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Review of the doctoral dissertation of Hubert Wyszkowski "Role of J-domain proteins in regulation of Hsp70-mediated protein disaggregation"

This work describes a very thorough biochemical characterization of representatives of two classes of J-domain proteins that assist the Hsp70 chaperone in the disaggregation of protein aggregates. This research is in an important field of chaperone proteins that play a fundamental role in maintaining protein homeostasis in the cell. The importance of these processes is underscored by the fact that dysregulation of protein homeostasis in humans leads to a variety of diseases, in particular neurodegenerative disorders with characteristic amyloid formation. The laboratory of this thesis advisor has made numerous contributions to the field of chaperone proteins. It is one of the leading laboratories studying these proteins and excels in careful biochemical studies of their properties.

The contents of this thesis have been published in the prestigious Proceedings of the National Academy of Sciences, so this research has already undergone rigorous and thorough peer review. Publication in PNAS is a major accomplishment. This is one of the reasons why this review will be rather brief and the assessment of this thesis can only be positive. I will not describe the entire content of the thesis in detail. That will be done during the defense. I would like to highlight the elements of this research that I found particularly valuable.

The introduction presents the aspects of the field that are most relevant to the experimental part of the thesis. It gives a broad overview of chaperone proteins and their functions. It describes the mechanisms of protein aggregation and the properties of molecular chaperones, in particular Hsp70, its co-chaperones (J-domain proteins and nucleotide exchange factors) and Hsp100 disaggregases. J-domain proteins (JDPs) are the main regulators of the major chaperone system that includes Hsp70. They promote the interaction of Hsp70 with its substrates and stimulate its ATPase activity. Humans and yeast have 41 and 22 J domain proteins, respectively, which are involved in many processes. The classification of JDPs is still under debate. However, a division into three classes has been proposed. Among them class A JDPs contain a J domain, a G/F-rich region, C-terminal domain I (CTDI) with a zinc finger-like domain, CTDII, and a helical dimerization domain. Class

B JDPs lack the zinc finger-like domain. Deciphering the functions of these proteins is an active area of research. The representatives of class A and B JDPs from yeast are Ydj1 and Sis1, respectively, and their properties are the main subjects of this work.

Another element of the system studied are the disaggregases present in yeast, which form hexameric rings that undergo specific motions in the cycles of ATP hydrolysis and release, and these motions are used to translocate polypeptide chains through the central channel of the hexamer, which can be used to mechanically disentangle aggregates. Overall, the introduction provides a good overview of the topic, highlights the information relevant to the thesis, and explains well the rationale for conducting the studies described in the thesis.

The Methods section contains a thorough description of the experimental procedures and should allow repetition of the experiments performed. Hubert used a particularly well-suited methods to study this system, which allowed real-time observation of protein binding and disaggregation activities. This is perhaps the most innovative methodological element of the studies presented. In particular, the work relies heavily on biolayer interferometry methods. A comment on the methodological part of the thesis is that it would be important to present an analysis of the purified proteins used in this study (i.e. by SDS-PAGE), so that the purity of the proteins used can be assessed.

The starting point for this project was the initial characterization of the disaggregation activity of the Hsp70-Hsp100 system in the presence of Ydj1 and Sis1, performed in real-time experiments using luciferase or GFP aggregates. The results showed that reactions with Sis1 resulted in more efficient protein disaggregation than Ydj1. In addition, the Sis1 reaction started with a delay. These results suggested different modes of action for the two proteins. Next, Hubert used biolayer interferometry. He immobilized aggregates of luciferase, GFP or yeast lysate proteins on the sensor and monitored the binding of Hsp70-Sis1 and Hsp70-Ydj1. The results showed that much more of the Hsp70-Sis1 system was loaded on the aggregate than the Hsp1-Ydj combination. Hubert also showed that Ydj1 could bind aggregates on its own, while Sis1 bound weakly. The experiments included carefully designed controls to show that the observed effect was specific to the JDP present. Next, Hubert tested how the studied JDP-Hsp70 systems interact with the Hsp100 disaggregase (Hsp104). The docking of Hsp104 is enabled by the creation of its binding sites by Hsp70. The

Ydj1-Hsp70 system loaded much less Hsp104 on the aggregate compared to Sis1-Hsp70. This was consistent with an earlier finding that Sis1 loads more Hsp70 on the aggregates. These results provided the basis for a model in which Ydj1 binds protein aggregates more strongly, but is less effective at recruiting Hsp70 and is less efficient at promoting aggregate remodeling. Sis1 requires Hsp70 to bind aggregated proteins but is more efficient at loading Hsp70 onto the aggregate, remodeling it and allowing Hsp100 disagregase to do its job. In my opinion, this is the most important take-home message of this work.

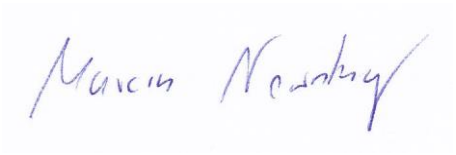
Additional work was done to gain further insight into the mechanism behind the different behavior of the JDPs studied. In particular, Hubert wanted to understand the different kinetics of disaggregation in the presence of the two studied JDPs. He used clever experiments in which he either performed two rounds of Hsp70-JPD binding or used aggregates cross-linked with glutaraldehyde. The results showed that the Sis1 complex remodels the aggregate (loosens its structure), while the Ydj1 complex does not. Experiments with a hyperactive Hsp104 disaggregase mutant that functions independently of Hsp70 showed that aggregate remodeling allows more efficient disaggregation.

In a series of elegant experiments, Hubert also showed that the EEVD motif of Hsp70 is required for the binding of protein aggregates by the Hsp70-Ssa1 system, but not by the Hsp70-Ydj1 system. A mutant of Sis1 that functions independently of EEVD binding was also tested and was partially functional with ΔEEVD Hsp70. These results and additional experiments confirmed that the interaction between the CTDI of Sis1 and the C-terminal EEVD motif of Hsp70 plays a role in aggregate remodeling and protein extraction from aggregates. Here I would like to ask Hubert to discuss the following points. There are two binding interfaces between Hsp70 and Sis1 (NDB - J domain and EEVD - CTDI). The model presented in the paper suggests that one Sis1 dimer will bind four Hsp70 molecules. Can one exclude the possibility that both interfaces are involved in the formation of a 1:1 Hsp70:Sis1 interaction? Is it sterically possible that such a simultaneous interaction of both J-domain and CTDI of Sis1 with a single Hsp70 molecule occurs? Are there any data on the stoichiometry of the Hsp70-Sis1 complex in vitro? What methods could be used to determine this stoichiometry? In addition, I would like Hubert to discuss whether the available structures of Sis1 and Ydj1 fragments provide any clues to explain their different mechanism of disaggregation.

The thesis also includes a rather preliminary characterization of human JDPs from two classes of these proteins. The human system is fundamentally different because of the absence of Hsp100 disaggregase equivalents. The results suggest a similar behavior of the human and yeast proteins, but further detailed studies will be needed to firmly establish this.

Overall, the results are very clearly presented. The schematics of the experiments are very helpful in understanding their design. The experimental part nicely combines real-time experimental methods for monitoring aggregate binding with a thoughtful use of available protein variants to provide a consistent picture of the mechanism and potential functions of representatives of two classes of JDPs. The discussion section is well written and nicely describes how the different properties of Sis1 and Ydj1 complement each other in maintaining protein homeostasis.

Overall, I rate the quality of the PhD student's research highly. He achieved the goal of deciphering the differences in the mechanism and properties of two classes of JDPs. The dissertation presented to me for evaluation describes research at an excellent technical and scientific level. I believe that the dissertation meets the conditions set forth in the Act on Higher Education and Science (Dz. U. z 2018 r. poz. 1668 z późn. zm.) and with full conviction propose to admit Mr. Hubert Wyszkowski, M.Sc. to further stages of the doctoral process. At the same time, taking into account the high level of the dissertation, an important field of research and the publication success, I propose to award the dissertation with a distinction.



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