Dissertation Thesis Report

Reviewer: Richard Stefl, CEITEC Masaryk University, Brno

Dissertation Thesis Title: Binding of eIF3 in complex with eIF5 and eIF1 to the 40S ribosomal subunit is accompanied by dramatic structural changes

*by **Mgr. Jakub Zeman***

In this PhD thesis, Jakub Zeman presents his work in elucidating the architecture of eukaryotic initiation factors (eIFs) bound to the 40S ribosomal subunit. In the introduction to his thesis, Jakub Zeman provides an overview of translation cycle and the role of eIF3 complex. He discusses the eIF3 complex architecture and function, and variations that occur in yeasts and mammals. The introduction illustrates the current understanding of the structure eIF3 complex in the free form and bound to 40S in great detail. Jakub Zeman then clearly describes the aims of his PhD and the ultimate goal of his study to determine the structure of yeast eIF3 complex and its interaction surface with the 40S ribosomal subunit. To study the eIF3 complex and its interactions, he employed x-ray crystallography, chemical cross-linking coupled to mass spectrometry, and various biochemical and genetic assays.

The results section comprises five papers, four published in Nucleic Acid Research, and one in RNA Biology. The major publication, in which Jakub is the main author, describes the binding of eIF3 in complex with eIF5 and eIF1 to the 40S ribosomal subunit and this work represents the core of this thesis. In this work, he improved the previously reported in vitro reconstitution protocol of yeast eIF3, which was consequently cross-linked and trypsin-digested to determine its overall 3D architecture by mass-spectrometry. Using this approach, Jakub mapped distances between the solvent-exposed parts of the free eIF3 complex and revealed that it has a rather globular 3D arrangement. Next, he used the same method to model the 3D structure of the eIF3–eIF1–eIF5 assembly, which revealed an extensive rearrangement of eIF3 upon binding to the small ribosomal subunit. As the ultimate goal of this work was to determine the structure of eIF3 and its complex with 40S/80S, the thesis should contain more information and discussion related to unsuccessful trials to obtain crystals suitable for X-ray diffraction analyses. Were the small or needle shaped crystals of the modified eIF3-Rack1 bound to 80S tested at suitable X-ray setups for small crystals (e.g. PSI Villigen)? If yes, what was the quality of measured diffraction data? What approaches were used to improve the quality of crystals? Were the cross-linked species used to grow crystals? This should perhaps be discussed in detail during defense.

In the other four papers, Jakub declares a minor contribution as he was involved in bioinformatics analyses, binding experiments, and manuscript preparations. The thesis contains well-written discussion on individual experimental parts of this work and I appreciate the critical view of Jakub concerning the limitation of individual approaches.

Taken together, the work presented in this thesis represents a significant advance in our understanding of the binding of eIF3 to eIF5 and eIF1 to the 40S ribosomal subunit. Mgr. Jakub Zeman showed that he is able to ask important scientific questions, design experiments to answer them and draw conclusions from these experiments. This thesis contains solid and creative research and I therefore ***recommend the dissertation for defense***.

Richard Stefl

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CEITEC – Středoevropský technologický institut
Masarykova univerzita
Kamenice 753/5, 625 00 Brno
www.ceitec.cz