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**Review of the PhD thesis of mgr Agnieszka Thompson**  
presented in order to obtain a PhD degree from the Faculty of Biology at the Adam  
Mickiewicz University in Poznań.

Thesis title: **Biogenesis and Function of tRNA-derived Fragments in *Arabidopsis thaliana***

In recent few years tRNA-derived fragments, their origin and function have attracted considerable interest of several researchers. However, the available data concern so far mostly these fragments in mammals and yeast, leaving tRNA-derived fragments in plants highly unexplored. Therefore, I found the topic of the Thesis very interesting and scientifically sound.

The research presented in the Thesis was conducted at the Department of Computational Biology, Institute of Molecular Biology and Biotechnology, Faculty of Biology, The Adam Mickiewicz University in Poznań, under the supervision of Professor Wojciech Karłowski.

In the work bioinformatics approaches were used to search for tRNA-derived fragments in a model plant *Arabidopsis thaliana*. A database of these fragments named tRex was developed and using built-in tools, biogenesis and functional potential of selected tRNA-derived fragments were characterized.

The Thesis is organized in a standard way and its main chapters are as follows: Introduction, Aims of the study, Materials, Methods, Results and discussion, Conclusions and perspectives as well as the References list containing approx. 220 positions. Three appendices are attached to the Thesis with detailed data derived from the research. The Introduction chapter (10 pages) provides basic information on tRNA molecules, their maturation, structure and

modifications. In the last part of the chapter databases and resources dedicated to research on tRNA-derived fragments are described in detail. This information is highly beneficial to the reader, since it places the major achievement of the thesis – the tRex web portal, among other similar databases and programs which are publicly available. The Introduction chapter is very condensed but it contains all the necessary information crucial to follow the Author's work and discussion.

The Materials and Methods chapters (14 pages) are complete and clearly presented. The tRex datasets encompassed 300 in-house and publicly-available sRNA-Seq libraries. The in-house libraries came from earlier studies on *Arabidopsis thaliana* which were conducted at the collaborating Department of Gene Expression (by Prof. Z. Szweykowska-Kulińska and Prof. A. Jarmołowski). In the tRNA annotation procedure the Author took advantage of the assistance of experienced colleagues from the Computational Biology Department. In the Methods chapter several computer programs, publicly-available and those based on in-house modified algorithms, are described.

The Results and discussion chapter (56 pages) is divided into three main parts. In the first part, the tRex web portal is described. Importantly, the portal is the first repository of tRNA-derived fragments in *A. thaliana*. Over 1.5 million tRFs have been deposited in the database. For the purpose of their better characterization the existing classification of tRFs is extended to nine groups. The portal is equipped with tools and predictions for exploratory analysis of tRFs in various genotypes, tissues, developmental stages and environmental conditions. In particular, the presence of predicted modification sites for all applicable samples is also incorporated in the tRex web portal, as well as putative target sites for tRFs in the *A. thaliana* genome. The tRex database structure is outlined in Figure 1, which is, however, very small in the hard copy of the Thesis, and therefore, it is hardly readable. I am curious whether the portal with its exploratory analysis devices can be expanded to also include tRNA-derived fragments from other plant organisms?

In the second part the exploratory potential of the tRex portal was used for elucidation of tRF's biogenesis pathways. The analysis involved the comparison of tRFs behaviour between different *A. thaliana* mutants and their wild-type controls. Biogenesis analysis workflow was elaborated and following a multi-step analysis several clusters were distinguished which produced similar profiles affecting a single family of tRNA. Subsequently, selected mutant

clusters were characterized in terms of enzymes possibly involved in tRFs biogenesis, their predicted nucleotide modifications and putative binding sites to *A. thaliana* RNA sequences. Several interesting hypotheses were formulated in this part of the Thesis, which undoubtedly are worth of experimental verification. In my opinion, in this part the Author documented a high level of expertise in the tRNA-derived fragments field. However, unclear to me is the attempt to investigate aspects of tRNA structure which affect tRF biogenesis which is based on the results of MFE analysis. I wonder, whether such conclusions are correct, taking into account that computer programs for prediction RNA secondary structures are still imperfect? Could the Author comment on this issue during the public defense of her dissertation?

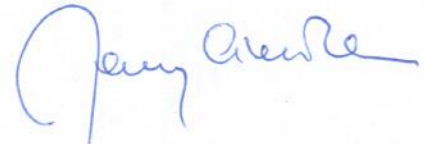
In the third part, in order to identify the stress-responsive tRNA-derived fragments, a differential expression analysis on sRNA-seq libraries from various stresses was performed. The strongest response to stress was observed under excess salinity (NaCl). Interestingly, a stress response towards dehydration was quite unexpected; a 20% drought produced many fragments exhibiting gain or loss of their amounts relative to the control while in 30% drought conditions no significant changes were observed between stressed and control samples. All the information obtained in this phase of the research was summarized in two tables (No 4 and 5) where 12 previously characterized and 109 novel, stress responsive tRFs were included. For selected tRFs the Author analyzed also their putative target sites. Analyzing the data for 20% and 30% drought stresses the Author formulates an attractive hypothesis on the role of nucleotide modifications in generating stress-responsive tRFs. It is of note that although several examples of tRF's modification are known they have not been reported in plants so far.

In the last chapter Conclusions and perspectives the Author collects all the hypotheses that came out from her bioinformatics studies. She is aware of the fact that they would need experimental verification, likely, with the involvement of at least a few PhD students. Which of these hypotheses, in the Author's opinion, would deserve experimental verification first?

To conclude this report, the results presented in the Thesis are novel and highly useful for plant biologists. The data described shed a new light on the origin of tRNA-derived fragments and their plausible biological functions. The tRex web portal has been described in a paper published in the *Plant and Cell Physiology* journal, with Agnieszka Thompson as the first author. She is also the second author in a paper that has been very recently published in *RNA*

Biology. These remarkable achievements and high competence of mgr Agnieszka Thompson in the area of RNA bioinformatics documented in her PhD thesis prompted me to ask for considering awarding her an appropriate scientific award.

Taking all the above into account, I recommend the Scientific Board of the Faculty of Biology, The Adam Mickiewicz University in Poznań, to proceed with further procedural steps to confer mgr Agnieszka Thompson the degree of Doctor.

A handwritten signature in blue ink, appearing to read 'Agnieszka Thompson', is written in a cursive style.