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

**Applicant:** Dr. Elizabeth Hughes

**Supervisor:** Dr. Edward Wallace

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

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

**Reasons for applying for a Fellowship**

The Daphne Jackson Fellowship with MRS presents an ideal platform 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 high-demand skills to help me develop as a person and move my career forward.

During my undergraduate degree I loved molecular biology and how it relates to the pathogenesis of disease. My PhD looked at the relationship of the HIV viral population to disease progression, primarily the evolutionary analysis of isolates infecting lymphoid and non-lymphoid tissues 1,2. This led to the discovery of a previously unknown dormant HIV population in the brain and a first author paper with over 100 citation 1.

Subsequently, I examined the replicative processes of Hepatitis C Virus (HCV) by determining whether the non-structural 5B protein (NS5B; predicted to possess an RNA dependent RNA polymerase activity) was capable of directing HCV replication. I optimized bacterial expression systems and purified the NS5B fusion protein.

I then investigated the structure/function of the major outer membrane proteins (MOMP’s) of *Chlamydia trachomatis* and *psittaci* where I cloned and expressed wild-type and short variable domain 4 (VS4) mutated proteins and functionally reconstituted them at the single-channel level. The VS4 domain was not required for pore formation but may help to form the channel3.

I then joined a leading bio safety testing facility, BioReliance, as the research and development scientist and developed a wide range of molecular based assays for clients as well as providing 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. Due to this 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 they are mature and resilient enough for me to return to my chosen career. I am excited to re-engage with the scientific community, even more so after visiting the Wallace lab in November 2018. I find the challenges associated with research both rewarding and enlightening and I look forward to returning to the lab environment.

Word count: 400 (400)

**Research Summary**

I will investigate the dynamic processing of mRNA and gene expression profiles of *Cryptococcus neoformans* in response to environmental stimuli. *C. neoformans* is a fungus that lives in the environment and infects people through inhalation of spores/desiccated yeast cells. Infection is rare in healthy individuals and most cases occur in individuals who have a weakened immune system. Infection of the lung causes a pneumonia-like illness, however, if it spreads to the brain, life-threatening meningitis can occur. It is estimated that 223 000 cases occur per annum world-wide resulting in approximately 181 100 deaths.

**Aim 1:** How does the rapid change in environment from soil/vegetation to a mammalian lung affect the gene regulation of *C. neoformans*? I would like to examine what happens when this fungus reactivates in a host environment alien to its normal life cycle.

**Aim 2**: Upon presentation to the lung *C. neoformans* will encounter a number of foreign bodies, some of which will be commensal and/or pathogenic bacteria. I would like to investigate the interaction with components of the bacterial cell wall to examine what effect this could have on the structure and infectivity of *C. neoformans*.

**Aim 3**: In the mammalian lung the first line of defense is the mucosal lining of the airways. This contains soluble effector molecules which help eradicate foreign bodies. One such molecule is SP-D which has anti-microbial properties and can regulate the immune response. SP-D will be one of the first effector molecules to interact with this pathogenic fungus and has been shown to help with its survival. I would like to investigate the binding of this molecule to *C. neoformans* to elucidate what changes occur in the gene expression during this event.

I will measure mRNA processing using RT-qPCR and further analyze with RNA-seq. Measuring the intermediate step between genes and proteins effectively bridges the gap between the genetic code and functional proteins. The number of transcripts can be quantified to provide information on the amount of gene activity under a given set of circumstances.

Word count: 335 (350)

**Abstract**

*C. neoformans* is an accidental pathogen of mammals. Its natural life cycle is that of an environmental saprophyte. The mammalian lung is an alien environment yet somehow this fungus can circumvent a myriad of defenses to cause disease. Upon inhalation into a host, *Cryptococcus* cells transition from spores/desiccated yeast into actively replicating virulent yeast cells. This transformation must be associated with dynamic regulation of mRNA and hence gene expression. What happens to *Cryptococci* when they reactivate within a mammalian host? I would like to investigate this phenomenon in detail during the early stages of infection. I will use quantitative and qualitative methods to examine differential gene expression of *Cryptococcus* under different environmental stimuli. Gene expression profiles represent a snapshot of cellular metabolism at the molecular level and may provide insights into novel therapeutic methods for drug discovery.

Word Count: 137 (150)

**Host Organization**

Edinburgh University is one of the world’s top universities, ranked 18th in the world, 5th in the UK and top in Scotland. As one of the UK’s leading research facilities Edinburgh University provides a state-of-the-art environment for cellular and molecular biology.

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. Included is the Edinburgh Genome Foundry (a world class facility for automated DNA design and assembly) and EdinOmics providing expertise in quantitative biochemistry including up-scaling and automation of RT-qPCR. There are facilities for cell imaging (LEAP and single cell analysis and microscopy groups) and for innovative data analysis and mechanistic modelling.

Dr. Edward Wallace, of the institute for Cell Biology, has agreed to be my supervisor for the duration of the fellowship. Dr. Wallace is a quantitative biologist specializing in fungal RNA processing. Working with him will allow me to build on my existing skills in the field of molecular biology whilst learning new techniques including high throughput RT-qPCR, cDNA library preparation and high throughput sequencing (RNA-seq), lab automation, bioinformatics, and working with 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. Dr. Rosey Bayne (PDRA/Lab Manager) has 30 years of experience in molecular biology techniques and will be invaluable during my re-training and introduction to new concepts and technologies.

Edinburgh University supports academic and personal development through the Institute for Academic Development. This program provides support, learning and research development opportunities throughout the year, including workshops, courses, online resources, networking and advice. These include workshops and courses on academic writing, career management and development, data management, funding opportunities, ethics, teaching and supervising students. During my fellowship I intend to make full use of these resources to enhance my personal and professional qualifications to help advance my career. Within the Institute for Cell Biology there is also a very dynamic seminar series covering a wide range of topics from internal and external speakers.

**Supervisor:** 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.

**Relevant collaborations:** Dr. Elizabeth Ballou. Sir Henry Dale Fellow and Lecturer. Institute for Microbiology and Infection, School of Biosciences, University of Birmingham.

Word Count: 399 (400)

**Retraining Programme**

\*Dr David Kelly, Wellcome Trust Centre for Cell Biology, University of Edinburgh runs the Wellcome Centre Microscopy course.

\*\*Edinburgh Genomics runs a Bioinformatics for Genomics 5 day workshop. Cost £750.

Word Count: 400 (400)

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

**Background:**

*C. neoformans* is an opportunistic facultative pathogen and the causative agent of cryptococcosis. 4,5*C. neoformans* is typically associated with pigeon guano, soil and decaying wood. It is a free living fungus with no requirement for mammalian virulence, however, it is able to adapt, survive and proliferate within a mammalian host to cause disease.

The natural ecology and disease progression of *C. neoformans* is well characterized, however, few studies have examined the differential gene expression and regulation of *C. neoformans* during early stages of infection. The ability to investigate the genetic response to environmental stimuli is a powerful tool to elucidate the adaptive response/responses required for this accidental pathogen to survive in a hostile environment. I would like to examine in detail what happens when this organism reactivates within the lung.

**Aim 1: What environmental stimuli in the host trigger an acute stress response in *C. neoformans*?**

Current knowledge of the early events in *C. neoformans* infection are based on research using animal models or *in vitro* culture methods, primarily in rich fungal support media such as YPD. While these culture methods have produced useful data about the infectivity and virulence of *C. neoformans,* they do not accurately reflect the lung environment, where nutrients are likely to be scarce.

Previous investigations were carried out by Dr. E Wallace, in collaboration with Dr. Elizabeth Ballou, designed to dissect the contributions of host factors and temperature in shaping initial growth. In this pilot study a distinct physiological response (capsule induction) and differential RNA abundance was documented between the different conditions (See Appendix 1).

**Objective:**

I would like to identify what causes this phenotypic shift by analyzing differential gene expression using RT-qPCR over a time-course to produce a snapshot of actively expressed genes under different environmental stimuli. This may shed light on the important steps for infection at early time-points.

**Method:**

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. I will examine the cells to determine any phenotypic changes and extract RNA for analysis using RT-qPCR (primers against differentially expressed genes previously detected by RNA-seq in the Wallace labs). I will compare results from these and the previous experiments to determine how the gene profile alters in relation to phenotype.

Analysis of this data will determine future studies. If the addition of serum to media is sufficient for capsule induction I will incubate GA-Cn-YPD in RPMI-1640 + purified albumin and RPMI-1640 + charcoal stripped FBS (CS-FBS). This will allow me to compare the effect of albumin and reduced levels of endotoxins and hormones (reduced in CS-FBS) on capsule induction. (Appendix 2).

If serum is not sufficient I will look at the impact removing phenol red from RPMI-1640 has on capsule induction. Phenol red has been described as a weak estrogen6 and such steroids have been shown to inhibit C. neoformans growth7 and have shown a synergistic/additive in vitro activity with drugs currently used to treat cryptococcosis 8,9. Also, recently the physiological relevance of culture media has been questioned where the electrolyte and carbohydrate concentrations may result in irrelevant changes in cell behavior10. For example, RPMI-1640 has very low concentrations of mg2+ and Ca2 and elevated levels of PO42-, Mg ions have been shown to act as a signal for capsule induction11. I will test MEM where these levels are close to that in human plasma and CSF (Appendix 3).

**Aim 2**: **Investigate how bacterial cell wall components influence gene expression in *C. neoformans*.**

Peptidoglycan and cell wall fragments are increasingly being recognized as important signaling molecules that can inhibit growth and virulence factors and influence morphology of other microbes. The addition of fetal calf serum (FCS) to media induces capsule formation in *C. neoformans*12. Bacterial cell wall components (in serum) have been shown to modify the morphology of *C. neoformans* from a normal yeast cell to a large polyploid titan cell 13. The peptidoglycan subunit muramyl dipeptide was identified as a component of serum associated with titan cell induction.

**Objective:**

I would like to dissect the role bacterial cell wall components play in the pathogenesis of *C. neoformans* in the lung. I will identify which components influence gene expression and examine changes in phenotype to determine the molecular mechanisms underlying these changes.

**Method:**

I will incubate growth arrested GA-Cn-YPD and GA-Cn-YNB in serum-free media with purified components of bacterial cell walls. Namely, peptidoglycan subunits N-acetyl glucosamine (NAG), N-acetyl muramic acid (NAM) and muramyl dipeptide found in gram positive and negative bacteria; lipopolysaccharide found in gram negative bacteria and teichoic acid (a major surface antigen) found in gram positive bacteria. These reagents are all commercially available. I will incubate cells at 25⁰C and 37⁰C if capsule was induced at both temperatures in aim 1. Otherwise, I will only carry out these experiments at 37⁰C. (Appendix 4). Depending on results from pilot studies I will further characterize the effect of lipopolysaccharide by incubating yeast cells with the subunits lipid-A (antigenic), core polysaccharide and/or o-polysaccharide.

I will examine the cells to determine any phenotypic changes under each condition. I will extract RNA for analysis using RT-qPCR and RNA-seq to identify unknown enriched genes or pathways.

**Aim 3**: **Investigate how SP-D influences gene expression in *C. neoformans*.**

Following inhalation, fungi will first encounter the mucosal surface of the lung which is covered in a film of surfactant that maintains surface tension during respiration and contains soluble effector molecules with antimicrobial properties, such as the collectins and host-defense peptides.

Collectins, surfactant proteins A and D and mannose binding lectins (SP-A, SP-D and MBL’s), have previously been shown to bind to *C. neoformans* 14–18. *In vitro* studies suggest SP-A has little effect on *C. neoformans* 19. In contrast, SP-D binds acapsular yeast with a high affinity (and capsular yeast with a lower affinity) causing profound aggregation, increased phagocytosis and enhanced fungal survival 15,16,20,21.Previous studies, using animal models, have suggested a protective role for SP-D in infection 22.

**Objective:**

I would like to investigate if binding of SP-D affects *C. neoformans* directly by analyzing fungal gene expression before and after binding of purified recombinant human SP-D (rh-SP-D). Direct binding of rh-SP-D may modulate gene expression of surface receptors in a synergistic or antagonistic way and could therefore reciprocally modulate virulence factors, such as capsule production/cell wall rearrangements, thereby enhancing fungal survival.

**Method:**

I will incubate rh-SP-D (commercially available) with yeast cells in a microtiter plate at various concentrations. I will analyze the plates for agglutination of *Cryptococci* and extract RNA for further qualitative and quantitative analysis using RT-qPCR and RNA-seq.

Functional profiling under differing stimuli may identify enriched pathways during the early phase of *C. neoformans* adaptation to the host lung. Analyzing the transcriptome of *C. neoformans* over a time-course under these selective environmental pressures may lead to micro-evolutionary adaptation of *C. neoformans* in the host lung and pave the way for possible new drug targets aimed at de-regulating virulence instead of directly killing the fungus.

**Challenges:**

Going from yeast cultures to identification of gene expression involves a multi-step approach including harvesting cells, RNA isolation, removal of genomic DNA, cDNA synthesis and RT-qPCR/RNA-seq. Although all these steps are established and routine in the Wallace lab, this multi-step approach can introduce inter- and intra-sample variation which must be normalized in order to make sense of the data produced.

Normalization is very important when comparing data from different experimental conditions in a quantitative and qualitative manner23. I will normalize global RNA levels by spiking in a 1:100 ratio of methanol-fixed *Schizosaccaromyces pombe* using three reference *S.pombe* genes. Analysis of RT-qPCR data can result in misrepresentation of the expression profiles obtained 24 I will overcome this by selecting 3-4 stable expressed reference controls and the geometric mean of these will be taken. These will allow me to normalize differences in the amount and quality of starting material as well as in the efficiency of the reaction. It is common to use ubiquitously expressed genes. RT-qPCR will be carried out under MIQE guidelines25.

Manipulation during the construction of the cDNA libraries can complicate the analysis of RNA-seq reads where PCR artefacts of short identical reads can be confused for genuine reflection of the RNA present. In order to overcome this limitation I will run 2 or 3 biological replicates and determine whether the same sequences are observed in each sample.

Normalization is an important prerequisite for any quantitative data analysis of gene expression. Different normalization approaches can have significant effects on the distribution and calculation of significant values (*P*-values)26,27. I will analyze RNA-seq data with DESeq2 in R. The package DESeq2 provides methods to test for differential expression by use of negative binomial generalized linear models.

During this project I will be dealing with many samples and carrying out RT-qPCR on many genes. In order to make this practical I will use the sate-of-the-art lab automation facilities at the Edinburgh Genome Foundry to automate RT-qPCR plate loading thereby reducing human error through repetition.

Word count: 1497 (1500)

**Ethical approval and licenses**

Awaiting information from Dr. E Wallace.

**Timetable**



1. Hughes ES, Bell JE, Simmonds P. Investigation of the dynamics of the spread of human immunodeficiency virus to brain and other tissues by evolutionary analysis of sequences from the p17gag and env genes. *J Virol*. 1997;71(2):1272-1280. http://www.ncbi.nlm.nih.gov/pubmed/8995651. Accessed November 20, 2018.

2. Hughes ES, Bell JE, Simmonds P. Investigation of population diversity of human immunodeficiency virus type 1 in vivo by nucleotide sequencing and length polymorphism analysis of the V1/V2 hypervariable region of env. *J Gen Virol*. 1997;78(11):2871-2882. doi:10.1099/0022-1317-78-11-2871

3. Hughes ES, Shaw KM, Ashley RH. Mutagenesis and functional reconstitution of chlamydial major outer membrane proteins: VS4 domains are not required for pore formation but modify channel function. *Infect Immun*. 2001;69(3):1671-1678. doi:10.1128/IAI.69.3.1671-1678.2001

4. Park BJ, Wannemuehler KA, Marston BJ, Govender N, Pappas PG, Chiller TM. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. *AIDS*. 2009;23(4):525-530. doi:10.1097/QAD.0b013e328322ffac

5. Harrison TS. The burden of HIV-associated cryptococcal disease. *AIDS*. 2009;23(4):531-532. doi:10.1097/QAD.0b013e328322ffc3

6. Berthois Y, Katzenellenbogen JA, Katzenellenbogen BS. Phenol red in tissue culture media is a weak estrogen: implications concerning the study of estrogen-responsive cells in culture. *Proc Natl Acad Sci U S A*. 1986;83(8):2496-2500. http://www.ncbi.nlm.nih.gov/pubmed/3458212. Accessed December 10, 2018.

7. Welshons W V., Wolf MF, Murphy CS, Jordan VC. Estrogenic activity of phenol red. *Mol Cell Endocrinol*. 1988;57(3):169-178. doi:10.1016/0303-7207(88)90072-X

8. Butts A, Koselny K, Chabrier-Roselló Y, et al. Estrogen Receptor Antagonists Are Anti-Cryptococcal Agents That Directly Bind EF Hand Proteins and Synergize with Fluconazole In Vivo. *MBio*. 2014;5(1):e00765-13. doi:10.1128/MBIO.00765-13

9. Mohr JA, Long H, McKown BA, Muchmore HG. *In vitro* susceptibility of *Cryptococcus neoformans* to steroids. *Med Mycol*. 1972;10(2):171-172. doi:10.1080/00362177285190331

10. McKee TJ, Komarova S V. Is it time to reinvent basic cell culture medium? *Am J Physiol - Cell Physiol*. 2017;312(5):C624-C626. doi:10.1152/ajpcell.00336.2016

11. Rathore SS, Raman T, Ramakrishnan J. Magnesium Ion Acts as a Signal for Capsule Induction in Cryptococcus neoformans. *Front Microbiol*. 2016;7:325. doi:10.3389/fmicb.2016.00325

12. Zaragoza O, Fries BC, Casadevall A. Induction of capsule growth in Cryptococcus neoformans by mammalian serum and CO(2). *Infect Immun*. 2003;71(11):6155-6164. doi:10.1128/iai.71.11.6155-6164.2003

13. Dambuza IM, Drake T, Chapuis A, et al. The Cryptococcus neoformans Titan cell is an inducible and regulated morphotype underlying pathogenesis. Mitchell AP, ed. *PLOS Pathog*. 2018;14(5):e1006978. doi:10.1371/journal.ppat.1006978

14. Walenkamp AM, Verheul AF, Scharringa J, Hoepelman IM. Pulmonary surfactant protein A binds to Cryptococcus neoformans without promoting phagocytosis. *Eur J Clin Invest*. 1999;29(1):83-92. http://www.ncbi.nlm.nih.gov/pubmed/10092994. Accessed November 22, 2018.

15. van de Wetering JK, Coenjaerts FEJ, Vaandrager AB, van Golde LMG, Batenburg JJ. Aggregation of Cryptococcus neoformans by surfactant protein D is inhibited by its capsular component glucuronoxylomannan. *Infect Immun*. 2004;72(1):145-153. doi:10.1128/IAI.72.1.145-153.2004

16. Schelenz S, Malhotra R, Sim RB, Holmskov U, Bancroft GJ. Binding of host collectins to the pathogenic yeast Cryptococcus neoformans: human surfactant protein D acts as an agglutinin for acapsular yeast cells. *Infect Immun*. 1995;63(9):3360-3366. http://www.ncbi.nlm.nih.gov/pubmed/7642263. Accessed November 22, 2018.

17. van Asbeck EC, Hoepelman AI, Scharringa J, Herpers BL, Verhoef J. Mannose binding lectin plays a crucial role in innate immunity against yeast by enhanced complement activation and enhanced uptake of polymorphonuclear cells. *BMC Microbiol*. 2008;8(1):229. doi:10.1186/1471-2180-8-229

18. Chaka W, Verheul AF, Vaishnav V V, et al. Induction of TNF-alpha in human peripheral blood mononuclear cells by the mannoprotein of Cryptococcus neoformans involves human mannose binding protein. *J Immunol*. 1997;159(6):2979-2985. http://www.ncbi.nlm.nih.gov/pubmed/9300722. Accessed November 22, 2018.

19. Giles SS, Zaas AK, Reidy MF, Perfect JR, Wright JR. Cryptococcus neoformans is resistant to surfactant protein A mediated host defense mechanisms. *PLoS One*. 2007;2(12):e1370. doi:10.1371/journal.pone.0001370

20. Geunes-Boyer S, Oliver TN, Janbon G, et al. Surfactant protein D increases phagocytosis of hypocapsular Cryptococcus neoformans by murine macrophages and enhances fungal survival. *Infect Immun*. 2009;77(7):2783-2794. doi:10.1128/IAI.00088-09

21. Geunes-Boyer S, Beers MF, Perfect JR, Heitman J, Wright JR. Surfactant Protein D Facilitates Cryptococcus neoformans Infection. Deepe GS, ed. *Infect Immun*. 2012;80(7):2444-2453. doi:10.1128/IAI.05613-11

22. Geunes-Boyer S, Beers MF, Perfect JR, Heitman J, Wright JR. Surfactant protein D facilitates Cryptococcus neoformans infection. *Infect Immun*. 2012;80(7):2444-2453. doi:10.1128/IAI.05613-11

23. Chen K, Hu Z, Xia Z, Zhao D, Li W, Tyler JK. The Overlooked Fact: Fundamental Need for Spike-In Control for Virtually All Genome-Wide Analyses. *Mol Cell Biol*. 2015;36(5):662-667. doi:10.1128/MCB.00970-14

24. Vandesomlele J. Accurate normalization of real-time quantitative RT-PCR data.pdf. 2002:1-12. doi:10.1186/gb-2002-3-7-research0034

25. Bustin SA, Benes V, Garson JA, et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin Chem*. 2009;55(4):611-622. doi:10.1373/clinchem.2008.112797

26. Aigner TMD, Sc D. Normalization strategies for mRNA expression data in cartilage research. 2008. doi:10.1016/j.joca.2007.12.007

27. Winata C, Moen LF, Østrup O, et al. Normalization of RNA-Sequencing Data from Samples with Varying mRNA Levels. 2014;9(2):1-7. doi:10.1371/journal.pone.0089158

**Future planning**

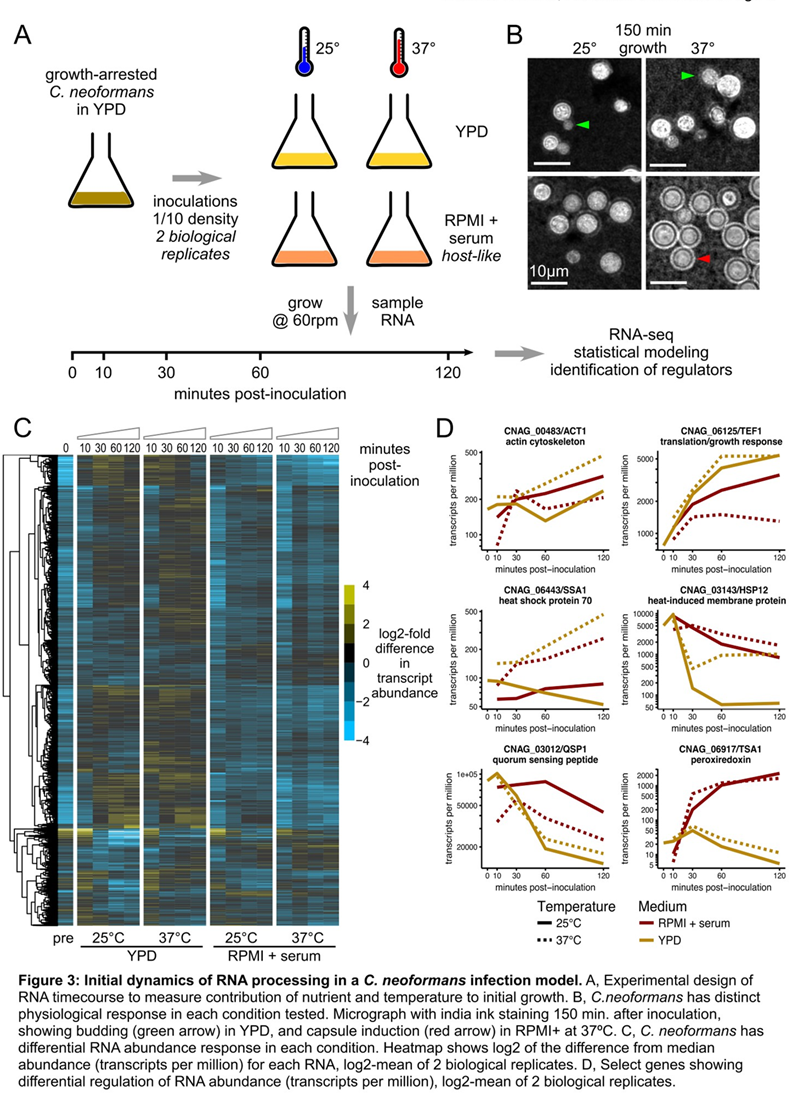
During my career re-entry fellowship I would like to be able to immerse myself in the lab, update my existing skills and learn new ones within the first year to enable me to develop as an expert in my field and build my confidence. In the subsequent two years I would expect to produce peer reviewed papers to consolidate my credentials which I could then use to secure further funding in order to continue my research and develop my independence.

I will use my time during this fellowship wisely to build my CV and boost my research and publication portfolio while working alongside world class researchers at Edinburgh University. It is essential to network and build relationships during this fellowship as part of my career development to find out about potential opportunities and to help me frame the kind of position that would suit me. 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 building up my scientific profile by participating in conferences, attending seminars and continuing to educate myself.

Following this fellowship I would like to continue in research, with a view to gaining a deeper understanding of the grant proposal process. Having spoken to Dr. E Wallace, my first choice would be to apply for a researcher co-investigator grant through the MRC or alternative funding bodies to continue my research in *C. neoformans*. Further studies to elucidate the early stages of infection would include studying bi-microbial cultures and the effects small signal peptides produced by microbes have on *C. neoformans*, gene expression in titan and daughter cells and the environmental impact of spores/desiccated yeast cells upon entry to the lung. I will remain open to PDRA positions in industry and biotechnology, however, my long term goal would be to remain in research. I believe my love for research, self-motivation and hunger for knowledge will really help me in this aim.

Word count: 361 (400)

**Appendix 1:** Contributions of host factors and temperature in shapinginitial growth.

**Appendix 2:** Aim 1 experimental plan: Dissection of serum components

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Media | No Serum  (Serum starvation) | FBS | Charcoal stripped FBS | Albumin | 25⁰C | 37⁰C |
| RPMI-1640 | ✓ |  |  |  | ✓ | ✓ |
|  |  | ✓ |  |  | ✓ | ✓ |
|  |  |  | ✓ |  | TBD | TBD |
|  |  |  |  | ✓ | TBD | TBD |
| YPD | ✓ |  |  |  | ✓ | ✓ |
|  |  | ✓ |  |  | ✓ | ✓ |
|  |  |  | ✓ |  | TBD | TBD |
|  |  |  |  | ✓ | TBD | TBD |

**Appendix 3:** Aim 1 experimental plan: Dissection of media components

|  |  |  |
| --- | --- | --- |
| Media | 25⁰C | 37⁰C |
| RPMI-1640 | ✓ | ✓ |
| RPMI-1640  (no phenol red) | TBD | ✓ |
| MEM | ✓ | ✓ |

**Appendix 4:** Aim 2 experimental plan.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| RPMI | Supplemented with | GA-Cn-YPD  (fast growing) |  | GA-Cn-YND  (starved) |  |
|  |  | 25⁰C | 37⁰C | 25⁰C | 37⁰C |
|  | NAG | TBD | ✓ | TBD | ✓ |
|  | NAM | TBD | ✓ | TBD | ✓ |
|  | Muramyl dipeptide | TBD | ✓ | TBD | ✓ |
|  | LPS | TBD | ✓ | TBD | ✓ |
|  | TA | TBD | ✓ | TBD | ✓ |