A perspective piece on my virology lab research experience through the Undergraduate Research Opportunity Scheme

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Abstract

This article is a perspective piece on my experience undertaking an Undergraduate Research Opportunities Scheme (UROS) project. This is a scheme designed to provide experiences and research skills to students in their area of interest. My project explored the interaction of the Herpes Simplex Virus 1 (HSV-1) with cellular defences and how this might affect the production of new virus. Throughout the project, optimisation was needed, with additional tests and experiments performed to allow my academic supervisor and I to produce results we were confident in. In this paper, I have outlined the skills of problem solving and optimisation, and address how they have impacted upon my research. The discussion will further explore my work in an active research lab, and how this has impacted my perception of professional lab research. This project not only changed my perspective, but allowed me to gain independence with my research, while working with an academic supervisor to develop my interest in virology. UROS helped me develop specific practical lab skills that will benefit me going forward in my studies and later, my career. This experience has solidified my interest in virology and molecular biology, but more importantly, research, allowing me to be certain that an M.Sc. or PhD is the right path for me.

Keywords: Virology, cellular defences, viruses, experience

Introduction

The Undergraduate Research Opportunities Scheme (UROS) is a scheme that encourages undergraduates to engage in, and experience, research at the University of Lincoln (UoL). There is a focus on the concept of 'Student as producer', a hands-on approach to learning that is reflected in the teaching at UoL. Students can explore their area of interest whilst developing key skills and gaining experience. The scheme is competitive, and students apply with an academic supervisor with whom they will work while conducting the research. Successful students receive a bursary allowing them to fund their project. Once the research is finished, students publish a blog report and present their findings at the annual UROS Exhibition Showcase. My area of interest is virology and as my application was successful, I conducted my research in the summer of this year (2022). My project focused on the interaction between HSV-1 and cellular defences.

Project background

Viruses have evolved to overcome cellular antiviral defenses to ensure their replication. For a virus to successfully express its genes, it must take over the host's cellular machinery to allow it to synthesize viral mRNAs and proteins. Host cells however have mechanisms that attempt to stop infection, for example through the activity of restriction-factors as part of the intrinsic immune response, or the innate immune response triggered by interferon signaling (Alandijany, 2019; Lanfranca et al., 2014). In order to replicate, viruses must overcome these defenses through the action of antagonists, but is it possible these responses may not be as detrimental as they seem? Prior student research had suggested that this might be the case and so we set out to investigate this gap in current understanding more thoroughly.

Literature Review

Herpesviruses are ubiquitous and cause a large range of diseases in many species. HSV-1 infections vary from asymptomatic infections, where an individual may present without any typical symptoms or knowledge of infection, to characteristic cold sores, ocular keratitis or lethal encephalitis (inflammation of the brain). This group of viruses cause lifelong infections, which can reactivate after initial infection, such as shingles reactivating after primary chickenpox infection by the related Varicella zoster virus. Shingles is characterised by a painful blistering rash and can cause further conditions such as postherpetic neuralgia (PHN) which causes lifelong disability(Kawai et al., 2014).

HSV-1 is very prevalent with more than half the human population having been infected (Kumar et al., 2022). It is commonly associated with cold sores or fever blisters, but can cause keratitis, and is the leading cause of infectious blindness in industrialised countries, or life-threatening outcomes like encephalitis especially in immunocompromised individuals (Alandijany, 2019). It is therefore important to try to understand this virus in order to ultimately develop new therapies. Herpesviruses establish latency meaning they lie dormant and undetectable to the immune system, and this contributes to the hosts inability to clear infection. Evolution has shaped the interaction between virus and host during replication to run smoothly (Grinde, 2013). Over years of coevolution, Herpesviruses have developed ways to surmount the many defences put up by the host against the virus (Full and Ensser, 2019). I wished to explore in more detail how the virus interacted with these cellular defences and if all such interactions were detrimental to the virus.

Methodology

Methodology used one-step real time quantitative polymerase chain reaction (RTqPCR). This technique allowed us to specifically measure the levels of target mRNAs within infected cells, by recording amplification of PCR products through detection of fluorescent signal the strength of which corresponds to the abundance of the mRNA (Smith and Osborn, 2009). In this case, it was used to measure relative viral mRNA abundance at specific times after infection, comparative to a cellular control gene, to ensure what is seen can be correlated between different experiments, in cells that either had an impaired or fully functional response to infection.

While conducting this research we met some challenges. Our first test indicated that optimization was needed to allow us to specifically measure the signal from mRNA. Our initial tests showed contaminating viral DNA to be present in our RNA samples, which could act as a template for PCR and thus be detected in addition to signal due to the viral mRNA. After finding this, multiple different runs were conducted changing various parameters of the experiment to try and remove this contaminating template. In doing this we were able to achieve results that were much more reliable and represented the viral mRNA rather than a hybrid signal of viral mRNA and viral DNA (vDNA).

Results

Due to the potential that our results may contribute to a publication in a subject specific journal in the future, I am not able to go into detail regarding our results. However, I can share that during our project, we had several rounds of optimization to achieve results void of contaminating viral genomic DNA that could act as a template, in addition to the viral mRNA, for qPCR detection and quantitation. In order to be confident in these results, there needed to be just the mRNAs we were aiming to amplify being present. Initially, we tried a different manufacturer for the RNA prep kit, this did not change the outcome of the amplification, suggesting viral genomic DNA was carried over in this preparation method also. We found that using a 1/100 dilution of RNA and treating twice with DNase, a treatment used to degrade contaminating DNA so it cannot act as a template, shifted the amplification curve for a control test where only contaminating DNA would be measured to where signal from that DNA could be considered insignificant. This meant that our results only measured our intended viral RNA target rather than this and viral DNA. The pattern found in our final results did not mirror the previous work.

Key Areas of Development

This project has helped me to develop experience of what it is really like to work in a research lab. During my UROS project, I undertook roles as a researcher to prepare and plan every part of an experiment. This included preparing solutions, using a pipette properly and measuring out very small amounts of components for a mixture that is usually preprepared when in a teaching lab. These skills are fundamental to progressing if the preparation is executed correctly and will ensure there is less variability in the results. This experience also opened my eyes to the amount of the work that goes into preparation and ensuring all parts of the experiment are consistent.

I also learnt how to develop and adapt experiments, as well as what to do if your results are not what you expect. There were multiple occasions during the project where we had to change plans and re-strategize. Optimization and problem solving are key aspects of research, and these skills are relevant beyond the lab. These skills have been useful in my own research during overseas field work in Ecuador. The nature of biological lab work means that not everything is going to turn out how you would expect, and the important thing is how you respond to these unforeseen outcomes. I have come to realize that optimization and problem solving are what is at the core of research, and in my case, what interests me.

This research experience also developed my confidence and brought me a sense of independence within the lab while working alongside an academic. My academic supervisor gave me responsibilities to fulfill and allowed me to carry out complicated processes with support and guidance along the way. I experienced the “Student-as-producer” framework, as I was a part of the research project every step of the way. Carrying out the experiments allowed me to understand what I was doing and engage with what I was doing, and this hands-on attitude is something I will bring with me to my future lab experience such as my third-year research project. I will go into this project with more of an idea of what is involved in ‘real’ research and how to use some of the equipment including more sensitive equipment. Knowing how to use some of these complicated pieces of equipment gave me the confidence that I *can* do this type of research, especially with the support of my academic supervisor.

Challenges and Lessons learnt

Challenges come with every research project, and we were met with a few challenges throughout our research, mainly resulting in us optimizing our conditions for the experiment. Removing background interference allowed for a clearer picture of what is going on, with results that can be compared and most importantly

recreated. Through this, the importance of re-strategizing and the ability to be adaptable was highlighted to me.

Another challenge that presented itself was the precision and discipline required to ensure that your experiment is not contaminated. This skill develops with practice and by the end of our project, this awareness of not contaminating and working as clean as possible was instilled in me as a discipline to take through my career in the lab.

Conclusion

Though this experience has been very beneficial for development of transferable skills and lab techniques, I think the greatest benefit for me is my newfound perspective on what my future may look like. The certainty of my decision to pursue a career in research that the UROS bursary has provided me with is invaluable. In the future, I hope to pursue a master's degree in research and possibly a PhD, and I believe this experience has solidified that choice, while also allowing me to have some practical experience to convey during interviews for further study or a job. I have gained a perspective of what it is like working in a lab and feel a lot more prepared to go forward in my education and further lab experience. I cultivated an array of transferable multidisciplinary skills, as well as some specific to my area of study, allowing me to explore the reality of what a life researching in this field might look like. It has shown me exciting new ideas and concepts I would not have thought possible. I would encourage undergraduates to apply to the UROS if they want to experience what it is like working alongside an academic, researching in an area they have an interest in and working within the 'Student as producer' philosophy.

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