Załącznik nr 4
do Regulaminu postępowania kwalifikacyjnego na studia doktoranckie
w ramach międzynarodowego programu KNOW „Poznańskie Konsorcjum RNA”
na Wydziale Biologii Uniwersytetu im. Adama Mickiewicza w Poznaniu

Projekt rozprawy doktorskiej planowanej do realizacji w ramach międzynarodowego programu studiów doktoranckich KNOW „Poznańskie Konsorcjum RNA” w latach 2015-2019 na Wydziale Biologii Uniwersytetu im. Adama Mickiewicza w Poznaniu

1. **Wnioskodawca** (kierownik projektu): prof. UAM, dr hab. Krzysztof Sobczak

2. **Tytuł projektu**: Rola helikazy DDX5 w dojrzewaniu końca 3' transkryptów zachowawczych histonów rdzeniowych w komórkach ludzkich

3. **Dyscyplina naukowa** (właściwą podkreślić): biologia, biochemia, biotechnologia

4. **Krótki opis projektu w j. angielskim** (maksymalnie 1 strona; Autorzy zakwalifikowanych projektów zostaną poproszeni o przygotowanie streszczeń w j. angielskim oraz j. polskim, które zostaną zamieszczone na stronie internetowej Wydziału Biologii):

I. Research project objectives/Research hypothesis

The aim of the project is to describe the role of DDX5 (p68) protein in the canonical core histone pre-mRNA 3’ end processing in human cells. DDX5 is well known ATP-dependent RNA helicase, which is involved in many cellular processes, especially in those requiring secondary structure remodeling, like pre-mRNA splicing, pre-rRNA or miRNA processing. Recently, using affinity chromatography and immunoprecipitation it was shown that helicase DDX5 can also interact with U7 snRNP. Moreover, pull down experiments and yeast two-hybrid assays showed that this interaction occurs between DDX5 and the U7 snRNP-specific protein, Lsm10. U7 snRNP is an essential component of the histone transcripts 3’ end processing machinery. In the Metazoa cells, those replication-dependent mRNAs are neither spliced nor polyadenylated. U7 snRNP binds to histone transcripts by RNA:RNA basepairing: the 5’ end of U7 snRNA hybridizes to the histone downstream element (HDE) sequence which is located few nucleotides downstream of the cleavage site. The U7 snRNA:HDE duplex formation recruits many other proteins of the histone cleavage complex (HCC), including the CPSF73 endonuclease.

The research problem of this project concerns still not fully elucidated steps of histone pre-mRNA 3’ end processing that can be mediated by RNA helicase DDX5, according to well-known functions of its activity. Thus, we assume that DDX5, due to its helicase activity, mediates formation of a duplex between the 5' end of U7 snRNA and HDE sequence at the 3' end of histone pre-mRNA. We also suggest, that RNA helicase DDX5 can be involved in dissociation of U7 snRNA from HDE after the 3’ end cleavage, resulting in liberation of the U7 snRNP from the HDE. Furthermore, we speculate that DDX5 could mediate in presenting of the histone pre-mRNA cleavage site for CPSF73, by some rearrangements within the HCC complex. We will also try to establish, among others, which domain of the DDX5 helicase is required for interaction with Lsm10, and whether this role of DDX5 requires the RNA helicase DDX17.

II. Research project methodology

To verify proposed hypothesis, we will conduct the following experiments:

- To test, whether DDX5 protein mediates formation of a duplex between the 5' end of U7 snRNA and HDE, RNA fragments containing 3’ end of histone transcripts will be synthesized by in vitro transcription and bound to the streptavidin beads via biotinylated oligonucleotide. Next, they will be incubated with the protein extracts isolated from HeLa cells or DDX5 knock

down cells. Proteins eluted from the resins will be tested by Western blot using specific anti-Lsm10 and anti-Lsm11 antibodies.

- To examine, whether RNA helicase DDX5 is involved in dissociation of U7 snRNA from HDE, we will check the stability of U7 snRNA:HDE duplex in HeLa cells in comparison to cells where DDX5 is knocked down or overexpressed. Resin-bound 3' end of histone transcripts will be incubated with the protein extracts during defined periods of time, then cross-linked by UV light in the presence of Trioxsalen and analyzed on denaturing polyacrylamide gel.
- To test, whether DDX5 could mediate in presenting of the histone pre-mRNA cleavage site for the endonuclease CPSF73, we will analyze the ability for cleavage of the 3' end of histone transcript in protein extracts isolated from HeLa cells and cells where DDX5 has been knocked down or overexpressed. The RNA fragments will be labelled by an radioisotope for this experiments. Cut and uncut RNAs will be analyzed in polyacrylamide gels.
- Furthermore, to examine whether the RNA helicase DDX17 is also involved in the 3' end processing of histone transcripts, similar experiments will be performed in the presence of protein extracts isolated from cells with DDX17 knock-down or overexpression and expressed in bacteria recombinant DDX17 protein.
- To test, which part of the DDX5 helicase interacts with Lsm10, different parts of human recombinant DDX5 protein will be prepared and their binding affinity to the Lsm10 protein will be analyzed in pull down experiments. Experiments in which recombinant DDX5 mutants with impaired ATPase (NEAD) and helicase (LGLD) activity will be added to the samples, will give additional information whether these activities are crucial or not for the DDX5 function in the 3' end processing of histone pre-mRNA.

III. Expected impact of the research project on the development of science, civilization and society

The obtained results will provide, new important information about still unknown steps of the 3' end processing of canonical core histone pre-mRNAs in animal cells. Furthermore, they expand the list of functions performed by RNA helicases DDX5 and DDX17 which activity is well known in various biological processes, but has not been studied yet in the context of the histone pre-mRNA processing. The tangible effect of this studies will be publications in the international journals with high impact factor and presentation of the results on national and international conferences.

5. **Źródła finansowania badań w ramach proponowanego projektu rozprawy doktorskiej (**wskazać źródło finansowania, okres i miejsce realizacji, charakter udziału w projekcie oraz budżet projektu):

- źródło finansowania: Projekt Preludium NCN nr UMO-2013/11/N/NZ1/00010
- okres realizacji: wrzesień 2014 – sierpień 2017
- miejsce realizacji: Zakład Ekspresji Genów, IBMIB
- charakter udziału w projekcie: doktorant, główny wykonawca
- budżet projektu: 149 640,00 zł na 3 lata

6. **Lista najlepszych publikacji kierownika projektu z ostatnich 5 lat** (5 publikacji wraz ze wskaźnikiem oddziaływania [IF] i liczbą cytowań bez autocytowań).

Sobczak K, Michlewski G, deMezer M, Kierzek E, Krol J, Olejniczak M, Kierzek R, Krzyzosiak WJ.
Structural diversity of triplet repeat RNAs.
J. Biol. Chem. **2010**; 285:12755-64. (IF: 4.9, cyt: 39)

Sobczak K, Wheeler TM, Wang W, Thornton CA.
RNA Interference Targeting CUG Repeats in a Mouse Model of Myotonic Dystrophy.
Mol. Ther. **2013,** 21:380-7. (IF: 6.4, cyt: 15)

Childs-Disney JL*, Stepniak-Konieczna E*, Tran T, Yildirim I, Park H, Chen CZ, Hoskins J, Southall N, Marugan JJ, Patnaik S, Zheng W, Austin CP, Schatz GC, Sobczak K, Thornton CA, Disney MD. *co-first author
Induction and reversal of myotonic dystrophy type 1 pre-mRNA splicing defects by small molecules.
Nature Commun. **2013**, 4:2044. doi: 10.1038/ncomms3044. (IF: 11.0, cyt: 11)

Konieczny P, Stepniak-Konieczna E, Sobczak K.
MBNL proteins and their target RNAs, interaction and splicing regulation.
Nucleic Acids Res. **2014,** 42:10873-87. (IF: 8.8, cyt: 2)

Wojtkowiak-Szlachcic A, Taylor K, Stepniak-Konieczna E, Sznajder LJ, Mykowska A, Sroka J, Thornton CA, Sobczak K.
Short antisense-locked nucleic acids (all-LNAs) correct alternative splicing abnormalities in myotonic dystrophy.
Nucleic Acids Res. **2015,** 43:3318-31. (IF: 8.8, cyt: 0)

7. Oświadczenie kierownika

a) Wyrażam zgodę na zamieszczenie moich danych osobowych wymienionych we wniosku oraz streszczenia niniejszego projektu na stronie internetowej Wydziału Biologii.
b) Oświadczam, że powyższe informacje są prawdziwe, kompletne, rzetelne oraz zostały przekazane zgodnie z moją najlepszą wiedzą i przy zachowaniu należytej staranności.

Miejscowość i data

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podpis kierownika projektu