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**REPORT ON THE DOCTORAL THESIS OF MSC WIKTORIA SZTANGIERSKA**

PhD thesis title: **Role of yeast nucleotide exchange factor (Sse1) in functioning of Hsp70 chaperone system in protein disaggregation**

PhD candidate: **mgr Wiktoria Sztangierska**

PhD thesis supervisor: **prof. dr hab. Krzysztof Liberek**

PhD thesis assistant supervisor: **dr Agnieszka Kłosowska**

The PhD thesis presented by Wiktoria Sztangierska is aimed at determining the role of yeast nucleotide exchange factor Sse1 in functioning of Hsp70 chaperone system in protein disaggregation. The doctoral studies were carried out in the Laboratory of Protein Biochemistry at the Intercollegiate Faculty of Biotechnology, University of Gdańsk and Medical University of Gdańsk under the supervision of Professor Krzysztof Liberek and under the co-supervision of Dr. Agnieszka Kłosowska.

Proteins are essential components of all living cells. Since proteins fulfill a myriad of biological functions in all organisms, the balance within the proteome must be tightly controlled to ensure the cellular and organismal well-being and integrity. Protein synthesis, modification, folding, assembly and transport, and finally elimination, are error-prone processes. Moreover, during their whole lifetime, proteins are exposed to many stress conditions. The unfavorable processes and conditions may lead to protein misfolding and aggregation. The topic of the presented dissertation is related to the important problem of how the cell counteracts the toxic effects of protein misfolding and aggregation. Living cells developed various strategies to restore misfolded or aggregated proteins their native shapes and functions. One of the strategies relies on protein disaggregation, where proteins trapped in aggregates are rescued by molecular chaperones. In yeast, the disaggregation system comprises an ATP-dependent chaperone Hsp70, its partnering co-chaperones: an Hsp110 nucleotide exchange factor and a J-domain protein, and a disaggregase from the Hsp100 family. The doctoral research concerns new aspects of the protein disaggregation process, precisely the involvement of the yeast Hsp110 nucleotide exchange factor Sse1 in protein disaggregation, and the problem of how the Hsp70 activity is influenced by Sse1, when paired with different J-domain protein classes (class A or class B).

The doctoral dissertation has a standard layout, and it contains the following sections: Abstract (Polish and English version), Abbreviations, Introduction, Aim of the project, Materials, Methods, Results, Discussion and References. In general, the dissertation is clear, coherent and well structured. I found only a few minor editorial errors, which in no way detract from the readability of the work (e.g. missing panel letters in the figure captions - Figure 30).

The Introduction section sets up the topic of the research and provides basic information on the mechanisms underlying protein aggregation, chaperone network in the proteostasis collapse, chaperones and co-chaperones involved in protein disaggregation. Descriptions of proteins and the mechanisms in which they are involved are accompanied by helpful illustrations adapted from the published literature; all of them are adequately referenced. In general, the Introduction provides a good background for the doctoral research, though, in my opinion, it could benefit from a table summarizing the chaperones and co-chaperones mentioned in this section (a summary of known bacterial, yeast and human homologs). At the end of the section, Mrs. Wiktoria Sztangierska emphasizes that the involvement of Hsp110 in protein disaggregation, considering the mechanism of interaction with Hsp70 and different J-domain protein classes, has not been investigated. On this basis, in the next section, the aim of the project was formulated; it is clear and precise.

The Introduction and Aim of the project sections are followed by a description of the materials and methods that have been used during the experimental parts of the doctoral thesis. To answer the clearly defined research questions, the PhD candidate used a variety of techniques from the area of molecular biology and biochemistry, including plasmid DNA preparation, protein production using *E. coli* system, protein purification, protein-protein interaction studies by bio-layer interferometry (BLI), luminescence-based assays, fluorescence microscopy imaging and Western blotting. Descriptions of the applied protocols are clear and legible.

The section describing the experimental results consists of nine subsections relating to the formulated research aims and objectives. Each subsection starts with the scientific rationale that guided the experiments. In general, the data collected by Mrs. Wiktoria Sztangierska strongly indicates that the yeast nucleotide exchange factor Sse1 plays a major role at the initial stages of protein disaggregation, exerting beneficial impact on Hsp70 (Ssa1) binding to the aggregates particularly in the presence of the J-domain protein from class B (Sis1). More precisely, Mrs. Wiktoria Sztangierska demonstrated that Sse1 enhances the remodeling activity of the Hsp70 system, specifically with class B J-domain protein Sis1, by causing major changes in the morphology of the aggregates (aggregates were modified into smaller aggregate species). She also found that the class B-specific binding of Sis1 C-terminal domain with the specific amino acid motif (EEVD) of Ssa1 is a key issue in the Sse1-dependent stimulation. Moreover, the PhD candidate showed that the high affinity of the yeast nucleotide exchange factor Sse1 for Ssa1 (Hsp70) enables strong stimulation of Hsp70-dependant disaggregation only at very low concentrations of Sse1; high Sse1 concentrations negatively affected the recovery of the aggregated substrates. This inhibitory effect could be explained, at least in part, by competition between Sse1 and Sis1 for Ssa1 binding (Sse1 restricted Ssa1 from binding to Sis1). Thus, the results presented by Mrs. Wiktoria Sztangierska highlight the significance of maintaining a delicate equilibrium between Sis1 and Sse1 for the efficient Hsp70-dependent disaggregation. Finally, Mrs. Wiktoria Sztangierska showed that human Hsp110 follows similar trends in regulation of Hsp70-dependet disaggregation as the yeast Hsp110 Sse1. The results of the doctoral research were presented in the form of numerous graphs and images. Overall, this section is well organized and

clear, though I have one minor comment: Figures 27-29 would be easier to follow, if their captions included detailed information on the concentrations of the titrated Sse1. I also appreciate that Mrs. Wiktoria Sztangierska precisely indicated which experiments were performed by other members of the research group involved in the project concerning the interplay between Hsp110, Hsp70 and J-domain proteins in aggregate disassembly (I have no doubt that the PhD student's participation was dominant). I also have a question: since the PhD candidate did not present gel images of the purified proteins, I would like to ask about the homogeneity of the proteins used in her studies?

In the Discussion section, the PhD candidate refers to the defined research aims and questions. The obtained results are discussed in the context of other findings in the field. Both older publications presenting the first reports on the discussed research topic and more recent ones are cited. The PhD candidate speculates about future directions and proposes solutions for the identified research problems. While I appreciate the scheme presenting a proposed mechanism of action of Hsp110 and its impact on Hsp70-dependent disaggregation (Figure 42), in my opinion, it would be beneficial, after the Discussion section, to summarize the key findings from the doctoral research in the form of bullet-points.

To summarize, I would like to underline that the study presented by Wiktoria Sztangierska provides an important and new insight into the mechanisms of cooperation between the yeast chaperone Ssa1 (Hsp70) and its partnering co-chaperones: nucleotide exchange factor Sse1 (Hsp 110) and class A J-domain protein Ydj1 or class B J-domain protein Sis1, during protein disaggregation. The collected results demonstrate that Sse1 increases Hsp70 recruitment to protein aggregates. The obtained data may be of broad interest and scientific significance to researchers working in the protein homeostasis field. Taking into account the practical aspect of the presented research, understanding the molecular pathways associated with protein disaggregation is essential to developing therapeutic strategies for diseases linked with protein misfolding and aggregation, including neurodegenerative disorders; e.g. Parkinson's, Alzheimer's or Huntington's disease. There is no doubt that it would be of great importance to uncover to what extent the yeast and human protein disaggregation mechanisms are conserved.

Here, I also have some general questions to the PhD candidate:

- What happens in case of chaperone misfolding or aggregation? Do other chaperones help to restore misfolded or aggregated chaperones their native shapes and functions?
- What is a half-life of the chaperone proteins? Does it depend on stress conditions?
- Are there any known naturally occurring mutations in human chaperone proteins that may affect their functioning or cause disease?

Taking all the above into account, I state that the doctoral dissertation by Wiktoria Sztangierska certainly meets all the conditions specified in the Journal of Laws of the Republic of Poland (Dz. U. 2018 poz. 1668. z późń. zm.) for the doctoral dissertations. Therefore, I recommend that the Scientific Council of Biotechnology, Intercollegiate Faculty of Biotechnology UG&MUG would admit Mrs. Wiktoria Sztangierska for the subsequent stages of the doctoral proceedings.

Z pełnym przekonaniem stwierdzam, że przedstawiona mi do oceny rozprawa doktorska Pani Wiktorii Sztangierskiej spełnia warunki określone w ustawie Dz. U. 2018 poz. 1668. USTAWA z dnia 20 lipca 2018 r. Prawo o szkolnictwie wyższym i nauce z późniejszymi zmianami i wnioskuję do Rady Dyscypliny Biotechnologia Międzyuczelnianego Wydziału Biotechnologii UG i GUMed o dopuszczenie mgr Wiktorii Sztangierskiej do dalszych etapów przewodu doktorskiego.

Moreover, I would like to emphasize that I appreciate the in-depth analysis of the functional cooperation between the yeast nucleotide exchange factor Sse1 and class B J-domain protein Sis1 in the protein disaggregation. Given the high quality of the research and generated knowledge providing new data on functioning of the yeast Hsp70 chaperone system in protein disaggregation, I recommend that the research effort made by Mrs. Wiktoria Sztangierska should be awarded with distinction.

Podpisany certyfikatem wystawionym dla Anna Kurzyńska-Kokorniak (Certyfikat kwalifikowany). Utworzony w dniu: 2024-08-30 10:26:26 +0200

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