**Daphne Jackson Trust Fellowship application, Oct 2018**

**Applicant:** Dr. Elizabeth Hughes

**Supervisor:** Dr. Edward Wallace

**Host Organization:** Institute for Cell Biology, School of Biological Sciences, Edinburgh University

**Project Title:**  Dynamic mRNA processing in response to environmental stimuli in the fungal pathogen *Crytococcus neoformans*.

During my undergraduate I was drawn to molecular biology and how it relates to the pathogenesis of disease. My PhD looked at the infecting HIV viral population and its relationship to disease progression, primarily the evolutionary analysis of isolates infecting lymphoid and non-lymphoid tissues. This led to the discovery of a previously unknown dormant HIV population in the brain and a first author paper with over 100 citations.

Subsequently, I examined the replicative processes of HCV by determining whether the NS5B protein (predicted to possess an RdRp activity) was capable of directing HCV replication. I optimized bacterial expression systems and purified the NS5B fusion protein.

Following this I investigated the structure and function of the major outer membrane proteins (MOMP’s) of Chlamydia where I cloned and expressed wild type and VS4 domain mutated proteins and functionally reconstituted them at the single-channel level. Reconstitution in planar lipid bilayers showed that the VS4 domain was not required for pore formation but may help to form the channel vestibule where it may interact with other protein loops.

I then joined a leading bio safety testing facility as the research and development scientist and developed a wide range of molecular based assays for clients, and provided technical training and support for colleagues.

I chose to take a career break to raise my family. My husband works in the marine industry as a consultant in risk assessment/safety management and this job takes him away from home on a regular basis. Taking this into account and the prohibitive costs of childcare we decided it would be best if I put my career on hold to raise our family. During this time I have taken over the administrative side of our business.

Both of my children now attend high school and I am confident that they are mature and resilient enough for me to return to my chosen career. I am excited to re-engage with the scientific community. I find the challenges associated with research both rewarding and enlightening and I look forward to returning to the lab environment.

The Daphne Jackson Fellowship with MRS presents an ideal platform for me to resume my biomedical research career. The provision of mentoring, support and retraining are invaluable. This fellowship will give me the opportunity to update my existing skills while learning new invaluable ones to help me develop as a person and move my career forward.

Word count 398

**Dynamic mRNA processing in response to environmental stimuli in the fungal pathogen *Crytococcus neoformans*.**

**Research Summary**

*Cryptococcus neoformans* is a major human pathogen causing pneumonia and cryptococcal meningitis in immunocompromised individuals. Both the basidiospore and desiccated encapsulated yeast cells are postulated to act as infectious propagules. This fungus is found in the environment typically associated with pigeon guano, soil and decaying wood, however, somehow it is able to adapt, survive and proliferate within a mammalian host. Within the basidiomycetes, pathogenic *Cryptococcus* species are the only fungi known to grow well at 37 ⁰C. The route of infection is inhalation of either desiccated yeast or spores from the environment. Although the ecology and life cycle of *C. neoformans* is well characterized few studies have examined the response of this fungus when it is presented with the hostile environment of a lung. I would like to examine in detail what happens when this organism reactivates within the host environment

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1. To date most *in vito* studies have been carried out in rich media which does not accurately reflect the conditions *C. neoformans* will encounter upon infection of the lung. I would like to design an *in vitro* system which more closely mimics *in vivo* conditions of the lung/alveoli. To do this I will test different cell culture conditions and establish a reproducible minimum growth medium and conditions for yeast cells and spores. Having established a reproducible *in vitro* system I will compare yeast cells and spores under different environmental conditions and examine the pattern of gene expression to generate a comprehensive picture of what genes are active in each species. This will help me to gain a deeper understanding of what transcriptional activity may contribute to infection.
2. Interaction with other organisms have been shown to effect infectivity and virulence. For example, bacteria can stimulate spore germination and, previously, bacterial cell wall components have been shown to modify the morphology of *C. neoforman*s yeast cells from normal cells to a titan cell. I would like to examine the changes in gene expression during this event to gain a deeper understanding of what transcriptional activity contributes to this morphological change.

I will construct cDNA libraries to give a snapshot of actively expressed genes under different environmental stimuli and use qPCR for quantitative gene expression analysis. qPCR is one of the most powerful and sensitive gene analysis techniques available. The sheer volume of expression data produced will necessitate sophisticated computational methods for analysis.

Modular cloning for sysnthetic biology

CRISPR transformation

High throughput sequencing library preparation

Lab automation

Scientific abstract.

Section 4: Host Organisation

Dr. Edward Wallace.

Sir Henry Dale Fellow (Wellcome Trust/Royal Society Early Career Research Fellow).

Institute for Cell Biology, School of Biological Sciences, Edinburgh University.

Project funding remit: The lab is funded by Wellcome Trust/opal Society. Innovative research on fungal pathogens falls under the MRC’s health strategic aim.

Dr. Edward Wallace, of the institute for Cell Biology in Edinburgh University, has agreed to be my supervisor for the duration of the fellowship. Dr. Wallace in a renowned RNA scientist and working with him will allow me to build on my existing skills in the field of molecular biology but also learn new techniques including modular cloning for synthetic biology, CRISPR transformation, high throughput sequencing and library preparation, lab automation and computational data analysis of large data sets. This will provide me with crucial work experience in one of Scotland’s world-class universities and equip me with specific skills in high demand in biomedical research and biotech industries.

Relevant collaborations?

How well equipped is the organisation to support the proposed research plan?

Names of people involved in retraining.

Section 5: Retraining program

Describe retraining program. Include planned research methods/techniques and personal development.

Distinguish between new and refreshed skills and include as many specific details as possible.

Section 6: Proposed Research plan.

Dynamic mRNA processing in response to environmental stimuli in the fungal pathogen Cryptococcus neoformans.

C. Neoformans is an opportunistic and facultative pathogen that is the number one cause of fungal meningitis worldwide. C. Neoformans primarily infects immunocompromised individuals and is one of only a few fundal species that have been shown to cross the blood-brain barrier leading to meningitis that is fatal if left untreated. An estimate one million cases of crypto meningitis are reported every year with 600,000 resulting deaths. (Lancet infectious diseases2017 may 5, rajasingham r eat al)

The onset of the AIDS epidemic in the 1980’s was accompanied by a surge in cryptococcosis cases world wide. In more recent years antiretroviral therapy and antifungals have reduced the number s of fatal cryptococcal meningitis. However, C neoformans remains a serious concern for immunocompromised individuals including AIDS, cancer, organ transplant and SCID patients. Cryptococcus meningitis is also a major problem in resource-limited countries where HIV prevalence is high and access to health care and appropriate drug regimes is limited. In addition a recent outbreak of cryptococcosis in immunocompetent individuals has been reported.

Disease occurs when either dessicated yeast or spores from the environment are inhaled, proliferate in the lung and become disseminated to the central nervous system causing life threatening meningitis. Although the disease and life cycle of C. Neoformans is well characterised few studies have examined C. Neoformans gene expression and regulation upon initial infection in the lung. An opportunistic pathogen such as C. Neoformans must undergo a rapid change in gene expression upon presentation in a foreign environment such as the lung. Indeed not many fungal pathogens can grow at 37 ‘C which is a characteristic virulence factor of C. Neoformans. The ability to investigate the genetic response of a pathogen in the host environment is a powerful tool to elucidate the adaptive response/responses required for an opportunistic pathogen to survive in the hostile environment of the host.

Very little is know about the initial infection of the lung by C. Neoformans. It is proposed that either dried yeast or spores infect the lung following inhalation although to date it is not known which or indeed if both are inhaled. Also, once in the lung alveoli little is known about what the yeast/spore may encounter and how it responds to environmental stimuli. For example bacterial proteins present in the hot lung. Studying the interaction of bacterial proteins and fungi living within the host may provide insight into the pathogenesis of the infectious disease. Understanding how and with what C. Neoformans may interact/anatagonize in specific niches may help identify potential targets for drug development.

I would like to examine in detail what happens when this organism reactivates within the host environment, which is alien to it’s natural environment, it is found in the soil, associated with trees and in bird guano, and normal life cycle. When an organism steps out of it natural environmental niche into an alien environment a great deal must go on I order for the organism to adapt to the unexpected circumstances in which it finds itself. Indeed C neo is one of only a few fungi that can exist at 37 degrees.

Previously C neo has been grown in vitro in primarily rich media and much has been extrapolated about the infectivity and virulence of c neo from this system. I would like to design an in vitro system which more closely mimics the in vivo conditions of the lung/alveoli to enable me to elucidate the important steps for infection in a reproducible in vivo like system. I would like to test different cell culture conditions to establish an appropriate growth medium for yeast and spores to enable me to study each of the possible infecting species more closely. Specifically I would like to look at the host-pathogen interaction and decider the cell surface modifications that allow C neo access to the host, the effect of interactions between C neo and native bacteria residing within the lung, as it has previously been shown that bacteria can stimulate spore germination, and the effect the host immune system has on the infection process in the lung.

The infection process of any organism is a complicated process. When the organism steps out of it’s natural environment and enters an alien one this process must be compounded by additional pathogen response. I would like to examine the host-pathogen interaction comparing yeast and spores under previously optimized conditions and look at the cell surface modifications that occur. I would like to compare host epithelial and alveoli macrophages during the infection process and compare the resulting gene expression profiles to delineate what is up or down regulated and from this to infer what may be important at the point of infection.

Interactions with host factors may effect the infectivity and virulence of infecting organisms. For example bacteria can stimulate spore germination. I would like to examine the effect bacteria residing in the lung may have on infecting yeast or spores by co infecting with live and dead bacteria. Previously bacterial cell wall components have been shown to modify the morphology of C neo from a normal yeast cell to a titan cell. I would like to examine the gene expression during these events to attempt to put forward a hypothesis for the intital infecting mechanisms.

I would also like to examine the effect of the immune system on this process. Add murine sera to yeast and spores and see what happens.

1. specifically looking at the gene expression of cell surface molecules that allow *C. neoformans* access to the host.

Hypothesis to be tested.

Research methods to be used.

Describe how your retraining will be useful for the project.

Describe the risks involved and how you will overcome them.

Section 7: Ethical approval and licences.

Not Applicable.

Section 8: Timetable

Simple diagrammatic workplan in a Gants chart or table illustrating research and retraining elements. Columns as months (1-36) rows as tasks/actions.

Indicate major outputs and landmarks.

Section 9: References

Use smaller font to keep to one page

Section 10: Future planning