ABSTRACT

of PhD thesis titled

"Identification of novel modifiers of noncanonical biosynthesis of toxic polyglycine protein from mutant *FMR1* mRNA" authored by Katarzyna Tutak

Fragile X-premutation-associated conditions (FXPAC) are genetic diseases caused by dynamic mutations of the fragile X messenger ribonucleoprotein 1 gene (*FMR1*) located on the X chromosome encoding fragile X messenger ribonucleoprotein 1 protein (FMRP). The gene usually contains 25–35 CGG repeats in the 5'-untranslated region (5'UTR). However, these triplet repeats are highly polymorphic and tend to expand. The premutation (PM) state of CGG expansion (CGGexp) corresponds to 55–200 repeats and is associated with multiple FXPAC such as a late onset neurodegenerative disease called fragile X-associated tremor/ataxia syndrome (FXTAS) and fragile X-associated primary ovarian insufficiency (FXPOI). On the contrary, when CGGexp exceeds 200, it is called full mutation and underlies the pathogenesis of neurodevelopmental disease named fragile-X syndrome (FXS), the most common form of inherited intellectual disability.

In case of FXPAC, FMRP protein is produced despite the presence of CGGexp in 5'UTR of *FMR1*, whereas, in FXS, methylation of *FMR1* promoter leads to the gene silencing. It is postulated that the interplay between three major molecular pathomechanisms drives FXPAC. At first, RNA with CGGexp is toxic and forms a secondary hairpin structure that sequesters RNA binding proteins into RNA foci leading to their functional depletion. Second, co-transcriptional formation of R loops, a DNA:RNA hybrids which triggers DNA breakage and compromises genomic stability. Third mechanism involves repeat-associated non-ATG initiated (RAN) translation, which leads to the production of toxic and aggregation-prone proteins called RAN proteins, which contain long tract of a repeated monoamino acid, that can be either polyglycine, polyalanine or polyarginine depending on the open reading frame, however polyglycine-containing proteins (FMRpolyG) predominate. Toxic FMRpolyG aggregates and forms intranuclear inclusions in patient's brain, a hallmark of FXTAS pathology.

Mechanistic insights into RAN translation remain elusive, therefore we sought to identify novel RAN translation modifiers, which constitutes the main part of the PhD thesis. We applied an in cellulo RNA tagging system combined with mass spectrometry (MS) based protein identification and discovered more than 60 proteins that bind to 5'UTR of mutant

FMR1 mRNA containing CGGexp. Gene ontology analysis performed on identified proteins revealed that majority of them represent RNA binding properties, are involved in ribosome biogenesis, translation or mRNA processing. Some of identified proteins overlapped with already identified interactors of FMR1, however our dataset contains newly identified factors.

Among identified proteins, we selected ten candidates and we verified their RAN translation regulatory properties using small interfering RNA. As a result, we identified few proteins, which depletion affected the level of FMRpolyG. For instance, depletion of small ribosomal subunit protein eS26 (RPS26), a component of 40S subunit which contacts mRNA sequence during translation, significantly impeded RAN translation in multiple tested models. In addition, insufficiency of two RNA helicases, ATP-dependent RNA helicase DHX15 (DHX15) and Nucleolar RNA helicase 2 (DDX21) as well as THO complex subunit 4 (ALYREF) negatively affected biosynthesis of FMRpolyG. Additionally, silencing of RPS26, DDX21 and ALYREF did not affect the level of FMRP indicating specificity of regulation towards FMRpolyG frame. Importantly, we showed that depletion of RPS26 decreases the amount of aggregates formed by FMRpolyG and alleviated their toxicity in cellular model. In addition, using quantitative MS approach, we found that the number of proteins produced by RPS26-sensitive translation is limited and that the 5'UTRs of the mRNAs encoding these proteins are rich in guanosine and cytosine, similar to *FMR1* mRNA.

In order to gain mechanistic insights into RPS26-sensitive translation regulation, we verified the function of RPS26 chaperone, Pre-rRNA-processing protein TSR2 homolog (TSR2). We demonstrated that TSR2 positively regulated the production of FMRpolyG. Finally, we verified that another component of the 40S subunit, Small ribosomal subunit protein eS25 (RPS25) also regulates CGG-related RAN translation.

To sum up, performed MS-based screening provided a unique and valuable source of information about *FMR1* interacting proteins in cellulo, which may be implicated in the biology of this molecule. The main achievement of this study is the identification of five novel RAN translation modifiers and the proposal of a concept suggesting that the composition of the 40S subunit plays a pivotal role in regulating noncanonical CGG-related RAN translation in FXPAC.