined in Prescott et al. 2015, indicate that NCCs can be derived from all 3 types of pigs.

The main goals of my project were:
1. To characterize the iPSC-NCC differentiation process by imaging and RNA expression analysis
2. To assess the differentiation potential of the NCCs I created
and
3. To optimize the differentiation protocol by testing different seeding densities.

Following the procedure in Prescott (2015), I plated each of the iPSC lines in neural induction media (N2B27 + FGF + EGF) to encourage differentiation. The cells aggregated to form embryoid bodies (EBs), which then plated down, and cells began to migrate out of them. At day 15, I dissected the EBs off the plate, leaving behind the migratory cells that are thought to represent NCCs. At

day 21, I removed the growth factors from the media to further encourage NCC differentiation. Whereas differentiated red river hog cells survived throughout the procedure, only a few wild boar EBs plated down and by day 21 had produced few migratory cells. The domesticated pig migratory cells did not survive differentiation after day 21.

As seen in **Figure 1**, I imaged the iPSCs during their differentiation and analyzed expression of a variety of genetic markers by RT-qPCR. These genetic markers were for NCCs, Cranial NCCs, trunk NCCs, and pluripotency markers. The pluripotency marker *REX1* was significantly downregulated, consistent with the onset of iPSC differentiation. Expression of NCC markers such as *ALX4*, *TFAP2a* and *SOX9* was upregulated over the course of the differentiation. Cranial NCC marker *DLX4* and trunk NCC marker *HOXA1* were expressed consistently at relatively low levels over the course of the differentiation, giving little information on the NCC-type that had been created.

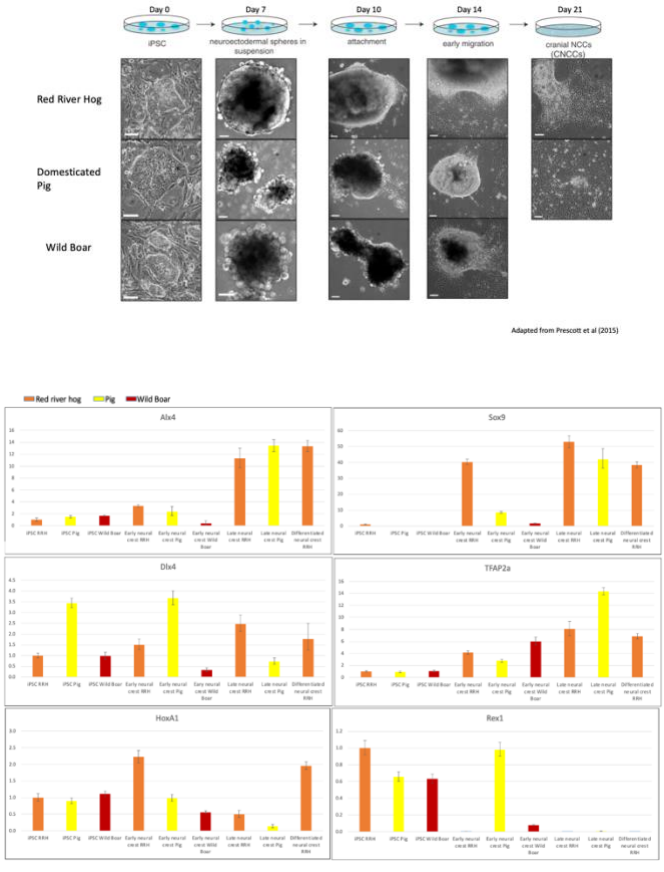


**Figure 1.** (A) Time course by photos of the different cell lines. At day 0 the media was changed from stem cell media to NIM (N2B27 + FGF + EGF) to force the cells to differentiate. They then formed embryoid bodies (EBs) as seen on day 7, and then plated down and cells began to migrate out (day 10 and 14) until the EBs were dissected off the plate leaving behind the neural crest-like cells. (B) RT-qPCR results of some of the analyzed genes at T0 (iPSCs, day 0), T1 (early neural crest-like, day 10), T2 (late neural crest-like, day 21) and T3 (differentiated neural crest-like cells, day 32). *ALX4*, *TFAP2a* and *SOX9* are known NCC markers. *DLX4* is a CNCC marker. *HOXA1* is a trunk NCC marker. *REX1* is a pluripotency marker.

I also tested the optimal seeding density for making neural crest cells by using a 96-well plate to make specific sized EBs. I made 2000, 40000, and 6000 cell EBs, with the best density being 4000 cell EBs. At this density, the EBs were large enough to develop and plate down efficiently, but not so large that they became inefficient at making NCCs. My last days in the lab were spent doing antibody staining on my remaining red river hog cells. We stained cultures with the Tuj1 antibody recognizing BetaIII tubulin to identify neurons. Based on staining and cell morphology there were neurons on the plate. To stain for a NCC marker, we chose *SOX9*, which, from the RT-qPCR results, should have been present in the cells. We also stained with DAPI to visualize the cell nuclei. Although neurons were present, no *SOX9* was detected (which would have shown up as green). This is likely because the level of *SOX9* expression was too low or that the antibodies did not work against the red river hog *SOX9*.

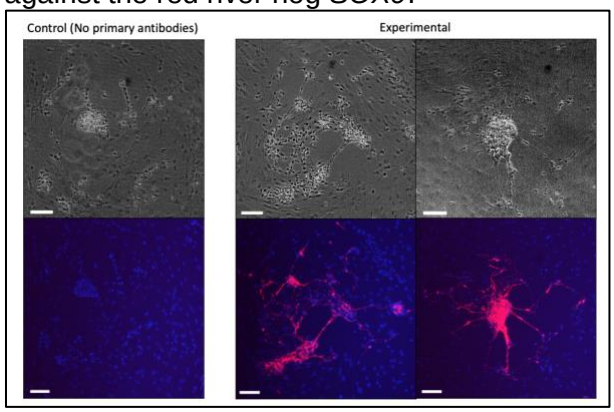


**Figure 2.** Day 32 post-differentiation red river hog cells, fixed and stained for nuclei (blue) and neurons (red). Also stained for Sox9, however there is no visible staining seen on the plates.

Based on the data collected I believe I was successful in making neural crest cells. However, there was insufficient data to

classify them as either cranial or trunk neural crest as all the markers for both were expressed at low, relatively unchanging levels throughout the course of the experiment. Given more time I would have liked to determine specifically which type of neural crest cell we were making. I would have also worked to optimize the pig and wild boar cell lines, as neither survived to the end of the experiment. For the pig cells, some of the procedures were likely too harsh, so there was too much cell death which led to a density that was too low for the cells to survive. For the wild boar, the specific clone line we used seemed to be the problem, so I would have liked to repeat the experiment on another clonal line. Despite these difficulties and remaining unknowns, I thoroughly enjoyed this opportunity to work with the Burdon and Schoenebeck groups on this project. They have provided me with valuable research experience that I can apply both in and out of the lab. I am so grateful for both the labs and the Gurdon studentship for making this project possible.

***Elizabeth Krull***

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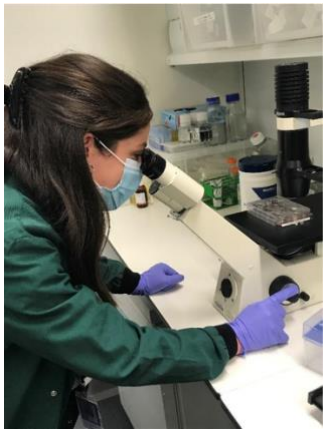
**Investigating the role of protein stability in developmental tempo at the Crick.**

Throughout my undergraduate degree in Developmental Biology I have become progressively more amazed at how fundamental an understanding of development is to the life sciences and biological research in general. Having spent my final year in lockdown and missing out on a summer graduation, a studentship facilitated by the British Society of Developmental Biology felt like the perfect way to soothe those wounds and spend some time in an exciting laboratory instead.

The Briscoe lab at The Francis Crick Institute wants to understand how the right number of cells develop at the right time and in the right place. Different species use closely related processes and molecular regulation to generate tissues, but often develop at markedly different tempos. The Developmental Dynamics laboratory utilises the well characterised development of the neural tube where distinct transcription factor expression patterns generate discrete classes of cells throughout the neural tube. The spatial and temporal control of gene expression is critical for normal development. When comparing the generation of motor neurons, one of the spinal cord neuronal types, human motor neurons take about twice as long to develop than mouse motor neurons. Previous work from the Briscoe lab found that human proteins are about twice as stable as their murine counterparts, and this can explain the similar difference in developmental timing (Rayon et al., 2020). Therefore, investigating protein stability in developing systems will contribute to the understanding of temporal control of development.

For my final year project in my undergraduate degree I had the opportunity to research protein stability during human pancreas development in the Papalopulu lab at the University of Manchester. As we found that the human protein we studied was about 2.5 times more stable than its murine counterpart, I thought a summer internship in the Briscoe lab would be the perfect way to build on my previous experience. During my 8 weeks in the Briscoe lab we continued some work done by a previous PhD student, Lorena Garcia Perez, into protein stability of the transcription factors IRX3 and OLIG2. *Irx3* is expressed along the dorsal-ventral axis of the neural tube. As the extent and duration of Shh signalling increases from the ventral region, *Olig2* expression is then induced. IRX3 represses *Olig2*



transcription whilst OLIG2 represses *Irx3* transcription, resulting in the mutually exclusive expression domains in space and time. Despite the cross-repressive interaction between IRX3 and OLIG2, how protein production and degradation interplay to control protein expression levels remains unclear.

Previously, Lorena had edited the IRX3 locus in mouse stem cells by inserting an HA and HaloTag by CRISPR/Cas9 gene editing to produce the IRX3-HA-HaloTag fusion protein suitable for antibody staining and manipulating protein stability. HaloTags are small enzymes that form a covalent bond with a specific HaloTag ligand like Janelia Fluorescent ligands (e.g. JF549 and JF646) and PROTACs that label the protein of interest upon ligand and PROTAC addition. A PROTAC is a proteolysis-targeting chimera that targets the fusion protein for proteasomal degradation via the recruitment of ubiquitination machinery, thus reducing the stability of the protein of interest (Buckley et al., 2015). Throughout the studentship, we performed several differentiations from mES cells to motor neurons following established protocols (Gouti et al., 2014). Conditions were subsequently changed to manipulate protein stability via addition of different concentrations of PROTAC to the differentiation media. We found that both 0.25μM and 1μM PROTAC was sufficient to reduce the stability of the IRX3 protein and accelerate the appearance of OLIG2 expression, further highlighting the role of protein stability in developmental tempo. We expected the levels of OLIG2 in the PROTAC treated cells to increase due to the reduction in IRX3-mediated repression. Interestingly, we found that the PROTAC treated cells had lower levels of OLIG2 than the control, suggesting that without IRX3 repression less OLIG2 is required to facilitate progression to the next phase of motor neuron differentiation.

Having previously measured protein stability in the Papalopulu lab by tagging the protein of interest with Dendra2, a photoconvertible fluorescent protein, and subsequent timelapse imaging with a confocal microscope, I was able to try a new method of quantifying protein half-life during my summer stay at the Briscoe lab. We took advantage of HaloTag technology and added the fluorescent JF ligand JF549 to the differentiation media for an hour to label all fusion protein present as a 'pulse'. Performing flow cytometry on cell collections throughout a timecourse, the 'chase', we quantified the reduction in fluorescence over time and consistently got similar results as had been previously calculated. This makes highly reproducible data and gives us confidence in our half-life value. Expanding on this pulse- chase experiment, we added another JF ligand (JF646) after the initial pulse to follow production of IRX3 fusion protein. These data will be used to quantify the production of IRX3 over time once degradation and saturation of the system have been taken into account.

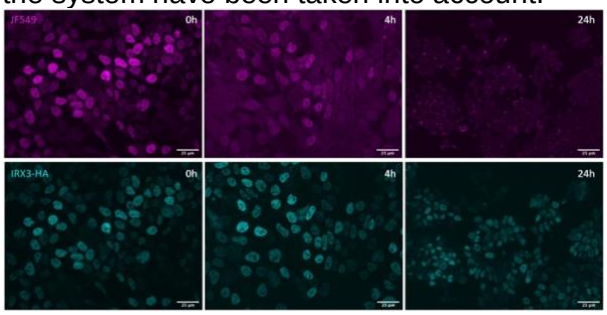



**Figure 1.** confocal microscopy images of degradation of JF549-labelled IRX3 protein over time (top) and of total IRX3-HA protein throughout (bottom). Initial distribution of IRX3 protein visualised using both JF549 and IRX3-HA fluorescence, where stronger JF549 fluorescence correlates with stronger IRX-HA fluorescence. Time is time after 1-hour 100nM JF549 pulse. Scale bar = 25μM

It has been a privilege to spend the last 8 weeks in the Briscoe lab. I have learnt incredible amounts, not just about the science itself but also about how the science world works. Everyone in the lab was unbelievably friendly and welcoming and I quickly felt a part of the lab. Having lunch together, morning coffees and the occasional drink at the end of the day in the canteen bar enabled me to integrate well and talk to all the lab members about their research and how they got there. I really can't fault the experience and feel extremely grateful to have been able to spend time around brilliant scientists like so many of those at the Crick. I am so pleased to have applied and to have been awarded the studentship. This experience will be invaluable for preparing me for whatever comes next, and I am so excited to see what interesting projects I'll be able to be a part of in the future.

***Florence Woods***

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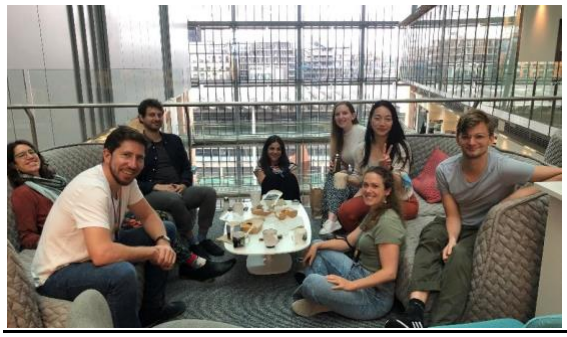

## Uncovering the Regenerative Capacity of *Araneus diadematus*

Regeneration is the mechanism by which a species can restore damaged or missing cells, tissues, organs or body parts. Different living organisms have vastly differing regenerative capabilities. Humans, while capable of regenerating some organs following damage or disease, most notably the liver, are very limited in their capacity. Other organisms, however, have remarkable regenerative capacities that facilitate re-growth of entire limbs and even parts of nervous systems, such as species of hydras or planarians.

Despite the relative prevalence of regeneration and the potential contributions of its research to modern medicine, the field is widely understudied.

This summer, the BDSB Summer Studentship gave me the opportunity to undertake an exciting research project, studying the regenerative abilities of the European Garden Spider, *Araneus diadematus,* within the McGregor Laboratory at Oxford Brookes, under the supervision of post-doc and spider expert, Anna Schönauer.

Fritz Volrath's (1990) paper outlines the remarkable ability of *A. diadematus* (Figure 1), to regenerate almost immediately functional legs, following loss**,** at the coxa trochanter joint (Figure 2). This functionality is crucial to their survival**,** as the spiders rely on the limbs' sensory-motor abilities for weaving their geometrically complex webs, which are intricately designed to facilitate prey capture (Reed *et al* 1965). Outside of Volrath's work, *A. diadematus* is an under-represented species within scientific research**,** despite its practical and scientific suitability as a model organism.

**Figure 1.** An Overview of a Fourth Instar Female *Araneus diadematus*, with Regenerated, Left First Walking Leg

**Figure 2.**The Coxa-Trochanter Joint of the Left First Walking Leg of a Fourth Instar Female

The aim of my project was to improve understanding of the regeneration of the first walking legs in the European Garden Spider (*Araneus diadematus*). Initially my objectives comprised:

1. creating a time series to document the species' post-embryonic development
2. comparing the regeneration of the first walking legs of two subsets of individuals within two separate instars
3. analysing the leg transcriptome of an *A. diademtus* leg, to compare with the pre-documented leg transcriptome of species unable to regenerate lost limbs, *Parasteatoda tepidarioum*.

Due to impediments imposed by Covid19, in addition to further challenges thrown up by the unpredictable nature of scientific research, the outcomes and overall procedure of my project ultimately deviated significantly from the parameters of my original aims. Covid regulations limited my lab access early enough in the year, and as a result, I was unable to accurately document the beginning of the time series for the species, obstructing my first aim. Nevertheless, this flexibility became an advantage and ended up being crucial in facilitating a level of adaptability, which made room for discoveries and inspired further investigations. The project was initially intended to last for eight weeks, but due to the interesting findings derived from the investigation of my second aim, the project was extended.

**Figure 3.** The Regenerated Left, First Walking Leg of a Third Instar Female, 21 Days (and 1 molt) After Leg Loss

The findings from the second objective, analyzing and comparing the regeneration and emergent legs (Figure 3) of individuals within the third and fourth instar, prompted a secondary investigation, examining the effects and implications of the leg regeneration of individuals within the fifth instar. Throughout these analyses, I thoroughly enjoyed the weekly imaging and recording of my experimental subjects' regenerative development, but nothing quite compared to the excitement of discovering the emergence of a new regenerated leg on my visits to the lab's spider room first thing in the morning.

The final objective (obtaining, analyzing, and comparing the *A. diadematus* leg transcriptome) is still ongoing and I am learning a lot about the patience required for bioninformatics!

Regeneration is understood to be an ancestral trait in arachnid species (Goss, 1992) and although some lineages have lost this ability (Vollrath, 1990), the evolutionary relationships giving rise to these differences remain unclear. Research into the development of closely related spider species, with and without regenerative capabilities, offers potential insight into the changes that have led to the loss of this trait. Further, as outlined in Karl Ernst von Baer's Laws of Embryology (Wanninger, 2015), studying the early development of a species offers essential contributions to the uncovering of evolutionary patterns and relationships of characteristics.

Acquisition of *A. diadematus* embryos, contributed an additional branch to the project. Embryos were frozen at different times in development, prior to being peeled, DAPI stained, and microscopically imaged (Figure 4).

The examination and comparison of the imaged *A. diadematus* embryos, with pre-existing images of the *P. tepidarioum* (Mittman and Wolff 2012) presents the opportunity to identify significant disparities within the development of the two species, with the potential to propose relevant evolutionary relationships of regeneration, signposted through embryonic development.

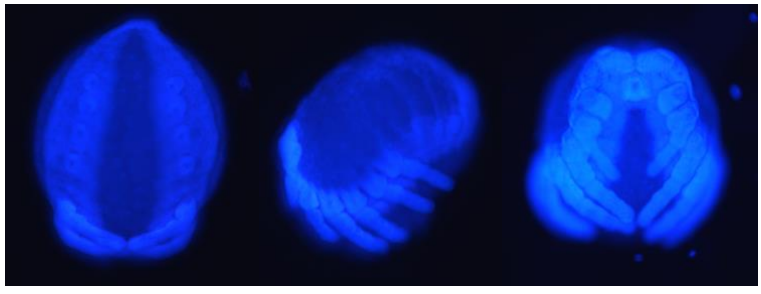

**Figure 4.** The Prosomal, Lateral and Frontal View (left to right) of a DAPI Stained *Araneus diadematus* embryo

My experiences in the lab have emphasized the fluid and unpredictable nature of scientific study, reinforcing the importance of patience, open mindedness, and flexibility. The Gurdon Summer Studentship has taught me so much that I would not otherwise have had access to in the ordinary course of my undergraduate program. I've been involved in lab meetings and journal clubs, worked alongside an amazing group of PhD students, and gained experience using cutting-edge equipment and techniques. I've developed lab skills, learned specialized spider husbandry techniques, and grown as a scientist, thanks to the lab team I have been privileged to be a small part of.

I did not underestimate the incredible opportunity being afforded to me and was excited before the project started, but I could not have imagined just how much I would enjoy the process. The unpredictable nature of the field of developmental biology has made for an exciting summer and I owe an enormous debt of gratitude to the BDSB. Further I am beyond grateful to Alistair McGregor and to Anna Schönauer for this incredible opportunity and for their support, encouragement, and training. Their passion for their subject is infectious and would inspire anyone to study further in the field of developmental biology!

***Georgia Henry***


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## Unravelling the genetic regulatory mechanisms behind cardiovascular development

Epithelial to mesenchymal transition (EMT) is an endogenous process that mediates embryonic development in a wide array of tissues, including the epicardium, the outermost layer of the heart. Epicardium-derived cells (EPDCs) undergo EMT, giving rise to diverse cardiovascular cell types and paracrine signals that are essential to normal development and growth of the heart, namely the formation of the coronary vasculature, valvulogenesis, and myocardium maturation (see Figure 1).[1] Once cardiovascular development is completed, EMT is silenced, and the epicardium remains dormant in adulthood. However, this process is reactivated is response to ischemic injury such as following myocardial infarction, in an attempt to replenish the degenerated cardiomyocytes. The extent to which EMT is activated following ischemia is, nevertheless, insufficient in promoting survival or restoring the original cardiomyocyte population and, therefore, most of the damaged cardiac muscle is replaced by non-contractile scar tissue, in a process termed fibrosis.[2] In fact, up to a billion cardiomyocytes can be loss during myocardial infarction, meanwhile EMT is only capable of regenerating roughly a few hundred cardiomyocytes.[3] The transcriptional regulation and signalling pathways that mediate epicardial EMT remain poorly understood. The objective of the Vieira lab is, therefore, to enhance our knowledge on the genetic regulatory mechanisms underpinning EMT, which will hopefully aid us in unravelling the regenerative capacity of the epicardium and identifying novel molecular targets for therapeutic cardiovascular repair.

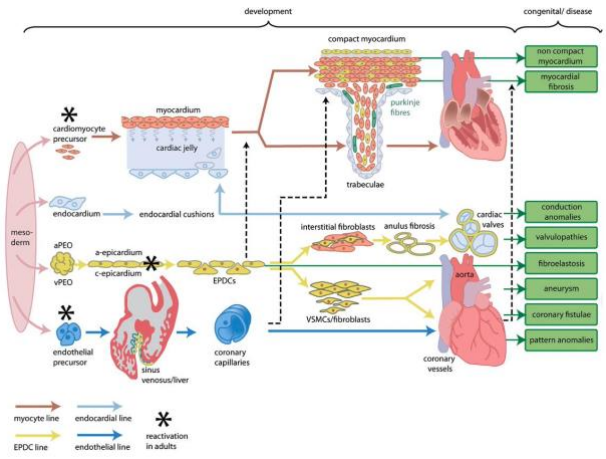

**Figure 1.** Schematic presentation of the cellular contribution to heart development with special focus on the role of the epicardium and epicardium derived cells (EPDCs) during normal development, disease and repair processes. Four mesodermal cell lines (cardiomyocytes, endocardium, epicardium, and endothelium) are considered to form the main building blocks of the heart. The differentiation of each line is depicted together with the main interactions with the other cell lines. The most frequent EPDC-related congenital malformations and (acquired) disease processes are boxed in green, while three cardiac (stem) cell populations, including EPDCs, that may become reactivated following ischemic injury are indicated by asterisks.[1]

This summer, I had the invaluable opportunity of partaking in this research at the Department of Physiology, Anatomy and Genetics in Oxford. I am very grateful to my supervisor, Dr Joaquim Vieira, who gave me this opportunity and despite his very busy schedule, allowed me to perform and learn numerous scientific techniques ranging from RNA isolation, cDNA synthesis, quantitative PCR, immunostaining, data analysis and visualisation (3D heart modelling), and even dissections of mouse embryonic hearts. Furthermore, I also had the opportunity to shadow during confocal microscopy imaging as well as High Resolution Episcopic Microscopy (HREM) studies, which was used to generate stacks of images capturing thin tissue slices containing the entire mouse foetal heart at embryonic day (E) 15.5. These images were subsequently processed to generate 3D heart models (below).

Within the scope of this project, I was primarily involved in phenotyping knockout mouse models that are carrying a deletion of enhancers located in intron 1 of the *Wilms' tumour 1* (*Wt1*) locus. These knockout mouse models were recently generated via CRISPR/Cas9 technology. It has been established by loss-of-function models that the transcription factor Wt1 determines the cell fate of EPDCs and hence, underpins the process of epicardial EMT. However, further upstream regulatory mechanisms underlying the activation of *Wt1* as well as the downstream transcriptional targets of Wt1 remain elusive. An initial characterisation of mutant embryos at E15.5 by HREM had been earlier performed in the Vieira lab. This phenotypic characterisation revealed that mutant embryos had thinner myocardial walls and that the development of cardiac vasculature was impaired, which is representative of disrupted epicardial EMT (Vieira et al., *unpublished*). I then performed data visualisation (3D modelling), processing and analysis of further HREM samples using a software platform called AMIRA so that all mouse models were assessed in terms of heart development (See Figures 2A-B). The rationale behind 3D modelling was to compare the volumes of each of the heart compartments including their vascular components (e.g., remodelling of the transverse aortic arch), cardiac valves (atrioventricular and semilunar valves), and ventricle and atrial chambers between control and mutant knockout mouse models. In addition, the analysis of HREM datasets with AMIRA enabled the identification of malformations such as abnormally shaped/sized valve leaflets or interventricular septal defects (See Figure 3), which are commonly observed in congenital

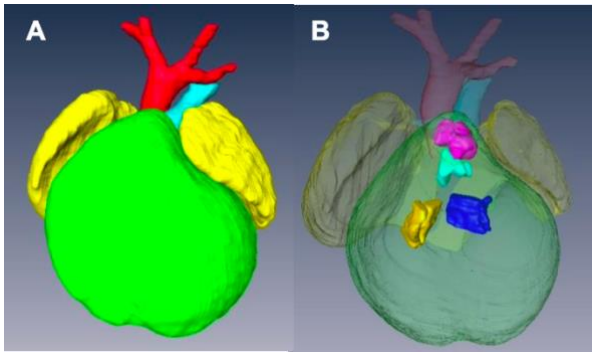


**Figure 2.** A-B – Representative 3D embryonic mouse heart model that was constructed using HREM samples. (A) External view showing the ventricles (green), atria (yellow), aorta (red) and pulmonary artery (light blue). (B) Internal view showing the aortic valve (pink), pulmonary valve (light blue), tricuspid valve (orange) and mitral valve (dark blue).

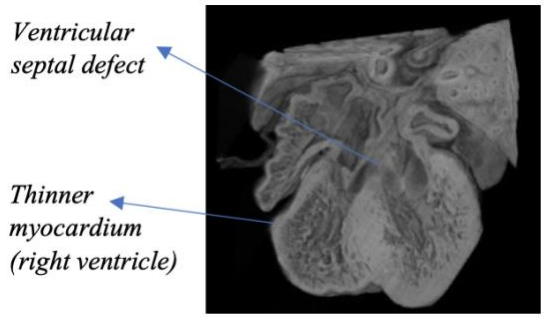


**Figure 3.** Showing a mutant heart with a thinner myocardium and ventricular septal defect (VSD).

Throughout the course of this internship, I was immersed for the first

time in the field of developmental biology with a focus on cardiovascular development. As heart development was not a topic that was covered in the first year of my undergraduate course, this
internship encouraged me to undertake my own personal research and learn beyond the scope of my course. In essence, it allowed to gain a further insight into the practical aspects of biomedical research that were not explored in my undergraduate course. One of the most exciting elements of this internship was performing dissections of embryonic mouse hearts at E13.5 and E16.5, and mouse embryos at E9.5 (See Figures 4A-D) using a stereomicroscope and thus, being able to personally observe the developmental trajectory of the mouse embryo. Likewise, I was able to observe the morphological events that take place during the development of the heart from E9.5 - the linear heart tube undergoing looping morphogenesis - until E16.5 – the formation of a fully developed, stereotypical four chambered organ (See Figure 5).

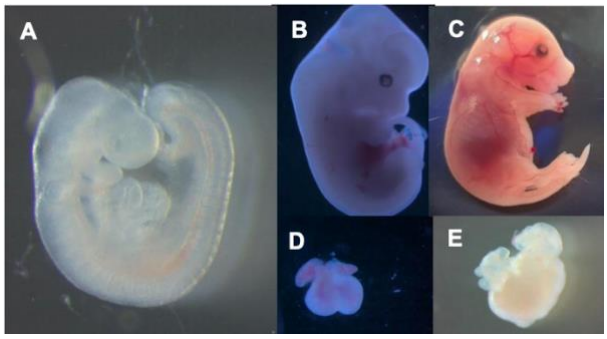


**Figure 4**. A-D Representative images of dissected mouse embryos at E9.5 (A), E13.5 (B), and E16.5 (C) and dissected mouse embryonic hearts at E13.5 (D) and E16.5 (E).

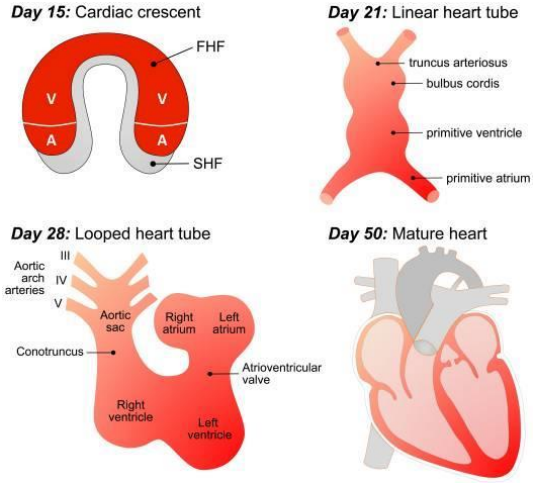


**Figure 5.** Depicting the developmental trajectory of a human heart.[4]

As a first-year student reading Biomedical Sciences, I am naturally fascinated by how our understanding of the molecular mechanisms behind human physiology can be applied in the treatment of disease. Some of my favourite lectures in the first year were on cardiovascular physiology and pharmacology, namely on positive inotropic drugs that are used to treat congestive heart failure, which can arise from conditions such as myocardial infarction. The idea of being able to pharmacologically modulate an endogenous biological process to mediate cardiovascular repair was very exhilarating and hence, it fuelled my desire to undertake this internship. Likewise, I genuinely enjoyed learning about the science behind cardiovascular repair and its implications on biomedical research and I was delighted that I could make a personal contribution to such research.

I would, therefore, strongly recommend undertaking a research internship via the Gurdon studentship scheme to anyone with an interest in developmental biology as well as to anyone, who is considering undertaking a PhD and wishes the explore biological/biomedical research in a laboratory setting beyond the scope of their undergraduate course.

***Karolina Zvonickova***

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## My glimpse into systems biology: Studying bipotent posterior progenitors in Cichlids

I was introduced to the ideas of systems biology during my first year of Natural Sciences at Cambridge University. The interplay between modelling and data collection was very appealing to me. Thanks to one of my supervisors - Tim Fulton (also a PhD student in the Steventon Lab, University of Cambridge)- I was exposed to it in the context of developmental biology. He helped me get in touch with Dr Berta Verd whose interdisciplinary approach to research enticed me. We talked a lot and came up together with my project. Due to Covid we had to modify it to include modelling, but I found it more rewarding that way!

I researched Neuromesodermal progenitor cells (NMps). They are a very interesting population of cells, persisting beyond gastrulation to generate both mesodermal and neural fates in the late embryo. This progenitor state is characterised by coexpression of two transcription factors - Brachyury (Tbxta) and Sox2 (Henrique et al. 2015). There are inter-specific differences in their proliferation dynamics - in chick and mouse embryos they proliferate, but in zebrafish they do not (Steventon and Martinez Arias 2017). This suggests they might be tuneable during the evolution of different axial elongation patterns (Sambasivan and Steventon 2021). My project was part of a larger effort in the Verd lab to see whether this might explain axial diversity observed in Lake Malawi cichlids.

I studied the NMps in *Astatotilapia calliptera* and *Rhamphochromis chillingali* - two species of cichlids from the Lake Malawi flock. They underwent a recent radial adaptation around a million years ago. Remarkably, the genetic differences between these species are very small (between 0.1-0.25% inter-specific divergence), while the morphological differences are immense (Malinsky et al. 2018). In particular, the vertebral count differs between those 2 species, making them a very suitable experimental system for studying the evolution of axial elongation.

I did *in situ* hybridisations of the fish embryos at various stages, with the help of Shannon Taylor - a PhD student in the Verd Lab. We used Hybridisation Chain Reaction v3.0 (Choi et al. 2018). Despite our best efforts we did not manage to get Sox2 to work in the tailbud. This meant I could not quantify NMps as one of the crucial markers was missing. This was a very humbling experience - experimental biology is much more capricious than I had thought. However, it also showed me what cooperation between labs can look like. Tim did a lot of HCR staining in zebrafish at the Steventon lab in Cambridge and so we asked him for advice. It turned out it took them a few months to get Sox2 working! This was very reassuring. Tim gave us some tips, but we only managed to try some of them. Figure 1. contains some of the best images we obtained.

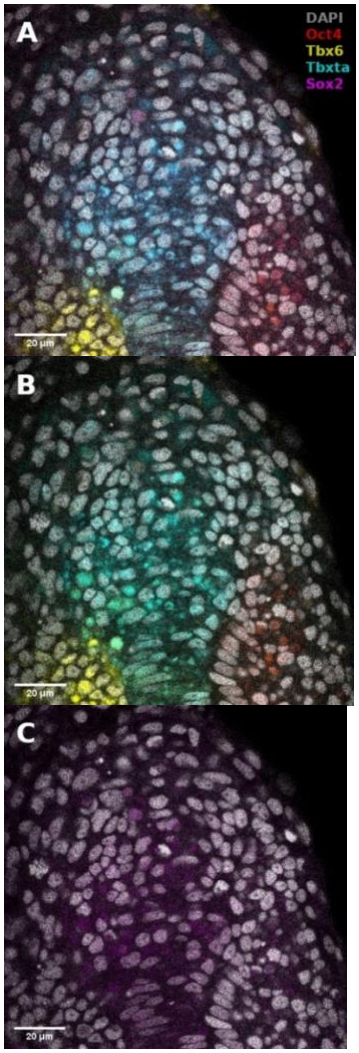


**Figure 1.** Dorsal view of a mid somitogenesis stage Astatotilapia calliptera tailbud. Top – posterior, bottom – anterior. A - all genes visible, B - only Oct4, Tbxta and Tbx6 visible, C - only Sox2 visible. Note that all the genes except for Sox2 localise mostly to the nuclei, indicating that Sox2 staining did not work.

In parallel to the experimental work, I investigated the effect of blebbistatin - a myosin II inhibitor - on somitogenesis and axial elongation in zebrafish. In collaboration with the Steventon lab I analysed almost 100 timelapses of zebrafish embryos in order

to determine if the rate of somitogenesis is influenced by blebbistatin. My analysis showed that it is not changed (Figure 2.). Other experiments with dye injection and cell tracking from the Steventon lab showed that the tail still elongates, but with limited cell mixing. This got us curious, how is that possible?

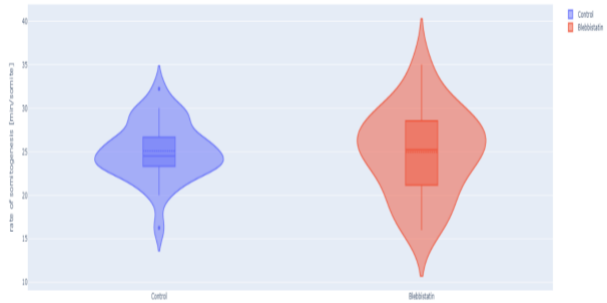


**Figure 2.** Violin plots of the rate of somitogenesis in minutes per somite in the control embryos (blue) and blebbistatin treated ones (red). Control n = 31, Blebbistatin n = 28.

In order to help address that problem I developed a conceptual model of the zebrafish Presomitic Mesoderm (PSM) elongation. I approximated the PSM as a uniform tissue in the form of a cut-off cone. To recreate the convergence-extension mechanism responsible for axial elongation in the zebrafish tail (Thomson et al 2021; Tada and Heisenberg 2012) I gave the cells two rules for movement: they have to stay a certain range of distances apart from each other, keeping the tissues continuous and preventing cells from occupying the same space; and the cells converge towards the x-axis, mimicking the convergence movements. I found that certain combinations of parameter values indeed lead to elongation without extensive mixing, which shows that – in agreement with our experimental observations - mixing itself seems to not be required for elongation but might rather be a side effect of a certain mode of elongation (Figure 3.).

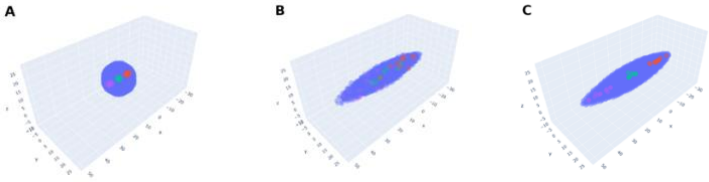


**Figure 3.** Modelling results (here starting from a sphere). The coloured spheres highlight the cell mixing. A - the starting shape, B - shape after 100 iterations, C - shape after 500 iterations, 5 times slower movement than in B.

This was my first time being fully immersed in the lab. I actively took part in the lab meetings and journal clubs which were just as edifying as research itself! Overall, this

was an incredible experience. It showed me that experimental biology is unpredictable and the relationship between results and time invested is non-linear. In contrast, computational biology has a much more linear relationship, almost always yielding something interesting! It also gives you the space to learn and think about underlying biological processes, how to recreate them *in silico*, consolidating your knowledge. This probably furthered my understanding of development the most! I am adamant I want to incorporate both experimental and computational approaches in my future research. I also gained much more understanding and appreciation of developmental biology and I want to specialise in it. I want to thank Berta, Tim, Shannon, Charlotte, Georgina, James, and Callum for welcoming me into their lab and helping me with the project, as well as BSDB for funding it.

***Maciej Żurowski***

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## Canine Somatic Cell Reprogramming and Its Facilitation through Inhibition of Endogenous p53

Numerous efforts have been made to establish *bona fide* iPSCs from companion animals such dogs and cats. Generation of iPSCs from companion animals would provide useful unrestricted cell resources with a vast scientific potential. To name a few applications, they can be exploited as new models for regenerative medicine and as therapeutic veterinary tools to replace tissues; in veterinary pharmacology for drug development assays, and to elucidate function(s) of genetic variants that are associated with disease.

While protocols for producing human and mouse iPSCs are established, protocols for derivation of iPSCs from domestic animals are slowly developing due to difficulties encountered presumably in their reprogramming process. Only a few studies have indeed focused on the possibility of producing iPSCs from these companion species, and despite some describing their production, the burden of proof is largely lacking.

As an undergraduate student at the University of Edinburgh, this summer despite the current pandemic situation making it harder to find a lab-based studentship, I was lucky enough to have the opportunity to work in Drs. Schoenebeck's and Burdon's labs. The Gurdon/BSDB award allowed me to spend two months at Roslin Institute, a pioneering center for genetics and stem cell studies, collaborating with research groups with extensive experience in stem cell research (Dr. Tom Burdon's) and canine genetics and genomics (Dr. Jeffrey Schoenebeck's). During my time at Roslin I contributed to the research of an efficient protocol to derive canine iPSCs, supervised by the joint effort of these two excellent lab groups.

Based on the findings that iPSC generation is enhanced by *P53* suppression and replacement of *L-MYC* with *C-MYC* (Okita et al., 2011) in the set of conventional reprogramming factors (OCT4, SOX2, KLF4, and *C*-MYC, collectively termed "OSKM" factors); recently, Yoshimatsu et al. (2021) have presented a study which provides insights on the possibility to facilitate canine cell reprogramming. They provided evidence

of reprogramming somatic fibroblasts from a canine using an integration-free method. Their 8 episomal (**Figure 1**) vectors contain the OSKM factors including *L-MYC*, other pluripotency genes (*LIN28* and *NANOG*), genes that have been shown to facilitate reprogramming (*GLIS1* and *KDM4*), and a dominant-negative form of the mouse *TRP53* (*mP53DD*), which was shown to suppress endogenous *P53* expression in human cells, and presumably should operate the same in canines.

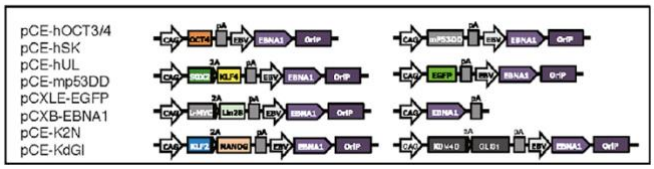


**Figure 1.** Schematic of the plasmid mixture used for vector transfection. (Adapted from Yoshimatsu et al., 2021)

culturing techniques and practiced such skills on mouse feeder cells, I expanded canine fibroblast from testis in feeder medium prior to transfection of the EBV-based vectors. I then electroporated such cells with 2 different mixtures of vectors, one consisting of the 8 plasmids including the dominant-negative *P53* (*+mP53DD*), and the other without it, consisting of 7 plasmids (*-mP3DD*). Right after transfection the medium used to feed the cells was changed to M10. Since one of the transfected vectors carried *EGFP*, I took GFP imaging to directly assess if the transfection was successful, comparing the transfected fibroblast with the non-transfected control (NTC). Images (**Figure 2**) show a high extent of cell death following electroporation of the cells, while GFP expression in a high proportion of the survived cells indicate uptake of the vectors. Cell recovered and showed prolonged GFP expression until day 14.

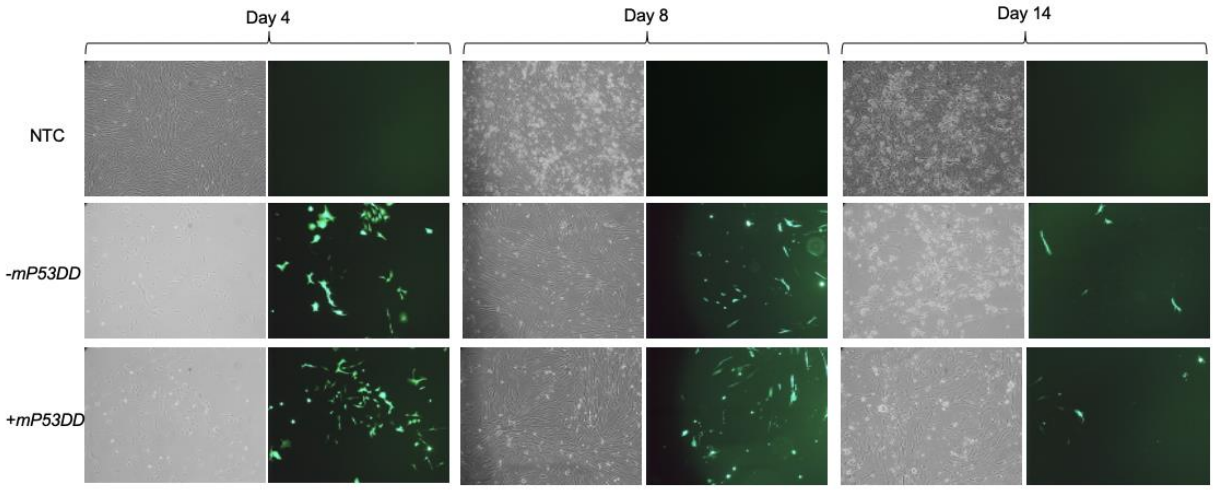


**Figure 2.** Bright field (left) and GFP (right) images of canine fibroblast at days 4, 8, and 14 after transfection.

The episomal vectors contain OriP/EBNA1 sequences derived from Epstein-Barr virus (EBV), which ensure the stable extrachromosomal replication of the vectors, hence high expression of the reprogramming factors carried along, which facilitate the production of iPSC. However, the full applicability of this EBV-based system is still unclear as only two dogs were used to prove its actual functionality.

The aim of my project was to assess the ability of the aforementioned system to reprogram canine fibroblasts, testing the capability of facilitating reprogramming by the inclusion of dominant-negative *P53*. After being introduced to the fundamental cell

Following pre-expansion for 8 days after transfection, fibroblasts were transferred onto STO feeder cells and changed medium with NSM for induction of iPCS colonies. During reprogramming I sampled cells periodically to harvest their RNA (days 4, 8, and 14). From such RNA samples I obtained cDNAs that I used to perform subsequent RT-qPCR analyses. Using canine specific primers - some of which I personally designed and formerly validated - for endogenous expression of pluripotency markers (*POU5F1*, *NANOG*, and *SOX2*) and other genes of interest (*CDH1*, *CD44*, *CDNK1A*), I was able to assess the reprogramming status of the cells during the process.

Changes in expression of two markers of reprogramming (*CDH1* and *CD44*) was consistent with what shown in another study (O'Malley et al., 2013). *CDH1* (E-cadherin) is upregulated (**Figure 3A**) indicating mesenchymal-to-epithelial transition which is a typical behavior of the cells entering reprogramming. Furthermore, consistently with O'Malley et al. (2013) *CD44* was found to be upregulated at day 4 and progressively downregulated passing the time (**Figure 3B**), the final population of iPSC are expected to be indeed *CD44*$^{-}$.

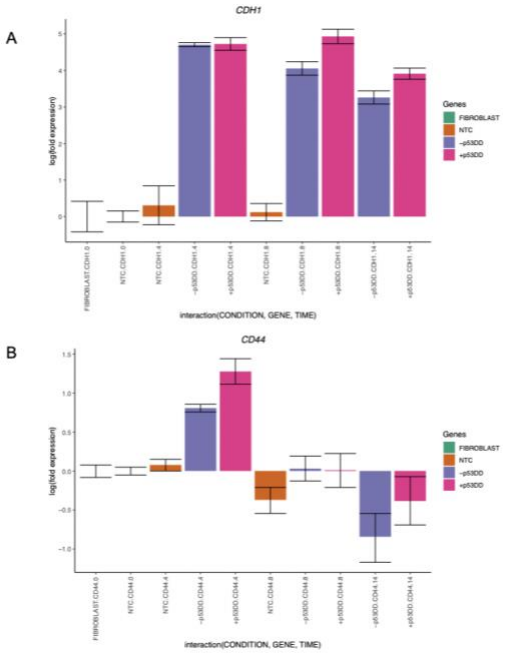


**Figure 3.**

Unfortunately, I was not able to identify any iPSC colony by day 14, as showed in the Yoshimatsu et al. (2021) study, or later in time under either condition (+/- *mP53DD*). Upregulation of the core pluripotency markers *POU5F1*, *NANOG*, and *SOX2* during the experiment, demonstrate the ability of the used EBV-based vector system to induce endogenous expression of pluripotency genes in canine cells; however, such expression dissipates throughout time (**Figure 4**). The reason I could not obtain any iPSC colony might be that this vector system was shown to not maintain sustained enough endogenous expression of the pluripotent genes to overcome the full barrier of reprogramming.

The two vector mixtures +/- *mP53DD* did not show distinguishable effects, since *CDNK1A*, direct target of *P53* showed no difference in expression levels between the two conditions (**Figure 5**). This suggest that either *mP53DD* was expressed at not effective levels or not at all, or that this dominant-negative form of *P53* do not interact with the canine form of *P53*.

Ultimately, this project surely helped to broaden my knowledge in stem cells and reprograming methods, as to learn numerous lab techniques fundamental to pursue hopefully my research career in the future.

***Noah Candeli***

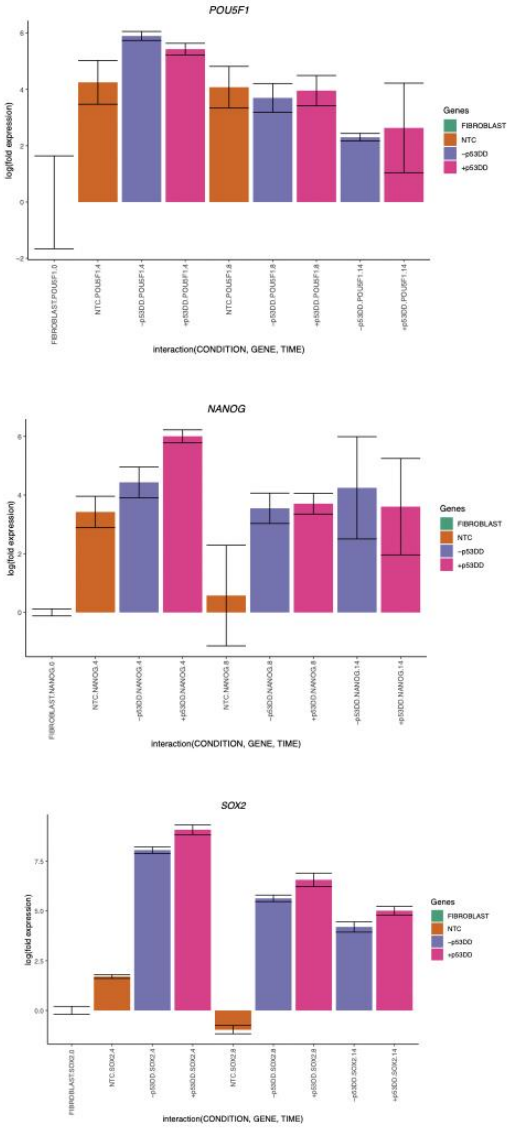


**Figure 4.**

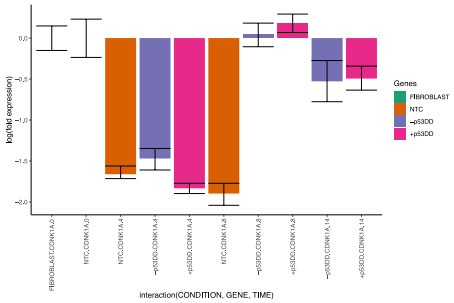


**Figure 5.**

**References:**

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## Does wnt promote the switch from cardiac to skeletal muscle programme?

Biomedical research tries to understand among other things, how during development, gene expression determines cell fates. One of the aspects that we look at is how cells are recruited to the heart and how to apply this knowledge to cardiac therapy. However, cell fate decisions in the head mesoderm, the tissue responsible for delivering the heart, craniofacial muscle, parts of the skull and vasculature, are poorly understood.

This Summer, I had the opportunity to work with Dr. Susanne Dietrich, who studies the formation of muscles in early development, including, but not only, genes responsible for committing cells into a mesodermal fate. I was part of ongoing research addressing this question, at her lab in Portsmouth.

The Dietrich lab has shown that initially, the entire head mesoderm has cardiac competence. However, at early neurula stages of development, the cardiac inducer Bmp2 fails to induce the cardiac programme and instead, it induces Msc, a craniofacial precursor marker. It is not yet clear how this switch in developmental competence is achieved.

We hypothesize that Wnt may be responsible for the switch, inducing an early expression of craniofacial precursor markers and downregulating cardiac markers.

To test this idea, I grafted heparin-coated acrylic beads soaked in recombinant Wnt3a, the Wnt inhibitor Sfrp2 and Bmp2 or bovine serum albumin as control, into HH7/8 embryos. Embryos were cultured for 6 hours, the time sufficient for Bmp to induce Msc. I then used *In situ hybridization* to analyse the expression of Msc, the cardiac marker Nkx2.5 and the Wnt responsive genes Pax3 and Axin2.

We found that Wnt did not upregulate and Sfrp2 did not downregulate Pax3 and Axin2 (data not shown), probably because it takes more than 6 hours to change the expression of these genes. However, Wnt3a did downregulate Nkx2.5 as expected (Fig.1). Nonetheless, Wnt did not upregulate Msc (Fig.1), suggesting that the concentration used and the 6-hour time period might not have been enough for Wnt to participate in the activation of Msc. This was against our hypothesis, and we wondered why that might be. So, we decided to test the effect of Wnt on the paraxial head mesoderm marker Cyp26C1, an inhibitor of retinoic acid signalling. We found that Cyp26C1 was suppressed (Fig.1). Thus, Wnt may in fact suppress paraxial head mesoderm features.

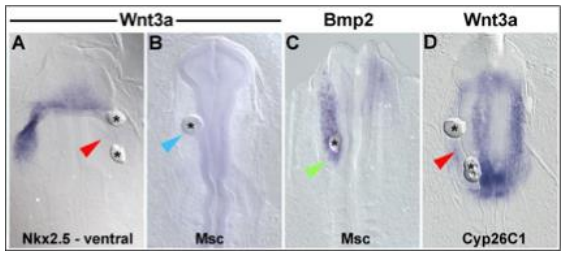


**Figure 1.** Dorsal view of whole chicken embryos at developmental stages HH7/8 (A, B and C) and stage HH9/10 (D). Wnt3a strongly downregulated Nkx2.5 (A) but did not upregulated Msc (B). Cyp26C1 was downregulated (D), suggesting a possible suppression of head mesodermal/rostral features.

I am now faced with new questions: What really is the role of Wnt? Does it suppress heart and paraxial mesodermal features because it posteriorizes the tissue? To answer this question, I will have to analyse if Wnt causes an ectopic expression of posterior information markers (e.g: Raldh2

and Hoxb1). And our original question is not answered: what facilitated the switch from cardiac to skeletal muscle competence? I am intrigued by these results, and I do want to find out the right mechanism that causes this switch. I will be working on this project throughout the next academic year, looking at different embryonic stages, different concentrations of Wnt, different Wnt inhibitors (e.g.: Dkk) and possibly, longer culture periods. I am hoping to find results that tell us if Wnt signalling is or not responsible.

If Wnt is not involved, what else could it be? Many other signalling cascades converge on the head mesoderm, and they could be tested using similar approaches. Alternatively, I could use small molecule inhibitors of signalling cascades on embryos cultured as Cornish pasties. We also have to consider that the epigenetic landscape might change over time, and cardiac genes might be put out of use. This would require a different approach, chromatin immunoprecipitation. I would love to learn about chromatin immunoprecipitation and work on this approach during my master's or PhD.

I want to continue working on these questions with Dr. Susanne throughout the next years of my academic life. I hope that with my 3rd year module "genes and development" I will gain more insight into new experimental methods used in developmental biology and maybe use them as an approach in my project. I would love to work with different model organisms and upgrade my knowledge with new techniques that may facilitate the research.

Working with Dr. Susanne and her team made me grow as a scientist. I remember the week before starting on my project I was so nervous I even had nightmares about it. But the people in the lab were very kind and helpful and they made me feel at home. During the summer, I was faced with some of the ups and downs of science. *In situs* that did not work, embryos that were accidentally lost, beads not sticking, and all of that (Particularly the last one), allowed me to develop my problem-solving skills and patience (especially while grafting beads). Being part of a research group made me realize that I do not see myself doing anything else. I love planning my experiments and I love the practical part. I am also very interested in presenting and explaining my results to other people.

I look forward to continue working on developmental biology throughout my studies. I plan on continue my education with Dr. Susanne, working on finding the mechanism behind this cardiac to skeletal muscle switch, and other projects.

***Petra Mendes Vieira***

**Mechanical regulation of cell division in developing tissues: Speed Vs Strength**

During embryogenesis, dynamic mechanical forces act on developing tissues, inducing cellular mechano-responses. These changes in cellular behaviours such as cell division, adhesion, and motility are a vital aspect of tissue morphogenesis and homeostasis.

This summer, I was given the opportunity to work under Dr. Woolner at the University of Manchester's Division of Cell Matrix Biology & Regenerative Medicine. Using a multidisciplinary approach, Dr. Woolner's lab examines the cellular response of developing tissues to an applied mechanical force and seeks to identify the underlying molecular basis. This placement was an incredible and unique opportunity for me, as I was able to receive training and experience in a variety of new techniques used in biomechanics, mathematics, biomodelling and developmental biology. Previous work by the Woolner lab demonstrated that the rate of cell division increases in epithelial cells following the application of a low-magnitude, uniaxial tensile force[1]. Work in other systems has shown that similar mechanically-induced increases in proliferation occur due to upregulation of the ERK1/2 pathway downstream of the stretch-activated

**Figure 1.** Selecting embryos at 2-cell stage for mRNA microinjection

calcium channel Piezo1, culminating in an upregulation of cyclin B[2]. Additionally, it is known that the orientation of cell division aligns with the axis of stretch[1].

However, all current studies investigating the cellular response to tensile force involve rapid, instantaneous tissue stretching. Under physiological conditions, changes to mechanical tension in the developing embryo occur over a period of minutes to hours rather than seconds. In tumourigenesis, mechanical changes may take place over years. It is not currently known how cell division rate differs between fast and slow stretch regimes. Preliminary work suggests that slow-stretch regimes may not elicit the same division responses that are seen with instantaneous stretching.

My project aimed to help shed light on whether the speed or strength of an applied mechanical force is the major factor in altering cell division rate.

Using a tissue stretching apparatus, we applied an instantaneous, uniaxial stretch with reduced strength to tissues. For these experiments, *Xenopus laevis* embryonic tissue was used. *Xenopus laevis* embryos are a robust model organism for use in biomechanical research as they are large, develop externally and are easily visualised. I was very grateful for the opportunity to shadow members of the lab working with the *Xenopus* colony throughout the project. They are a unique model animal (I also have a few as pets!) and it was great to see how they are cared for and used responsibly in a research setting.

In order to visualise the cell edge and nucleus, *Xenopus* embryos were injected at 2-cell stage with GFP-tubulin and Cherry-histone RNA. Straight away I was given the chance to jump in and get involved with the experiments, as I helped Gina (the Woolner Lab's Research Assistant) with DNA miniprep and mRNA preparation. We proceeded with microinjection, which involved inserting a microscopic needle tip into each cell under an optical microscope. This was a very tricky procedure at first but by practicing alongside Gina, I was eventually able to go from struggling to inject 10 embryos in an hour to injecting over 50 in half the time!

Following overnight incubation, embryos were staged at early gastrula and the animal caps were dissected. Isolated animal cap explants are a versatile tissue able to survive and develop *ex-vivo*, making them ideal for live imaging. Dissecting the animal cap was done through an optical microscope using two sets of forceps. This was the most technically challenging aspect of my lab work, as it required a steady hand and patience but couldn't be done too slowly or the embryos would become too developed. It was very rewarding to eventually get a perfect set of animal cap explants.

Following incubation on a fibronectin-coated silicone membrane, the animal caps were stretched and imaged. Shown in Figure 2 is a single frame from one of our live movies captured using confocal fluorescence microscopy. This was great experience, as imaging science was always of great interest to me but I had never previously had the chance to put my theoretical knowledge into practice. I also used image analysis software to calculate the mitotic index, as well as try cell population tracing. The Woolner lab uses tracing alongside vertex modelling[3,4] to measure cell shape and infer mechanical stress across the tissue. The data collected during my project will be used to determine whether an increased cell division rate acts to relieve tensile stress across the tissue.

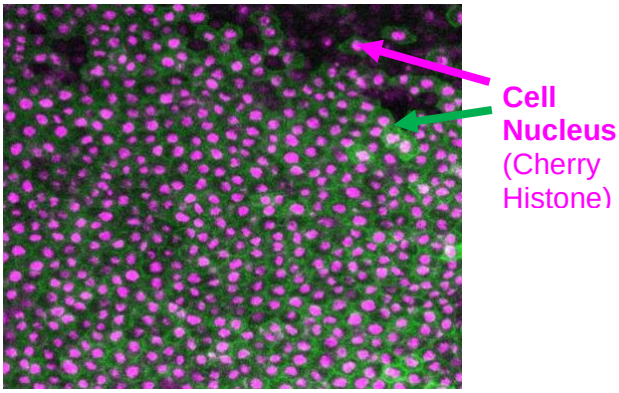


**Figure 2.** Fluorescent image of a Xenopus embryo animal cap explant experiencing a uniaxial stretch.

Visualisation of the cell nucleus (magenta) and cell edge (green) allows image analysis techniques and cell population tracing to be performed. This was performed to calculate the mitotic index and biophysical properties of the tissue.

Alongside my core project work, I also successfully titrated the CDK-1 inhibitor RO-3306 to find the optimal concentration for cell division inhibition in *Xenopus* embryos. It is currently known that mechanical tension may

increase cell division in fast-stretch regimes by promoting G1 to S phase transition[5], which the Woolner lab will be investigating in slow-stretch regimes using a Fucci probe coupled with RO-3306 inhbition.

Towards the end of my studentship, I was really grateful to have the opportunity to attend the 18th International Xenopus Conference. This was a great chance to discover the wide array of biomedical research using *Xenopus* currently being conducted worldwide and make valuable connections.

**Figure 3.** *Xenopus laevis* produce large, externally developing embryos which are easy to collect, visualise and manipulate.

These properties make them particularly suitable for tissue stretch experiments.

I would like to thank everyone for all their support, guidance, patience and coffee & cake sessions throughout the internship. I am very grateful that I was able to receive the Gurdon/BSDB Summer Studentship and would recommend any student interested in developmental biology research to apply. Gaining first-hand lab experience in this field has given me invaluable skills and insight and has opened many doors for my future career.

***Stephanie Leadbitter***

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## What I Learned from Making a Website and a YouTube Channel for the Lab

I recently finished a summer project in a neurobiology lab. Contrary to popular belief, an internship in a science lab does not necessarily mean operating high-tech machines wearing a lab coat. My project, for example, is for science outreach.
Nowadays, more and more researchers are realising the importance of public outreach, as science itself becomes less of an exclusive domain for scientists, and more of public interest. My project, I believe, is among the first few outreach projects that have successfully drawn funding. It is kindly sponsored by the BSDB Gurdon/The Company of Biologists Summer Studentships.

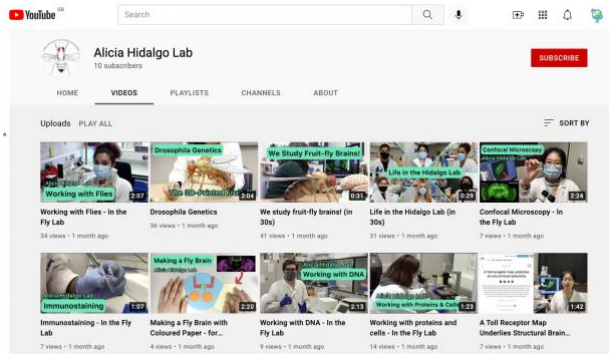
### The Project

I want to become a serious academic one day, but I enjoy gossiping about science so much that I just couldn't help seeking out ways to spread my passion for it. So after finishing my BSc dissertation project in the Alicia Hidalgo Lab in the School of Life Sciences, University of Birmingham, I stayed over the summer to help with the outreach.

Professor Alicia Hidalgo and I on Graduation Day

For this project, I designed a website for the lab and produced 20 educational videos about

research done at the bench, featuring people in the lab.
The videos were published on a YouTube channel we set up for the lab and got over 500 views in the first month. They show details and the rationale behind the research projects they've been working on. The topics include why study fruit flies, summaries of recent research papers by the lab, and how neuroscientists work with DNA, proteins, cells, and lab animals.

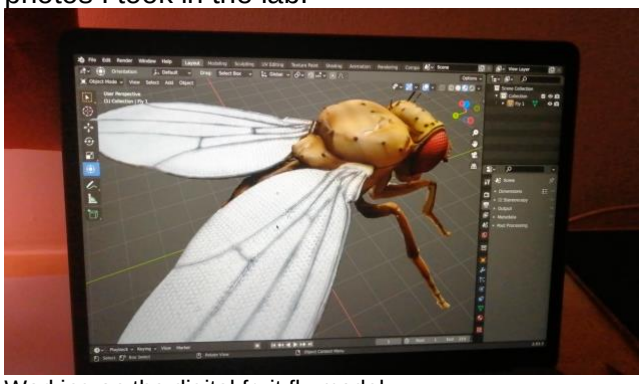


The lab's YouTube Channel with 20 videos I made

As for the lab website, it's primarily a summary of the lab and its work over the years, with a strong emphasis on education and science outreach. We have embedded videos, clickable 3D objects, and other interactive elements for interested people around the world to explore.


Alicia and the 3D-printed fruit fly

**What I've Learned**
When I first started, I was as clueless as you probably are right now about how to make videos and websites. Over the two months, however, I taught myself the necessary skills, including making animations, building a website with WordPress, and filming educational videos in a lay-people-friendly way.
Towards the end of the project, I had another idea. A friend of mine created a digital 3D object using 2D photos of a fossil, as an assignment in class. So I asked her for advice and set out learning how to do it myself. A week later, I created a clickable digital 3D model of the fruit fly *Drosophila*, using 600 photos I took in the lab.


Working on the digital fruit fly model

Having just graduated, I was reflecting on my uni experience throughout the summer, and doing the outreach project helped me figure it out.
I used to think that knowledge is what I'd gain from higher education, but despite getting a First Class Honours degree, I could barely recall 20% of my class notes. Apparently, detailed knowledge isn't the most important thing I gained in these four years. Then what is? While doing this project, I realised that there are three key things I've learned.

**1) - Insight: life in the lab.**
During my four-year degree, I worked on three research projects and got an inside look at the workings of research labs. I got to learn about collaborations, group dynamics, and stories of people at various stages of their academic careers. This leads me to an MRes degree in Experimental Neuroscience, (very likely) a PhD and beyond.
**2) - How to learn something from the scratch by myself.**
The ability to learn anything using the internet is a crucial skill that got me through uni and especially this project. With information overflow versus a limited lifetime, we need to quickly locate what we need from the Internet and master the necessary skills. Being able to teach ourselves anything unleashes a lot of potentials, and opens up many opportunities we never thought possible.
**3) - How to communicate with different audiences.**

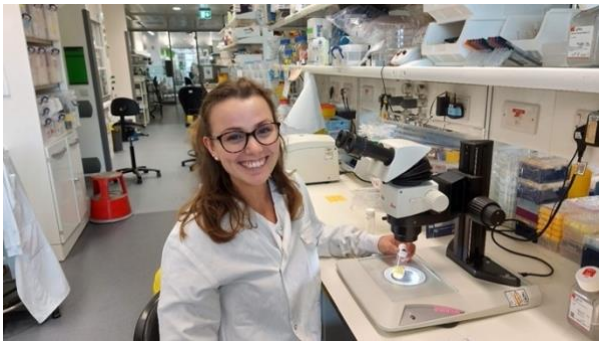
Communication, be it verbal or non-verbal, online or face-to-face, is a transferable skill across all careers. Learning how to present and promote one's work online is arguably the most important skill in the modern age. By learning to promote the lab's work online, I realised that I don't need to be a professional YouTuber or influencer to get my voice heard. So I started gossiping about things I'm passionate about - neuroscience, productivity hacks - to people around me. During my summer project, I started blogging, made a personal website, and even started two podcasts with friends! Instead of passively interact with my phone when I'm bored, I now initiate deep conversations with people and share what I've learned with others around the world.

How can this information help *you*, a fellow *scientist*?
Well, for starters, no matter which career stage you're in, face this reality - if your work isn't online, it doesn't exist. Digital journals have made it easier for us to share our work, but we also need to promote our research in other ways like seminars and conferences. Successful scientists seek to actively promote their work by taking part in interviews, which are published as articles, videos, or podcasts. Never before has there been so many brilliant ways to promote our work, but never before has it been so difficult to compete with other voices to make sure that ours get heard. Creating a lab website and a YouTube channel will be a good start.

**What Next?**
The insights and skills I gained this summer are invaluable. As I'm starting my Master's degree, I want to continue blogging, try making more outreach stuff for future labs, and I'm considering a career in academia with teaching elements. Maybe I'll be a professor, or a public influencer, or both, to inspire curious minds around the world.
Finally, I want to thank the Alicia Hidalgo Lab and the BSDB studentship for their support throughout my project. I would definitely recommend future students to apply to the studentship, not only for lab projects but outreach projects as well.

***Yuqian Ye***

**Roles of amino-acid transporters in the developing brain**



After a year of lockdowns and virtual classes at Bangor University, the opportunity to do a real lab project this summer at the Francis Crick Institute was definitely not one to miss. Under the patient supervision of Adrien Franchet and Sebastian Sorge in Alex Gould's lab, I set out to explore the roles of some amino acid transporters during the development of the genetic model organism *Drosophila*.

This two month summer project was my first opportunity to gain hands-on experience doing hypothesis-driven science and to interact with many talented researchers at the Crick. As an undergraduate, my only previous exposure to fruit flies was from reading published papers but, right from day one, I got stuck in to the nitty gritty of *Drosophila* developmental biology and larval dissections.

The Gould lab are interested in figuring out how the neural stem cells of the developing CNS are so highly protected against environmental stresses such as nutrient restriction (NR) and hypoxia. This process is a key part of brain sparing, which involves sustaining the growth of the CNS at the expense of other organs such as adipose tissue. In mammals, brain sparing is commonly observed in neonates following intrauterine growth restriction. However, the key signalling and metabolic pathways underlying brain sparing are still unclear.

Amino-acids are key signals for growth and they are also critical for protein synthesis. The uptake of amino acids by tissues involves a large number of different amino-acid transporters and I set out to decipher whether two of these transporters (AAT1 and AAT2) are required in the neural stem cell niche (glia in *Drosophila*) or in adipose tissue (fat body in *Drosophila*) for CNS and body growth. My project stemmed from Adrien's and Sebastian's recent RNA interference (RNAi) screen of amino acid transporter candidates. I followed up two of their screen hits (AAT1 and AAT2) using UAS-RNAi knockdowns lines crossed with Gal4-driver lines specific for glia (*repo-Gal4*) or fat body (*Cg-Gal4*). The goal was to measure the phenotypic effects of these cell-type genetic manipulations during standard fed development and also during severe NR on an agar-only diet. Phenotypes were measured for larval and pupal weights using an accurate microbalance. I also quantified CNS phenotypes from confocal microscopy images by measuring CNS area and also neural stem cell (neuroblast) proliferation via the incorporation of a labelled nucleotide analogue (EdU).

I found that RNAi knockdowns of either AAT1 or AAT2 produced more severe phenotypes in glia compared to fat body (Figure 1). Hence, larval pupal and adult weights were largely normal with the fat body knockdowns (Figure 1A, 1C). However, both glial knockdowns gave modest changes in body weight at the larval stage but, by the pupal stage, these only remained significant for AAT2 (Figure 1B, 1D). I also noticed that glial knockdown of AAT2 eventually resulted in adult lethality, shortly after eclosion, with flies displaying very severe locomotor defects.

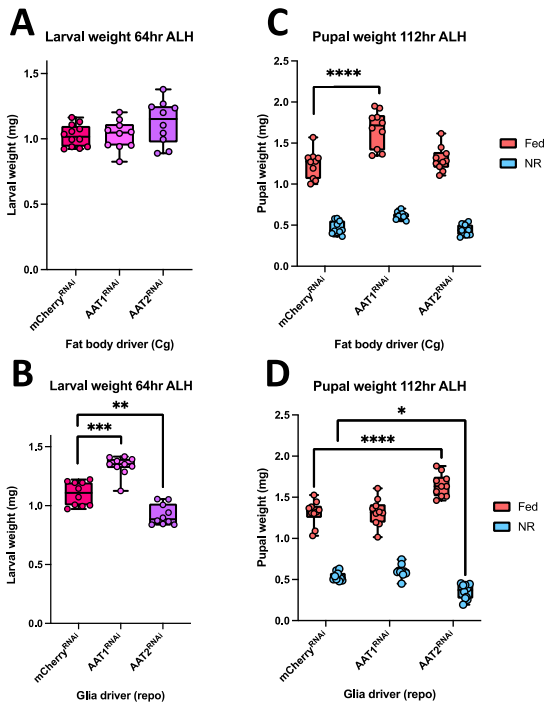



**Figure 1. Larval and pupal weights of AAT1 or AAT2 knockdowns.**
*Larvae were raised on standard lab diet (Fed) or nutrient restriction (NR). (A,B) Larval weights of fat body (Cg-Gal4) knockdowns (A) or glia (repo-Gal4) knockdowns. (C,D) Pupal weights of fat body (C) or glial (D) knockdowns. mCherry$^{RNAi}$ is a control RNAi line and times refer to hours after larval hatching (ALH). Statistical significance (asterisks) was determined using Tukey's Multiple Comparison Test.*

In a parallel set of experiments, I investigated the effects of the AAT2 knockdowns on the growth of the developing CNS and on the proliferation of neural stem cells. To do so, I dissected brains from fed larvae and from larvae exposed to one day of NR. I then performed an *in vitro* EdU incorporation assay as an indicator for neuroblast progression through S-phase of the cell cycle. I found that the fat body manipulations had no significant effect on CNS size or on neuroblast proliferation. In contrast, the glial manipulations revealed that AAT2 is required in glia for proper growth of the larval brain, as the CNSs of *repo-GAL4; AAT2$^{RNAi}$* larvae were strongly reduced in size and likewise the EdU incorporation was much lower than genetic controls (Figure 2A, 2B). This glial requirement for AAT2 for neuroblast proliferation occurred in both fed and NR larvae (Figure 2C, 2D). Thus, in conclusion, my project has revealed a constitutive function in glia for the amino acid transporter AAT2 during both normal CNS growth and brain sparing. It will be important in future to explore whether AAT2 is required in the surface glia of the blood-brain barrier or in the internal cortex glia that surround neuroblasts and their daughter cells. Equally importantly, it will be interesting to identify which specific amino acids are transported by AAT2.

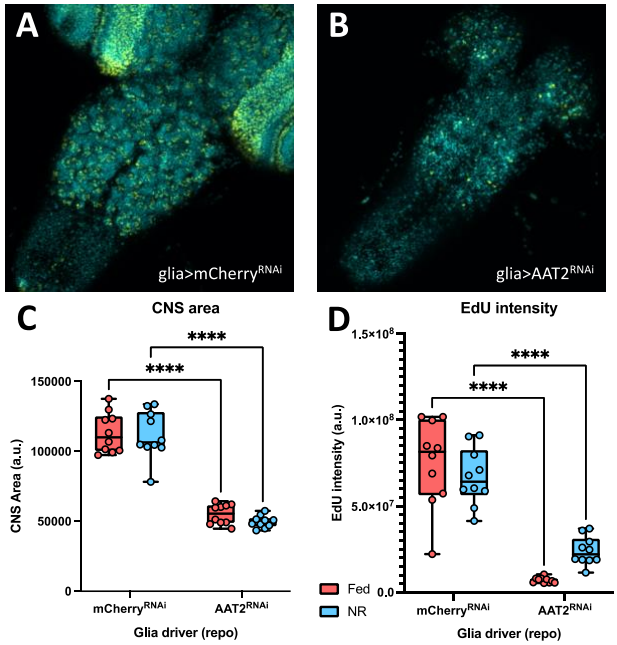
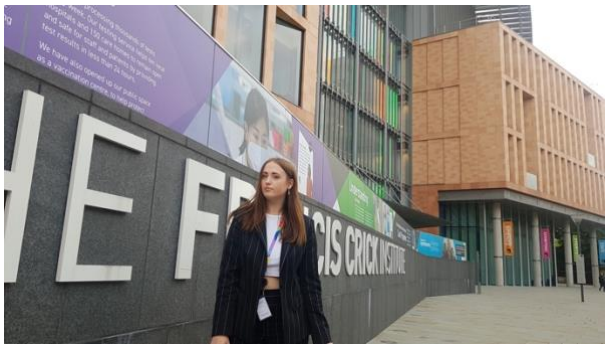



**Figure 2. Glial AAT2 is required for neuroblast proliferation in the larval CNS**
*Larvae were raised on standard lab diet (Fed) or nutrient restriction (NR). (A,B) Confocal images of the nuclear marker DAPI (cyan) and the proliferation marker EdU (yellow) for third-instar larvae expressing repo-GAL4 driving AAT2 RNAi and their genotype controls (mCherry$^{RNAi}$) (C,D) Quantitation of CNS area (C) and average EdU intensity (D) for control and AAT2 RNAi lines. **** Statistical significance determined using Tukey's Multiple Comparison Test.*

Overall, this fascinating project has given me a first taste of biological research at the bench and has also allowed me to develop critical thinking and data processing skills. I am indebted to Adrien Franchet and Sebastian Sorge for their fantastic direction, and to Alex Gould and all of his lab for their encouragement throughout. I would also like to thank the Francis Crick Institute for hosting me and the Medical Research Foundation Rosa Beddington Fund for supporting my project and allowing me to contribute to this captivating field of research.

***Charlotte Campbell-Broad***

**Testing constructs for CRISPR/Cas9 genetic modulation**
**Merissa Hickman**



This summer, I was given the opportunity to conduct research at the Francis Crick Institute in the Znamenskiy lab. The aim of the Znamenskiy lab is to understand the relationship between connectivity, gene expression and function of cortical neurons.

The neocortex is a region of the brain integral in performing higher cognitive functions. Neocortical projections can be divided into three broad classes. Corticothalamic (CT) neurons are located mostly within layer 6 and send axons to the thalamus. Pyramidal Tract (PT) neurons are nearly exclusively positioned within layer 5 and project to brainstem and spinal cord. Intratelencephalic (IT) neurons are distributed throughout all six layers and project to distant cortical areas (Kast & Levitt, 2019). The expression of transcription factors during development can affect projection patterns. For example, when *Fefz2* is deleted, the cortex no longer sends projections to the brain stem and instead sends projections to the thalamus or contralateral hemisphere (Kast & Levitt, 2019). This shows that genes expressed by a neuron during development play an important role in determining its wiring patterns.

Beyond these broad projection classes, the genetic basis underlying patterns of neocortical connectivity is little understood. The primary visual cortex (V1) is a region of the brain that is important for receiving, segmenting, integrating, and processing visual information relayed from the retinas. Subsequently, the processed information is then sent to other regions of the brain. This is a highly specialised process that allows the brain to recognise patterns quickly and with the absence of a conscious effort. The V1 provides a platform for understanding the neocortex due to its serially homologous structure, and therefore can be used as a model for neocortical projections. The V1 sends projections to several higher visual areas as well as many other areas of the brain such as the lateral geniculate and lateral posterior thalamic nuclei, superior colliculus, striatum, and other subcortical structures but little is known about how these connectivity patterns are established (Kast & Levitt, 2019).

To understand which genes are important for specifying long range connectivity patterns from V1, *in vivo* genetic manipulations using CRISPR/Cas9 can be used to determine what happens to connectivity patterns when the expression of target genes is altered. CRISPR/Cas9 is a simple, rapid method to modify gene expression which can be pooled together to look at many genes in parallel. As well as knocking-out the gene of interest using the prototypical CRISPR/Cas9 gene editing approach (Figure 1A), methods for modulating gene expression using catalytically inactive Cas9 fused to transcriptional modulators have recently been developed (Figure 1B-C). CRISPR activation (CRISPRa) allows functional analysis of redundant genes through overexpression, whereas CRISPR interference (CRISPRi) allows analysis of gene function by knocking-down gene expression at the transcriptional level and is thought to have fewer off-target effects **(**Gebre et al., 2018). The aim of my project was to perform preliminary experiments validating whether gRNA constructs designed to be used to examine changes in *in vivo* V1 connectivity patterns, using CRISPR knockout, CRISPRi or CRISPRa, altered gene expression *in vitro*. The first part of my project was to clone some of the gRNA CRISPR constructs, and the second part was to test constructs *in vitro.*

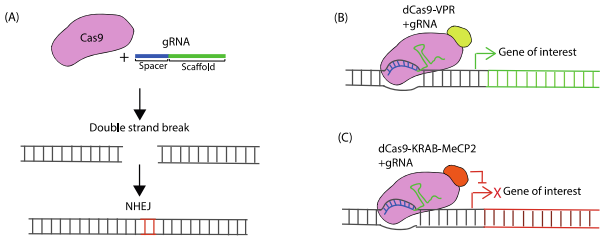



**Figure 1. Mechanism of CRISPR/Cas9 Genetic Modulation.** (A) CRISPR knockout involves co-expressing Cas9 and a gRNA in a cell. The Cas9 protein recognises a specific sequence called the scaffold sequence in the gRNA while another sequence within the gRNA called the spacer region determines the target site within the genome to be modified. The Cas9 protein generates double strand breaks in the gene of interest that are repaired through the non-homologous end joining (NHEJ) pathway that is prone to producing indel mutations (red bases here indicate an insertion) that can render genes non-functional when translated. (B) CRISPR activation (CRISPRa) constructs work via transcriptional activators fused to catalytically dead Cas9 (dCas9) which are targeted near transcriptional start sites of the endogenous gene of interest by the gRNA to induce their overexpression. (C) CRISPR interference (CRISPRi) constructs consist of dCas9 fused to transcriptional repressor domains that are recruited in proximity of the transcription start site of an endogenous gene to repress transcription.

gRNA constructs were tested along with corresponding Cas9s (SP-Cas9, dCas9-KRAB-MeCP2, and dCas9-VPR for CRISPR knockout, CRISPRi, and CRISPRa, respectively) to determine whether a change of expression in our genes of interest occurred within Neuro-2A (N2a) cells. Target genes for validation (Frizzled 1 (FZD1), Androgen Receptor (AR), Polycystic Kidney and Hepatic Disease 1 (PKHD1), and Anaplastic Lymphoma Kinase (ALK)) were identified due their established endogenous gene expression in N2a cells. To determine whether the gRNA constructs worked we co-transfected Cas9's with the gRNA construct into N2a cells and observed whether this altered expression of target genes by looking at endogenous protein levels through immunostaining. Endogenous protein levels in each condition were compared to a control plasmid without a gRNA insert. The results obtained from the quantification of the transfection and subsequent immunostaining are shown in Figure 3A-C. These results did not reveal expected differences in gene expression between gRNA constructs and further experiments need to be performed using alternative antibodies or staining conditions. However, the project has given me an insight into the molecular basis of developmental biology, and I thoroughly enjoyed learning the techniques and protocols required to complete the cloning process. During my research internship I was able to obtain applied, practical experience within the laboratory which due to the COVID-19 pandemic, has been limited during my undergraduate degree. I also was given a level of independence which I did not expect within the laboratory, completing the transfection of gRNA constructs was an engaging, albeit challenging process as my cells became contaminated during the passaging process. However, I was able to overcome this setback and build resilience. Overall, I really enjoyed my project, and it has encouraged me to pursue a career in scientific research.

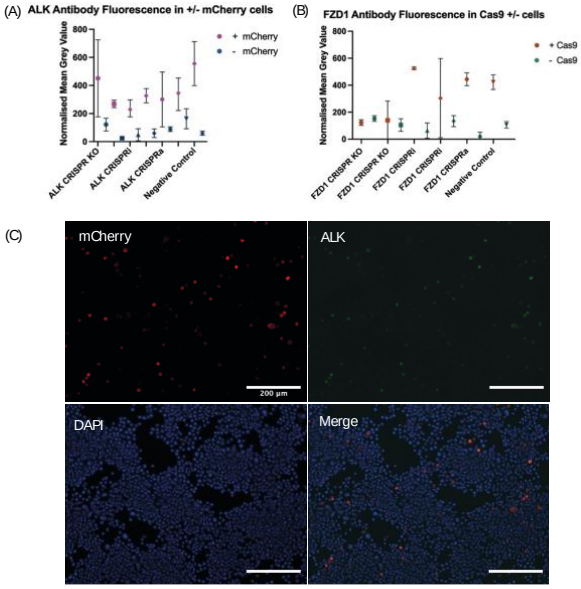



**Figure 2. Quantitative Immunofluorescence after transfection of Cas9 plasmids and mCherry expressing gRNA plasmids targeting either ALK or FZD1.** (A) ALK antibody fluorescence in +/- mCherry Cells. (B) FZD1 antibody fluorescence in + mCherry; +/- Cas9 Cells. KO/i/a – KO = Knockout; i= interference, a = activation. Each symbol shows the mean normalized grey value of N2A cells which reflects the level of fluorescence from antibodies targeting the endogenous protein-of-interest after immunostaining. The negative control used was a plasmid without a gRNA insert. SP-Cas9 was used for ALK/FZD1 KO & negative control, whereas dCas9-KRAB-MeCP2 was used for ALK/FZD1 CRISPRi, dCas9-VPR used for ALK/FZD1 CRISPRa. For the FZD1 transfection there was an unexpectedly low number of +Cas9 cells. (C) Immunohistochemistry staining against mCherry and ALK, as well as DAPI staining in N2a cells transfected with SP-Cas9a and a gRNA targeting *ALK*. mCherry is expressed by the gRNA constructs, staining this protein shows which cells were transfected with our construct of interest, whereas DAPI staining marks the nuclei of all cells. The overlap

in ALK and mCherry signals suggests further optimisation of immunostaining and imaging conditions is required to avoid bleed-through.

I would like to take this opportunity to thank the Francis Crick Institute, particularly the Znamenskiy lab for allowing me to undertake research at their facility, alongside my supervisor Benita Turner-Bridger for supporting me in my project. Furthermore, I would like to show my appreciation to the Medical Research Foundation and the Rosa Beddington fund which has provided the financial support for my project. It is an honour to have the opportunity to contribute to The Node and the British Society of Developmental Biology, and I would strongly encourage other undergraduate students to pursue a similar research project during their studies. This experience has been unlike any other.

***Merissa Hickman***

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# Conference and Travel Grants

## (1) BSDB Conference Grants

BSDB Conference Grants are available to cover registration and accommodation costs to attend BSDB-sponsored meetings. They are open to all BSDB members. Applications must be received by the abstract submission deadline of the intended BSDB meeting.

### To apply:

1. Download the **BSDB Conference Grant form** as a Word document or PDF
2. Return the completed form by email to **grants@bsdb.org**.
3. The application should include a letter of support and, if appropriate, the abstract of the poster or talk you intend to present
4. Applications should include details of the proposed visit and the breakdown of the amount of money requested

## (2) The Company of Biologists Travel Grants

These grants are **sponsored by The Company of Biologists**, the not-for-profit publisher of *Development*, *Journal of Cell Science*, *Journal of Experimental Biology*, *Disease Models & Mechanisms* and *Biology Open*.
The **Company of Biologists Travel Grants** are to attend meetings and courses outside the UK and pay for a proportion of the total cost, to a maximum of £600. We will accept applications for funds to attend online conferences and courses. These applications are not restricted to meetings and courses outside the UK.

### To apply:

1. Download The **Company of Biologists grant application form** either as Word document or PDF.
2. Return the completed form to **grants@bsdb.org**.
3. The application should include a letter of support and, if appropriate, the abstract of the poster or talk they intend to present
4. Applications should include details of the proposed visit and the breakdown of the amount of money requested

## (3) The Company of Biologists travel and attendance grants for practical courses

These grants are sponsored by The Company of Biologists, the not-for-profit publisher of *Development, Journal of Cell Science, Journal of Experimental Biology, Disease Models & Mechanisms and Biology Open.* The funding scheme supports the attendance at practical courses such as Woods Hole, Cold Spring Harbour and EMBO lab management courses. A proportion of the total cost can be applied for up to a maximum of £1000.

### To apply:

1. Download The **Company of Biologists grant application form** either as Word document or PDF.
2. Return the completed form to **grants@bsdb.org**.
3. Applications will be grouped and reviewed at the end of each month, so members should apply at least 2 months before they need the grant.
4. Applications for a refund on money already spent will be considered only under exceptional circumstances.
5. No more than one person from one lab and two people from one department will be supported for any one course.
6. Normally, and unless demand is low, a member will only receive a single award per year.
7. Only applications from fully paid up members will be considered.

## (4) The Company of Biologists Travel grants for group leaders and PIs

These grants are sponsored by The Company of Biologists, the not-for-profit publisher of *Development, Journal of Cell Science, Journal of Experimental Biology, Disease Models & Mechanisms and Biology Open.* This funding scheme allows group leaders and PIs without sufficient grant funding to attend meetings and courses outside the UK and pay for a proportion of the total cost (maximum of £1000 for meetings in USA and Asia, £600 in Europe). We will accept applications from Group Leaders / PIs to attend online conferences and courses. These applications are not restricted to meetings and courses outside the UK and should be for meetings or courses that will take place in 2020 or 2021. All other conditions of the application process remain the same.

### To apply:

1. Download The **Company of Biologists grant application form** either as Word document or PDF.
2. Return the completed form to **grants@bsdb.org**.
3. Applications will be grouped and reviewed at the end of each month, so members should apply at least 2 months before they need the grant.
4. No application will be considered for a refund on money already spent.
5. Normally, and unless demand is low, a member will only receive a single The Company of Biologists grant per year.
6. Only applications from fully paid up members will be considered.

## (5) Louie Hamilton Fund

Louie Hamilton, the distinguished developmental biologist who was disabled in the latter part of her life, left a bequest to the Society with the intention that the interests should provide travel support for members who are disabled. Applicants who qualify for this fund should email to **grants@bsdb.org**.

## (6) BSDB Childcare grants

The BSDB have set up a fund to help offset additional childcare costs incurred by participants or speakers when participating at BSDB Spring or Autumn meetings. Eligible costs include contributions towards fees for a baby-sitter or child-care facility, travel costs for a care giver, or travel costs for taking the child to the meeting etc. This fund is not strictly restricted to childcare – requests for costs related to other caring responsibilities will also be considered. Please note that (for now) this fund can only be used for attendance at BSDB meetings where the applicant will present their work as a poster or a talk.

**We will provide up to a maximum of £250 per applicant.**
**Applicants must be BSDB members.**
**The application deadline is 6 weeks prior the start of the relevant BSDB meeting. We aim to inform applicants of the outcome within 2 weeks of the deadline.**

### To apply:

Please email the meetings secretary Sally Lowell at meetings@bsdb.org with the following information:

* Your name and email address
* Your current place of work (Institution /City /Country)
* Which BSDB meeting you plan to attend
* Title and co-authors for the poster or talk that you will present at the meeting
* Confirmation that you are a member of the BSDB
* A justification for why the support is required (no more than 250 words)
* A breakdown of the costs requested (no more than £250)