Prof. Matthias Bochtler

International Institute of Molecular and Cell Biology &
Trojdena 4, 02-109 Warsaw, Poland
Institute of Biochemistry and Biophysics
Pawinskiego 5a, 02-106 Warsaw, Poland
Tel: 0048 22 5970732
Fax: 0048 22 5970715
e-mail: mbochtler@iimcb.gov.pl

Opinion on the PhD thesis by Wiktoria Sztangierska

Wiktoria Sztangierska has presented a PhD thesis entitled "Role of yeast nucleotide exchange factor (Sse1) in functioning of Hsp70 chaperone system in protein disaggregation". The thesis deals with the biology of chaperones and protein disaggregation, and more specifically, with the regulation of Hsp70, a ubiquitously expressed heat shock protein (the orthologue of bacterial DnaK).

To understand Wiktoria Sztangierska's work, it is necessary to recall the nucleotide cycle of Hsp70. In its ATP-bound form, Hsp70 adopts an open conformation, with low polypeptide affinity, and high association and dissociation rates. Binding of hydrophobic regions in client proteins stimulates nucleotide hydrolysis, and leads to a more closed conformation. Subsequently, nucleotide exchange, triggers rebinding of ATP and the release of the substrate. It is believed that the conformational change in the nucleotide cycle extends from Hsp70 to the substrate, and thus gives an Hsp70 client protein a chance to refold (this has been called the "entropic" pulling mechanism). Nucleotide exchange factors (NEFs) and J-domain containing proteins (JDPs) have distinct roles in the Hsp70 catalytic cycle. JDPs stimulate ATP hydrolysis by Hsp70, and thus promote the closed conformation. Therefore, they help with substrate *binding*. NEFs, on the other hand, trigger ADP to ATP exchange, and thus promote the open conformation. Therefore, they help with substrate *release*.

The NEFs that promote substrate *release* come in several families. The ones relevant in eukaryotes are the Hsp110/Grp170 proteins, the HspBP1/Sil1 proteins, and the BAG domain proteins. In yeast, there are altogether six NEFs. Three are cytosolic. They are termed Sse1 (Hsp110 family), Sse2 (Hsp110 family), and Fes1 (HspBP1 family). NEF activity in the cytoplasm is known to be essential, since double ablation of Sse1 and Sse2 is lethal. In addition to the three cytoplasmic NEFs, there are also three ER associated NEFs. These are termed Lhs1p (Hsp110 family), Sil1 (HspBP1 family), and Snl1(BAG family). For her studies, Wiktoria Sztangierska focused on the cytoplasm. In this compartment, Sse1 is ten times more abundant than Sse2, and five times more abundant than Fes1. On the assumption that abundance reflects physiological importance, it was therefore reasonable for Wiktoria Sztangierska to focus on Sse1 as the representative NEF. The lethality of the Sse1/Sse2 double KO strain may be used as an additional argument to support the choice, or at least as a justification for narrowing down the focus to Sse1 or Sse2. For the education of the reviewer, is it known whether ablation of the third cytoplasmic NEF, Fes1, is also lethal?

The JDPs that promote substrate *binding* also form a protein family. Even in simpler eukaryotes such as yeast, there are several paralogs. Common to all paralogs is the presence of a J domain at the amino terminus. In addition, there are glycine/phenylalanine-rich regions, two C-terminal β-sandwich domains, and a C-terminal dimerization domain. Class A JDPs also have an additional Zn-finger domain, which is absent in class B JDPs. Proteins that contain a J domain but do not fit into either

Prof. Matthias Bochtler

International Institute of Molecular and Cell Biology &
Trojdena 4, 02-109 Warsaw, Poland
Institute of Biochemistry and Biophysics
Pawinskiego 5a, 02-106 Warsaw, Poland
Tel: 0048 22 5970732
Fax: 0048 22 5970715
e-mail: mbochtler@iimcb.gov.pl

class A or B are grouped "leftover" class called C. For her work, Wiktoria Sztangierska chose Ydj1 as a representative class A JDP and Sis1 as a representative class B JDP. The PNAS paper co-authored by Wiktoria Sztangierska and the literature referenced therein show that the biological roles of Ydj1 and Sis1 are different. The class B JDP protein Sis1 is essential, whereas the class A JBP protein Ydj1 is only required for growth at elevated temperatures. In addition, the two JDPs differ in their preference for aggregate type (amyloid or amorphous). For a reader outside the field of Hsp70 biology, it is not entirely clear why these specific representatives were chosen for further study. How many class A and class B JDPs are present in yeast (S. cerevisiae)? And if there are several per class, how were the representatives picked? Was the choice guided by expression levels as a proxy for likely physiological importance, as for the representative NEF? If not, what other criteria were used to pick representatives?

Wiktoria Sztangierska's thesis addresses the question how NEFs affect Hsp70 (Ssa1) activity in the context of different JDPs. Her approach is largely biochemical and relies on a reconstituted yeast chaperone system, with aggregated luciferase and GFP as the test substrates. Her primary assay is biolayer interferometry. This assay is similar to surface plasmon resonance, but relies on different biophysical principles, and does not require microfluidics. As the system under study is complex, some of the experiments were difficult to understand for me.

After some struggle, I found a way to explain some of the data to myself in terms of the a "balanced stimulation" model. Hsp70 needs a JDP to promote the binding to substrates, and a NEF to promote release. Clearly, an excess of either "helper" should be detrimental to the reaction. On the one hand, if there is too much JDP, the system should be stuck in a substrate bound state. On the other hand, if there is too much NEF, release should happen too early, and the system should have trouble to bind any substrates. Among the JBPs, the class B ones turn out bind better to Hsp70 than the class A ones, because the C-terminal tail of Hsp70 can only bind to the B class (via the C-terminal domain of the B-class JDP), but not the A class JDPs. Since binding is presumably a requisite for JDP action on Hsp70, the implication is that a B-type JDP promotes binding more than an A-type JDP. The finding that "Sse1 (the NEF) restricts binding of Ssa1 (Hsp70) to Sis1 (the JDP)" (p. 67), suggests that NEF and JDP compete for Hsp70 binding. If this is indeed the case, then an excess of either would displace the other. In my eyes, this mechanism would additionally contribute to the requirement for "balanced stimulation", beyond the purely functional antagonism.

From the point of view of the "balanced stimulation" concept, one would expect that there is more room for the NEF to ramp up the activity of Hsp70 with the B-type JDP than with the A-type JDP. This is indeed observed, however, the difference is moderate in the absence of an Hsp100 (Figure 11 of the thesis). Once an Hsp100, which functionally cooperates with Hsp70, is added, the difference is very clear (Fig. 12 of the thesis). I am tempted to interpret this finding in terms of another rate limiting step in disaggregation in the absence of an Hsp100. Would Wiktoria Sztangierska agree with this interpretation? Many other results in the thesis are also consistent with the "balanced stimulation"

Prof. Matthias Bochtler

International Institute of Molecular and Cell Biology &
Trojdena 4, 02-109 Warsaw, Poland
Institute of Biochemistry and Biophysics
Pawinskiego 5a, 02-106 Warsaw, Poland
Tel: 0048 22 5970732
Fax: 0048 22 5970715
e-mail: mbochtler@iimcb.gov.pl

model. These include the finding (a) that Sse1 promotes modification of aggregates by Hsp70 (section 8.4), (b) that Hsp70 responds to Sse1 in a JDP dependent manner (section 8.6), (c) that too much Sse1 is detrimental for refolding (section 8.6), (d) that affinity of NEF to Hsp70 determines disaggregation efficiency and (e) that affinity deficits of a variant can be overcome (section 8.7).

However, there are also data that are harder to reconcile with the "balanced stimulation" model and that I still do not understand. Wiktoria Sztangierska shows experiments that suggest that Hsp110 helps to recruit Hsp70 to aggregates (in the presence of a B class JDP). The experiments use a fluorescently labelled variant of Hsp70 and are a rather direct measurement of Hsp70 recruitment to aggregates. I find it counterintuitive that a NEF (or its ATPase deficient variant), which should stimulate substrate release (i.e. disengagement of Hsp70 from aggregates) promotes binding of Hsp70 to the aggregates. Wiktoria Sztangierska suggests that the NEF has independent substrate affinity, and therefore helps to bring Hsp70 to substrates. How does she reconcile this with the accepted role of the NEF to promote substrate release?

Wiktoria Sztangierska's thesis is structured conventionally into Abstract, Introduction, Aims, Materials, Methods, Results, Discussion and References. The thesis is polished to a high editorial standard. The well-written Introduction gives a good overview over the field. The extensive reference list (~100 references) shows that Wiktoria Sztangierska is well versed in the field. The research question formulated in the Aims section is precise, and also timely. The Materials and Methods sections are fairly extensive, which should contribute to the reproducibility of the work. The number of challenging proteins and protein variants that had to be purified for the reconstituted chaperone system is certainly impressive. Results and Discussion are a complicated read for anyone outside the narrow field of Hsp70 biology, but they are certainly reasonable. Throughout, informative illustrations accompany the work. I am always uneasy about very complex models with many degrees of freedom that can be adjusted to fit the data. However, I do agree that such models may be the only option for a system as complex as the reconstituted Hsp70 chaperone system. Even though I struggled with the Results and Discussion sections, the experts apparently approve, judging from publication of part of the work in PNAS, and of the reminder (possibly) in ELife. The status of the Elife manuscript as a "reviewed preprint" is somewhat unclear to me. Does this mean that the manuscript is in revision? Or rather that it is accepted but not yet typeset?

In summary, Wiktoria Sztangierska has provided extensive data on the regulation of Hsp70. She obviously deserves a PhD for this work. My understanding is that a distinction requires an accepted first author paper. If the Elife paper is accepted, I would also support a distinction for the work.

With best regards

Matthias Bochtler