



Jan Černý
Associate Professor of Immunology

CHARLES UNIVERSITY, FACULTY OF SCIENCE
DEPARTMENT OF CELL BIOLOGY,
Viničná 7, 128 40 Praha 2, Czech Republic

EVALUATION REPORT OF Ph.D. THESIS

Title: MYOSIN-PIP2 INTERACTION IN THE CELL NUCLEUS

Author: Mgr. SUKRIYE YILDIRIM

Ph.D. programme: DEVELOPMENTAL AND CELLULAR BIOLOGY

Charles University, Faculty of Science, Prague

I. General Comments:

The submitted thesis presents study of complex molecular interactions between the nuclear components and myosins (Myo1C and NM1) – molecules involved in regulation of nuclear organization and function. Thesis deals with the general topic, how proteins derived from one coding sequence via alternative splicing share/complement their roles and functions in the cell, while possessing high structural and sequential similarity. The topic is fundamental in the understanding what is the difference “between human and drosophila” – having similar numbers of ORF, but substantially different capacity to “play” with them complicating “games”. Elucidation of rules for one such “game” is the topic of the dissertation – in the context of surprising phenomenon – involvement of phospholipid PIP2 in the nuclear (nucleoplasmic) structures, their interactions and regulations.

Thesis is based on three recent publications in peer reviewed biomedical journals, together with two manuscripts already prepared for the publication (in all cases is clearly depicted, what was the particular role of the student in the experimental setup, experimentation and also in the writing of the manuscript). Sukriye Yildirim is the first author of the most recent publication in the top class cell biology journal (Journal of Cell Science - IF: 6,111) where important discoveries postulating role of PIP2 in the regulation of RNA polymerase I activities are presented. All three publications are rounded up by the general title MYOSIN-PIP2 INTERACTION IN THE CELL NUCLEUS, general enough to cover the whole story. From the broad range of topics, models and methodologies mentioned in the thesis as experienced by the author is obvious, that Sukriye Yildirim obtained very general scientific education and was equipped with knowledge and skills necessary for successful cell biologist. Thesis is written in English of good quality, with minimum formal mistakes and editing errors. Short introductory part deals quite successfully on only 12 pages with hard task – to cover the topic of nuclear myosins from different points of view and dissect sometimes contradictory data in the literature. From my point of view, this part should be a little bit longer and more general, but 120 carefully selected references are properly cited thorough the text is number high enough to show author’s competence and orientation in the field of

the thesis. I appreciate also a crisp clarification of the author's role in the experimental design and performance in all publications. It is above any doubt that Sukriye Yildirimis methodically competent enough (site-directed mutagenesis, plasmid construction, FRAP, FCS, immunofluorescence, pull downs, protein expression and purification, binding assays) to pass the "student period" to postdoc level...

II. Specific Comments:

In my specific comments I would like to focus on the most recent publication INVOLVEMENT OF PIP2 IN RNA POLYMERASE I TRANSCRIPTION, where Sukriye Yildirim is the first author and performed critical experiments and wrote the manuscript and on the manuscripts prepared for the publication (where Sukriye Yildirim is claimed also as a first author).

Among the proteins co-purified with nuclear myosins is Lamin A, protein postrationally modified with farnesylation and subsequently cleaved. The cleavage (removing farnesylated peptide) is not performed in in case of particular mutations resulting in Hutchinson-Gilford progeria syndrome (HGPS) – could be of interest to study cells expressing farnesylated (uncleaved) form of HGPS derived Lamin A – one can speculate, that it can lead to the changes in the complexes you observe with potential link to the molecular mechanism of the syndrome. It is also described, that in nucleoplasm Lamin A and Lamin B form distinct (but inter-dependent) differently mobile fractions. Could be Lamin B one of the farnesylated co-isolated molecules? The co-purification shows complex composition of the isolated complexes – do you have any data/speculation about the size of the complex/possible heterogeneity of the precipitated complexes?

Do you have any information/data about trafficking/biosynthesis of the PIP2 inside the nucleus/into the nucleoplasm? Obviously PIP2 could be a constituent of the nuclear membrane – therefore close to the location observed, but embedded in the suitable highly hydrophobic environment. Is there any PIP2 specific transporter involved? In co-purification experiments, not only proteins (and PIP2), but also variety of other particular lipids were isolated and identified indicating probable trafficking origin of the lipoprotein complexes. Could be there any conceptual link to mitotic membrane disassembly or nuclear membrane invaginations?

The "primary" myosin function is actin and ATP-dependent conformational change resulting in various types movements in the cell. Is correct to interpret your data in a way, that role of myosins in the nucleus is to form scaffolds, like adaptor proteins elsewhere in the cell? Is there still any actin-dependent mechanical role for myosins in the nucleus?

III. Evaluation:

Ph.D. thesis written and submitted by Sukriye Yildirim is of high quality and can be accepted as a proof of author's scientific education above the threshold demanded by the Ph.D. Committee in Developmental and Cellular Biology. I personally wish the author successful scientific career, already started in prestigious laboratory under supervision of outstanding scientists.

Prague, 20th May 2013

Jan Černý
(signed)