Referee report for M.Sc. thesis

Jarmila Tvarůžková (2015) The role of CSL proteins in oxidative stress response of *Schizosaccharomyces pombe*

The work presented in the thesis expands our knowledge on the molecular players during oxidative stress response with a focus on the workings of the fission yeast CSL proteins. The aims are to identify upstream and downstream regulators of CSL transcription factors. The results obtained are very useful to the field. Interestingly, *cbf11* deletion mutant cells are hydrogen peroxide but not menadione resistant and their oxygen consumption rates when compared to normal wild-type cells is very low. Moreover, mitochondrial architecture is disrupted in the mutant cells. The thesis contains the construction of a comprehensive list of double mutants for investigating the molecular players required as well as mediating the *cbf11*-dependent peroxide resistance. Indeed, it is found that Sty1 and Pap1 are key regulators in the process while sulfiredoxin and catalase are crucial in the observed resistance. By utilising transcriptome analysis it is clearly shown that cells lacking *cbf11* downregulate genes that code for proteins involved in electron transport chain/respiration. In addition, genes related to glycolysis and gluconeogenesis are upregulated. The study fullfils its aims and importantly opens new interesting questions.

I think that, overall, the quality of presentation is excellent. The aims of the work are presented coherently and the scientific rationale is clear throughout. The writing style is very good and guides well the reader on key concepts, mechanisms, results and interpretations. The figures look professional, are properly annotated and summarise the data gathered clearly and accurately.

The introduction is detailed and comprehensively covers the required background for results presentation and discussion. The literature is properly cited and sufficient. Jarmila has utilised a big list of techniques and both classical and high-throughput approaches . Full details of all Materials and Methods are given. All the statistical data analysis as well as the experimental design is explained and well justified.

The results presented are solid and publication quality. The data are presented in a consistent format with no mistakes or mislabeling. Positive quantitative and qualitative results are properly covered. Nevertheless, negative results are often referred as data not shown.

The Discussion is extensive and shows clear thinking and sharp interpretation of the results. Importantly, it shows ability to form and shape new hypotheses in the field.

In summary, this report reads very well clearly describes the diverse procedures that have been carried out and maked a great contribution in the field of oxidative stress response. My suggested grade for the thesis is *excellent.*

I am not familiar with the Department's thesis guidelines and size limits. However, negative results could have been presented if not in the main part oft he thesis probably within an Appendix at the end. It is very useful to include such data for future reference.

Below are a few questions regarding the work. I would appreciate a short reply from Jarmila.

1. Transcriptome analysis shows that genes encoding ATP synthesis coupled transport genes are downregulated in the absence of *cbf11.* A heatmap is also provided. Is there a difference between the behaviour of mitochondrially vs nuclear-transcribed subunits?

2. While Sty1 is required for hydrogen peroxide resistance of *Δcbf11* cells, Atf1 is dispensable. This phenomenon is discussed and explained by utilisation of alternative transcription factors. Could you please comment whether Sty1 can act itself as a transcription factor or its workings might also be related not to transcription but to translation?

3. It is nicely mentioned that nutrient-dependent respiration defect of *Δcbf11* cells could be due to deregulation of PKA and TORC1 nutrient-responsive pathways. Could you propose two feasible biochemical experiments to investigate this scenarios?

4. The genetic interaction of *cbf11* and *prr1* is questionable. In addition, the contribution of *cbf12* in oxidative stress response seems to be true but subtle. Could you propose a more sensitive assay compared to spot testing for stress sensitivity that might include growth kinetics of cell populations?

General remarks

I think the work contributes to our understanding of oxidative stress response in eukaryotes. A significant value of this work is that it opens new avenues of research.

There's a poor correlation between respiration rates, ROS production and their impact and relationship on growth, viability and ageing. Although briefly mentioned, this topic is of great interest to diverse communities and fields and should be discussed further. I think that the tools to explore such relationships have been built through this work and could be further explored.

Mitochondrial retrograde signaling (or retrograde response) is a pathway of mito-nuclear communication from mitochondria to nucleus. This pathway influences cellular and organismal activities in both normal and pathological conditions. Normal mitochondrial function requires continuous information exchange of the organelles with the nucleus. Mitochondrial needs or dysfunction, therefore, should initiate re-adjustments in cellular energetics even by trancriptional means. In addition, retrograde response is linked to TOR signaling pathway. I find very exciting the scenario that the cbf11 protein participates in mitochondria monitoring and integrity by *informing* the nuclear genome. Cbf11 fullfils several intriguing criteria: There is genetic interaction with *sck1* and mutant cells have a growth defect (TOR connection). Mitochondrial architecture is disrupted but respiratory defects can be rescued by PKA or SAPK pathways (impaired mito-nuclear communication that can be mended?). A fraction of cbf11 protein is found in mitochondria while heterochromatin seems perturbed in the absence of cbf11. Possible future experiments will show whether cbf11 has such a role. Equally intriguing is the fact that the same mechanisms might be conserved among Kingdoms (mitochondrial ROS can modulate Notch signaling).

London, 5th June 2015

Dr Charalampos (Babis) Rallis
Associate Lecturer A (tenured, Assistant Professor level)
Birkbeck College, University of London
Research Associate
Department of Genetics, Evolution and Environment and Institute of Healthy Ageing
University College London, Darwin Building, Gower Street,
WC1E 6BT, London, United Kingdom