2: Why applying

I am a skilled and highly motivated molecular biologist with 10 years lab-based experience, 6 post-doctoral.

3: Summary

*Cryptococcus neoformans* lives in the environment growing on trees, pigeon droppings and in the soil.

How does *C. neoformans* adapt to the rapid change in environment from soil/vegetation to a mammalian lung?

Once *C. neoformans* has entered the lung it will encounter a number of microbes, some of which may influence its ability to cause disease.

Identifying unique or critical pathways Cryptococcus uses to cause disease will provide unique targets for drug design

Fungal infections are one of the hardest diseases to manage in humans.

4: Abstract

How does *C. neoformans* adapt to the lung environment?

5: Host Organization

The University of Edinburgh is ranked 18th in the world, 4th in the UK and the top university in Scotland.

The Centre for Synthetic and Systems Biology, in the Institute for Cell Biology, is a unique inter-disciplinary environment with a track record for multi-disciplinary research.

My primary supervisor, Dr. Wallace, is a quantitative biologist specializing in fungal RNA processing.

My collaborator, Dr. Ballou, is an expert in *C. neoformans* biology, including relevant culture conditions and models of host-pathogen interaction required for this proposal.

Dr. R Bayne has 30 years of experience in molecular biology techniques and will take a lead role in my technical/lab based training.

6: Retraining

In order to carry out the proposed aims of my fellowship I will vastly expand my knowledge of new exciting techniques like RNA-seq and CRISPR while updating existing ones such as qPCR.

This fellowship will increase my employability by bridging my skills gap using a framework for structured training of relevant skills in demand in today’s job market.

During this fellowship I will have access to 3 training courses from the Daphne Jackson Trust covering professional skills, how to publish and how to improve your confidence.

The Institute for Academic Development at the University of Edinburgh is a facility providing many opportunities for education and professional development.

During my training I will attend relevant conferences as these provide a national/international platform for sharing information and ideas and keeping up to date with the latest innovations and advancements.

7: Proposal: Intro

*C. neoformans* is an opportunistic facultative saprophyte and an important global human pathogen.

The long-term disease progression of *C. neoformans* is beginning to be characterized, however, there is very little understanding of the early stages of infection.

I will examine what happens when this organism reactivates within this alien environment by measuring differential gene expression under different conditions and relating this to crucial virulence factors, including capsule production.

By combining bioinformatics and experimental investigation essential genes can be identified and putative proteins can be characterized.

Aim1:

Previous investigations carried out by Dr. Wallace and Dr. Ballou, to dissect the contributions of host factors and temperature in shaping initial growth, found a distinct physiological response.

Current knowledge of the early events in *C. neoformans* infections are based on research using animal models or *in vitro* culture methods, primarily in rich fungal support media such as YPD during log phase growth.

The reactivation of Cryptococcus is radically different in rich media compared to cell culture media containing serum suggesting host-like media/serum induces capsule production.

In the first 4 months I will retrain in cell culture and microscopy techniques and apply this to growing and identifying yeast cell morphologies.

To determine whether the phenotypic change is due to serum or host-like media in the above experiment I will inoculate growth-arrested *C. neoformans* yeast cells grown in YPD (GA-Cn-YPD) into RPMI-1640 media and YPD + serum and incubate at 25⁰C and 37⁰C.

It is known that serum can induce capsule production in Cryptococcus11 but it is unknown which component(s) of serum cause this response.

If time allows I will test Minimum Essential Media (MEM) containing electrolyte and carbohydrate levels close to that in human serum and CSF.

Aim 2:

The Ballou lab have shown that the serum compound, Muramyl Dipeptide, induces a morphological change in *C. neoformans* from a normal yeast cell to a large polyploid titan cell.

How can bacterial cell wall components influence the behavior of co-infecting fungal pathogens?

I will examine the transcriptional response of serotypes A and D (H99 and JEC21) to bacterial cell wall components.

I will identify interactions between the fungal cell surface and bacterial cell wall components by incubating GA-Cn-YPD (fast growing) and GA-Cn-YND (starved) yeast cells in serum-free media (RPMI-1640 and/or MEM depending on results from Aim 1) with different concentrations of purified components of bacterial cell walls:

If time allows I will extend this study to other fungal species of the respiratory microbiome and other surfaces (e.g. *Saccharomyces cerevisiae* and *Candida, Aspergillus, Pneumocystis, Cladosporium, Eurotium, Penicillium sp)* and correlate the results to investigate whether expression of virulence factors is regulated by similar mechanisms/pathways.

Aim 3:

I will identify critical genes and probe their biological function in *C. neoformans* using simple models to mimic infection/host-like conditions.

Once high quality RNA-seq data have been obtained, reads will be trimmed, mapped to a reference genome, and quantified along individual annotated transcripts using software pipelines and genome annotations that are established in the Wallace lab.

Targeted gene mutations are necessary to study the function of genes and genome editing enables researchers to switch a gene of interest off, determine the change in phenotype and ultimately deduce the function of the gene product.

Impact:

This research is discovery driven and will advance knowledge regarding the fundamental biology of *Cryptococcus* and the initial stages of Cryptococcal infection in the lung.

Further work:

Using preliminary data produced during my fellowship I would like to investigate the possible relationship between fungi and bacteria in the microbiome to see if production of virulence factors in fungi are regulated or in some way modified by interactions with bacteria.

Future planning:

This fellowship will give me valuable exposure to the unique work environment and culture in research as well as the technical and soft skills needed to succeed in a research driven career.

By the end of the fellowship I would like to be in a position where I can be competitive by securing a good publication record and build up my scientific profile by participating in conferences, attending seminars and continuing to educate myself.

Following this fellowship my first choice would be to write a project grant as researcher co-investigator (BBSRC, MRC, Springboard Award, Wellcome trust, Leverhulme early career fellowship) with Dr. Wallace to continue my research in *C. neoformans* at the University of Edinburgh.

If I am unsuccessful securing funding to continue my research at the University of Edinburgh I will look for full time PDRA positions in an academic/research setting in central Scotland as my son will still be attending school in Lanark at this time.

My long term goal is to remain in research, however, if this is not feasible I will look to industry/biotechnology companies within central Scotland.