



Report

on MSc thesis

Title: The molecular mechanism of CSL protein participation in oxidative stress response in *Schizosaccharomyces pombe*

Author: Petr Daněk

As high levels of reactive oxygen species have harmful effects on many basic cellular functions and developmental processes, the topic of this thesis is worthy of exploration. Petr Daněk has done a very good job in the investigation of the role of the two putative counterparts of Metazoan CSL proteins of the fission yeast *Schizosaccharomyces pombe*, a model organism assumed to be closer to Metazoans than most other groups of fungi.

The author worked in a team and certain his results can only be evaluated as parts of the team's project. Therefore certain relevant experimental data produced by Dr Přeavorovský or other members of the lab are also shown in certain paragraphs. It must be noted for the author's benefit that he always indicates the source of such data.

The thesis is divided in 8 chapters. Chapter one introduces the subject by way of providing a short description of the response of the *S. pombe* cells to the oxidative stress and some earlier results of the author's research team which demonstrated that this organism possesses homologues of metazoan CSL proteins which are involved in the regulation of stress signaling.

Chapter two defines the objectives of the work: investigation of the role of these metazoan homologues in oxidative stress response and their functional interactions with the Pap1 and Sty/Atf1 pathways. The research tasks and problems are clear and carefully justified.

Chapter three provides a thorough and comprehensive overview of previous results obtained by scientists who have contributed to this research direction. The review of literature is adequate and quite exhaustive: 25 pages and 154 listed references. It perfectly proves the author's familiarity with the studied phenomena and the molecular biology of *S. pombe*.

The Materials and Methods chapter describes an amazingly broad spectrum of experimental methods and techniques. The qPCR and chromatin immunoprecipitation methods are particularly demanding for an MSc project. In spite of this, they were successfully mastered in the work.

The results (Chapter five) are presented in an interesting, clear and organised way. The structure is clear and logical, and the research process is easy to follow. The text and figures/tables constitute a harmonious entity, which allows a profound and credible interpretation. The figures and tables support the interpretation of results particularly well.

In Discussion, the author's data are interpreted and evaluated in relation to previous studies in a versatile manner. It is demonstrated that one of the two CSL-like proteins of *S. pombe*, Cbf11, is a negative regulator of several stress responsive genes and performs its

regulatory functions indirectly through interactions with the Sty1/Atf1 and Pap1 pathways involved in the oxidative stress response of this organism. The other protein, Cbf12, does not seem to be involved in the regulation of these genes.

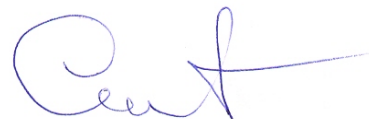
The summary is very precise and succinct. It focuses on the issues which were addressed and highlights the new information obtained.

Questions:

1. Are you sure that Cbf11 and Cbf12 are paralogues? Paralogues are genes/proteins related by duplication within a genome. Jensen, Genome Biology 2001: „Orthology and paralogy differ in that one proceeds from speciation and the other from gene duplication, but either evolutionary course of divergence has the same potential for acquisition of new properties”. Has anybody demonstrated that they were generated by duplication? The presence of a conserved domain in two proteins does not necessarily mean that the two proteins are products of an (ancient) duplication event.
2. In contrast to the Metazoan CSL proteins, Cbf11 appears to affect the activity of the stress-response genes indirectly, and Cbf12 seems to be inactive in the oxidative stress response. These differences indicate an enormous functional divergence between the animal and the Taphrinomycotina proteins. The author perhaps has a hypothesis about which evolutionary lineage might have evolved faster after their separation: which groups of proteins might be closer to those of the common ancestors of Metazoa and Fungi?

Overall, the thesis is well-structured, easy to read, the results are clearly presented and correctly interpreted. Brushing aside the minor typographical and editing deficiencies (e.g. non-specific description of the origin of strains) the thesis may be considered as a fine piece of work. It is undoubtedly of such a quality that it makes a significant contribution to the advancement of knowledge in the field. Considering also the enormous amount of work that has gone into the making of this thesis, I have no hesitation in recommending the thesis for acceptance for the award of the MSc degree in Biology to the author.

Debrecen, 06.09.2015.



Matthias Sipiczki
professor