Integrative Program *for* Biological & Genome Sciences *at* UNC Chapel Hill

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Narodowe Centrum Nauki,
Warsaw, Poland

Chapel Hill, August 23, 2014

Dear Sir/Madame:

I am very happy to support Prof. Michal Dadlez's application for a Maestro grant funded by Narodowe Centrum Nauki and express my great interest in continuing our effective, long lasting and valuable collaboration in the following years.

Our collaboration goes back to 2002 when we worked together on identifying proteins that interact with a highly conserved sequenced at the end of mature histone mRNA. Histone mRNAs are highly expressed only in S phase and used to generate massive amounts of histone proteins to meet the demand from the newly replicated DNA. Both the biogenesis and function of these mRNAs critically depend on the conserved terminal sequence and its interacting proteins. This was an important project as both replication of DNA and production of histone proteins, the two main components of chromatin, could be potentially targeted by small molecules aimed at slowing down or inhibiting cell proliferation. As a result of this collaborative effort, we identified a protein called 3'hExo that together with another protein forms a ternary complex at the end of histone mRNA. In 2003 we published a paper in Molecular Cell describing our findings and suggested a potential configuration of the ternary complex, which was confirmed by crystallographic studies published this year in Science. Subsequent findings from other groups on the involvement of 3'hExo in biogenesis of micro RNAi and human viruses triggered a growing interest in this protein and our study.

In recent years, we continued working together on multi-component assemblies involved in the biogenesis and function of histone mRNAs as part of the TEAM grant. We were particularly interested in using mass spectrometry in identifying subunits of a large complex that interacts with the protein FLASH, a key player in generating mature histone mRNAs. This part was completed in 2013 and published in the widely known journal Molecular and Cellular Biology. We also convinced a very talented and dedicated student, mgr Aleksandra Skrajna, to join our groups as a graduate student and to work on structural aspects of this complex. Aleksandra shares her time between our institutions in Poland and United States and her Ph.D. project is co-directed by me and prof. Dadlez. In Poland, A. Skrajna is mainly involved in applying hydrogen-deter exchange coupled with mass spectrometry (HDX/MS), a method mastered by Michal's team, to analyze critical interactions within the complex assembled on FLASH. This part is nearly completed and currently being prepared for a publication. A. Skrajna has already spent over 1.5 years at the University of North Carolina at Chapel Hill and proved to be a very productive and intellectually active member of the American group. Her broad knowledge, expertise in modern techniques of biology and other skills, work ethics and enthusiasm compare favorably with the best Ph.D. student of our University, a testimony to the high quality of education and growing interest in research in Poland. Her work here is mostly

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focused on isolating native protein complexes involved in generating mature mRNAs from large amounts of Drosophila cells (a cheap source of animal cells used as a substitute of very expensive and difficult to grow mammalian cells) and analyzing their composition in the facility in Poland. Her findings were recently published in RNA journal and were instrumental in defying U7 snRNP, a commonly known eukaryotic RNA/protein complex, as a specialized and multi-subunit RNA endonuclease controlled by the interaction with FLASH. At least three other projects should be completed in a relatively short time as a results of Aleksandra's work and are expected to make into papers soon, with two of them being coordinated by Dr. Igor Zhukow from IBB PAN, our active partner in the TEAM grant. During her time in the graduate school, Aleksandra attended three international meetings, including RNA Society conference in Quebec City for which in recognition of her work she was awarded a travel fellowship.

During her last visit to USA, A. Skrajna generated a set of very intriguing results that sparked our interest in two other proteins known to control expression of histone genes in S-phase: NPAT and YARP. These two proteins likely regulate their own function by directly interacting with each other and with FLASH. NPAT has been recognized for many years as a target for one of the key cell cycle-regulated kinases and we predict that heperphosphorylation of NPAT by this kinase breaks its contacts with FLASH, rendering both proteins active in generation of mature histone mRNAs at the onset of S-phase.

I consider my long term collaboration with Michal very successful and gratifying for the two parties and resulting in both significant scientific findings and expanding our usual areas of interest to other topics and methods. We constantly learn from each other and join forces in educating young generations of researchers. We are proud to see the rapid development of A. Skrajna into a mature and highly skilled scientist, a likely candidate for an excellent principal investigator and a leader in science in Poland in the near future.

Over the years, I and Michal worked out a model mechanism of collaborating in which important biological processes that are being discovered in our laboratory in the United States are analyzed from the structural point of view in Poland. In addition to having common scientific interests and using complementing approaches, we also share the same way of thinking and long lasting friendship. We would like to continue this mutually beneficial collaboration and extend it to the recently identified and biologically vital interactions involving NPAT, YARP and FLASH. I therefore strongly support Dr. Dadlez's application and express hope that our most important achievements and discoveries are ahead of us.

Zbigniew Dominski,
Prof. of Biochemistry and Biophysics,
University of North Carolina at Chapel Hill