Investigation of the nuclear import and function of Moesin

Ph.D. thesis summary

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Introduction and background

ERM (Ezrin-Radixin-Moesin) proteins are involved in the formation of interactions between the cortical actin network and transmembrane proteins, thereby they play an important role in T cell activation, lymphocyte migration (Neetha et al. 2013), and the regulation of signaling pathways (Neisch et al., 2011). In recent years, it has become evident that members of the ERM family are present also in the cell nucleus (Batchleor et al., 2004; Vilmos et al., 2009).

Our group described earlier that the only ERM protein of *Drosophila melanogaster*, Moesin (Moe), can be detected in the nucleus and it co-localizes with the actin cytoskeleton around the mitotic spindle during cell division (Vilmos et al., 2016). Studies in our laboratory have shown also that Moesin is involved in the nuclear export of mRNAs. This conclusion was supported by the findings that Moesin co-localizes in the mRNP complexes with the nuclear RNA export proteins Rae1 and pABP, and the silencing of the *nup98* and *rae1* mRNA export genes - similarly to other mRNA export factors, leads to the nuclear accumulation of Moesin. Additional evidence

for the role of Moesin in the transport of mRNP complexes was provided by mRNA accumulation in the nucleus caused by reduced Moesin level (Kristó et al., 2017).

Our current knowledge about the nuclear function of Moesin and other cytoskeletal proteins is based mainly on biochemical approaches, and the strains available at the Bloomington Drosophila Stock Center are not suitable to study nuclear Moesin, since in these lines the cytoplasmic functions of Moesin are also disrupted. We believe, that the methods we used and the *Drosophila* lines generated by us enable the *in vivo* study of the nuclear functions of Moesin.

Materials amd methods

In our work, we characterized the nuclear import of Moesin with the help of the S2R+ Drosophila cell line by monitoring the nuclear accumulation of different Moesin protein isoforms with *rae1* RNA interference.

To better understand the nuclear function of Moesin, we used *Drosophila melanogaster* as a model organism. Due to sterility and developmental malformations observed in the mutant flies generated by the CRISPR-

Cas9 system, most of our studies were performed on embryos and ovaries using live microscopy, immunostaining and fluorescent *in situ* hybridization.

Transgenic and mutant *Drosophila* strains were used for the detailed characterization of the observed phenotypes. Traditional DNA cloning techniques, SLIC (sequence and ligation independent cloning), Gateway and Gibson Assembly methods were used to prepare various DNA constructs and transgenic *Drosophila* lines for our work.

Results

1. During the study of nuclear transport we demonstrated that the active import of Moesin is mediated by a conserved, bipartite nuclear localization signal (NLS) (**$K_{279}R_{280}$**ILALCMGNHELYM**$R_{294}RRK_{297}$**).
2. In the vicinity of the NLS we identified two potential phosphorylation sites (Y_{292} and T_{300}) with possible regulatory function. We changed these amino acids into aspartate which mimics constant phosphorylation state, or alanine which disables phosphorylation. The Moesin

mutant forms accumulated properly in the nucleus indicating that the Y_{292} and T_{300} residues have no regulatory function.

3. To investigate the connection between the activation state of Moesin and its nuclear import, we analysed the nuclear accumulation of the Moe-T_{559}D and Moe-T_{559}A isoforms. According to the literature, Moe-T_{559}D is considered a constantly active protein form, while Moe-T_{559}A is an inactive isoform. Our work revealed that the Moe-T_{559}A protein is able to accumulate in the nucleus similarly to the wild type Moesin, while the nuclear import rate of the Moe-T_{559}D form is lower as compared to the wild type. However, we think that the decreased nuclear accumulation of Moe-T_{559}D might not be the result of weaker import capacity, but rather caused by the strong interaction between activated Moesin and the actin filament which in turn inhibits the translocation of Moesin to the nucleus. The nuclear import of the inactive Moesin is further confirmed by the observation that the Moe-KA isoform, which has no phosphatidylinositol-4,5-bisphosphate (PIP_2) binding

capacity thus representing a bona-fide functionally inactive form, shows wild type nuclear import rate.

4. The region surrounding the NLS was suggested previously as a G-actin binding site (Roy et al.,1997). To test if monomeric actin binding can regulate the nuclear import of Moesin, we increased (R_{63}D actin) and decreased (Jasplakinolide) the intracellular G-actin level, and found that the nuclear import of Moe is not affected by the G-actin amount. Therefore, the ratio of intracellular monomer and polymer actin levels play no regulatory role in the nuclear import of Moesin. However, this result does not completely exclude the involvement of actin in the nuclear transport of Moesin.
5. To investigate the nuclear function of Moesin, we generated a *moe*[NES] mutant fly line using the CRISPR-Cas9 system. The mutant expresses a Moesin isoform which is equipped with a nuclear export signal (NES) and as a result, the protein is constantly cleared out from the nucleus while it can still perform its cytoplasmic functions. This enables the examination of the consequences caused by the decreased level of nuclear Moesin. The use of the NES tag was necessary

because our previous results demonstrated that the deletion of the Moesin NLS signal cannot eliminate Moesin from the nucleus.

6. Next, we performed the detailed phenotypic analysis of the *moe*[NES] mutant, and found that the cuticular malformations and the dominant sterility are of maternal origin, while the decreased climbing activity, genitalia rotation of the males, and decreased heat tolerance of both sexes have zygotic background. The embryonic and larval lethality are caused by both maternal and zygotic effects. In addition to these phenotypes, we detected mRNA accumulation in the salivary gland cell nuclei of *moe*[NES] larvae which reflects defects in nuclear mRNA export.
7. We also performed experiments that uncover whether the observed phenotypes are caused by impaired cytoplasmic function or failed activation of Moesin, or by the overloaded CRM1 transport pathway. Because Moesin is involved in the formation of the actin cytoskeleton and thereby in the proper localization of maternal factors, we examined the integrity of the actin network, as well as the distribution (*oskar* mRNA,

Staufen and Vasa-GFP protein) and amount (Vasa-GFP protein) of maternal factors in the ovary of *moe*[NES] mothers. The experiments have shown that the organization of the actin cytoskeleton and the localization of maternal factors are normal in mutant flies. The amount and distribution of Vasa-GFP measured in the embryos of *moe*[NES] females were also similar to that of the wild-type. We found that the CRM1 pathway recognizing the NES motif of the MoeNES protein is not overloaded in the mutant, it functions properly. In addition, the activation and localization of the MoeNES protein is identical to the wild-type Moesin. Based on these results we concluded that MoeNES can perform its cytoplasmic functions and consequently, all the phenotypes observed in the mutant are due to the lack of nuclear functions of Moesin.

8. To investigate if defective mRNA export or impaired transcription caused by the nuclear absence of Moesin, may contribute to the observed phenotypes, sequencing of total mRNA from the ovaries of *moe*[NES] mutant flies was performed. Out of the 13.000 genes examined,

371 showed increased and 315 exhibited decreased transcriptional activity. Based on the GO term data of Flybase, the majority of the genes with altered expression plays role in ontogenesis, transcription, and response to external stimuli. Among the genes with increased transcription level we found six genes (*bt* (*bent*), *Mhc* (*Myosin heavy chain*), *Mlc1* (*Myosin alkali light chain 1*), *Mlc2* (*Myosin light chain 2*), *Neurochondrin*, *up* (*upheld*) responsible for proper muscle function. Six *hsp* genes (*hsp70Aa, hsp70Ab, hsp70Ba, hsp68, hsp26, hsp23*) were identified among the downregulated genes which may be responsible for decreased heat tolerance as well as for sterility.

9. Previous work in our group demonstrated that Moesin is involved in the process of heat shock-induced transcription. This result is further confirmed by our observation that the decreased transcript level of *hsp* genes and the heat stress intolerance of *moe*[NES] mutants are due to the lack of nuclear functions of Moesin.

Summary and discussion

Our study of the nuclear transport of Moesin revealed that Moesin translocates into the nucleus with the help of a regulated, bipartite NLS. After describing the NLS, we plan to investigate further the nuclear transport of Moesin by using RNA interference to identify the transportin responsible for Moesin's nuclear import. We have shown in our previous work that in the absence of the NLS sequence, Moesin fails to accumulate in the nucleus upon various stimuli (heat shock, ecdysone, *rae1* and *nup98* RNAi). Therefore, we plan to generate a mutant *Drosophila* line in which the region encoding the NLS motif is deleted. This line would enable the analysis of mutant phenotypes which will be most likely the result of the reduced amount of nuclear Moesin.

We observed numerous phenotypes in the *moe*[NES] mutant generated for the *in vivo* study of the nuclear functions of Moesin. The identified phenotypes were characterized and grouped, and the cellular and molecular mechanisms behind them has been studied. In

accordance with previous observations obtained in our laboratory, we concluded that the nuclear deficiency of Moesin leads to impaired mRNA transport and transcription. We have previously demonstrated that Moesin accumulates at the transcriptionally active sites of heat shock (*hsp*) genes during heat shock treatment, so we believe that the *hsp* genes may be ideal candidates for a detailed study of the role of Moesin in transcription.

The dominant sterility observed in *moe*[NES] flies is a side effect of the NES motif. Dominant effects are usually caused by the change in molecular dimerization ability therefore, in order to more accurately describe the molecular activity of the MoeNES protein, its dimer forming properties must be investigated for example with the help of the micro scale thermophoresis (MST) method. These studies will provide valuable new information about the functions of the Moesin homodimer. However, we found no evidence for defects in the cytoplasmic functions of the MoeNES protein, so it is reasonable to assume that the observed dominant sterility is related to impaired nuclear function or dimer formation in the nucleus. In parallel with the dimerization studies of the MoeNES

protein, we plan to continue the examination of loss of germ cells which leads to dominant sterility. In particular, we will investigate whether the disappearance of the Vasa marker protein in the pole cells is the result of apoptosis or loss of germ cell identity.

Publications

MTMT number: 10052993

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Publications related to the thesis:

Nuclear actin: ancient clue to evolution in eukaryotes?
Bajusz Cs, Borkúti P, Kristó I, Kovács Z, Abonyi C, Vilmos P
Histochem Cell Biol. 2018 Sep;150(3), 235–244.
doi.org/10.1007/s00418-018-1693-6
PMID: 30019087, **IF: 2,64 (2018)**

Characterization of the nuclear localization signal of the actin-binding Moesin protein.
Bajusz Cs, Kristó I, Borkúti P, Kovács Z, Vilmos P
Biopolymers and Cell 2019; 35(3):201.
http://dx.doi.org/10.7124/bc.0009D2
IF: 0,3 (2018)

Investigation of the role in mRNA export of the actin binding protein, Moesin
Kristó I, **Bajusz Cs**, Borkúti P, Kovács Z, Pettkó-Szandtner A, Vilmos P
Biopolym. Cell. 2019; 35(3):219-220.
http://dx.doi.org/10.7124/bc.0009E9
IF: 0,3 (2018)

Testing the biological significance of the nuclear localization of actin.
Borkúti P, Bajusz I, **Bajusz Cs**, Kristó I, Kovács Z, Vilmos P
Biopolym. Cell. 2019; 35(3):204-204.
http://dx.doi.org/10.7124/bc.000A06
IF: 0,3 (2018)

The actin binding cytoskeletal protein Moesin is involved in nuclear mRNA export.
Kristó I, **Bajusz Cs**, Borsos B. N, Pankotai T, Dopie J, Jankovics F, Vartiainen M. K, Erdélyi, M, Vilmos P
Biochimica et Biophysica Acta - Molecular Cell Research 1864:10 pp. 1589-1604.,16 p.(2017)
https://doi.org/10.1016/j.bbamcr.2017.05.020
PMID:28554770, **IF: 2,68 (2017)**

Actin, actin-binding proteins, and actin-related proteins in the nucleus.
Kristó I, Bajusz I, **Bajusz Cs**, Borkúti P, Vilmos P
Histochem Cell Biol. 2016 Apr;145(4):373-88.
https://doi.org/10.1007/s00418-015-1400-9
PMID:26847179, **IF: 2,55 (2015)**

Other publication:

Drosophila Atg9 regulates the actin cytoskeleton via interactions with profilin and Ena.

Kiss V, Jipa A, Varga K, Takáts S, Maruzs T, Lőrincz P, Simon-Vecsei Z, Szikora S, Földi I, **Bajusz Cs**, Tóth D, Vilmos P, Gáspár I, Ronchi P, Mihály J, Juhász G

Cell Death Differ (2019)

https://doi.org/10.1038/s41418-019-0452-0

PMID:31740789, **IF: 8,086 (2018/2019)**