

Nuclear function for the actin-binding cytoskeletal protein, Moesin

Main points of the Ph.D. thesis

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Introduction and background

In the last few years, it has been shown that, besides the nuclear intermediate filament-forming lamins, several cytoskeletal components are also present in the nucleus including actin, motor proteins and crosslinking proteins (Simon and Wilson 2011). However, the existence of a structure mechanically and functionally analogous to the cytoskeleton in the nucleus is not known, and there is only little direct evidence proving that cytoskeletal elements are functional in the nucleus. Recently, these proteins, especially actin, actin-binding proteins (ABPs), and actin-related proteins (ARPs), receive particular attention because more and more data suggest that they are present in all major nuclear complexes (Visa and Percipalle 2010). These proteins such as Profilin, Cofilin, and the Arp2/3 complex may participate in nuclear actin polymerization and are also implicated in nuclear activities like DNA replication or active gene expression (Söderberg et al. 2012, Obrdlik and Percipalle 2011, Yoo et al. 2007). It has been shown that nuclear actin and actin-binding proteins are involved in the assembly of the transcription complex in the case of all three eukaryotic RNA Polymerases in *in vitro* experiments (Fomproix and Percipalle 2004, Philimonenko et al. 2004, Hofmann et al. 2004, Hu et al. 2004, Kukalev et al. 2005). Besides the initial steps, these proteins have important roles in transcription elongation, pre-mRNA processing and mRNA export as well (Percipalle et al. 2001, Percipalle et al. 2002), although, very little biochemical and *in vivo* data exist for the interaction of nuclear actin with specific nuclear proteins.

Members of the actin-binding Ezrin-Radixin-Moesin (ERM) protein family of vertebrates are major regulators of actin dynamics in the cell. They have pivotal role in cell adhesion, cell movements and intracellular membrane trafficking processes, therefore they are key players in cell polarity, morphogenesis and tumor metastasis which features attracted outstanding interest to this protein family in the past couple years. *Drosophila* Moesin is the sole representative of the ERM protein family in the fly. To the present view Moesin, similarly to its vertebrate counterparts, is responsible for the crosslinking of membrane proteins to the cortical actin network. However, several current data in the literature point to a nuclear function of Moesin and our laboratory was the first who demonstrated that *Drosophila* Moesin is indeed present in the interphase nucleus.

Aims

The investigation of the cell nucleus in the past decade provided increasing and surprising evidence that homologues, variants or fragments of numerous cytoskeletal proteins are present in the nucleus. Recently, one of the most surprising and interesting discovery was that the eukaryotic nucleus contains a regulated amount of actin. This result is closely related to our observation, that the important *Drosophila* actin-binding protein, Moesin is also present in the nucleus. In the cytoplasm Moesin primarily anchors various proteins to filamentous actin, however, the presence of long actin filaments in the nucleus is still not fully accepted. Therefore the question arises, whether the nuclear presence of Moesin has any biological significance and, if yes, what could be the function of Moesin in the nucleus. To answer these questions, during my PhD work I examined the localization of Moesin in the nucleus under different conditions (1), analyzed its nuclear import and export mechanisms (2), and investigated the possible nuclear function/functions of Moesin (3).

Materials and methods

During my work I used *Drosophila melanogaster* as a model organism, both living animals and S2R+ cultured embryonic cells. The salivary gland of the third instar larva has large polytene nuclei which provide an excellent tool to perform localization studies. First we generated flies with suitable genotypes by crossing transgenic or mutant strains together, then we dissected the salivary glands and used them either live in microscopic and FRAP experiments or fixed them for immunostaining.

We analyzed the effect of triptolide, ecdysone hormone and heat shock treatments on the nuclear amount and distribution of Moesin in salivary gland cells. For the chromosomal localization experiments we made giant polytene chromosome preparations from the salivary glands and immunostained them.

In a subset of experiments we used cultured S2R+ *Drosophila* cells because it is easy, fast and effective to transfect them with protein expression vectors or with dsRNA silencing constructs. After the transfections we performed immunostaining on the cells. We also carried out the heat shock experiments on S2R+ cells.

To generate the vectors for new transgenic fly strains and cell culture protein expression vectors we used standard cloning methods and the *Drosophila* Gateway system.

For the biochemical confirmation of our data obtained in the immunostaining experiments, we carried out protein co-immunoprecipitation and Western-blot experiments on protein samples isolated from S2R+cells.

Results and discussion

1, During the intranuclear localization studies we observed that both the endogenous and the epitope-tagged versions of Moesin show the same localization pattern in the nucleus which indicated that the protein tags do not influence the distribution, therefore most likely the function of the protein in the nucleus. Moesin was present at the nuclear envelope, in the nucleoplasm, exhibited a banded pattern on the chromosomes and appeared occasionally in the nucleolus.

2, To analyze the dynamic properties of nuclear Moesin, first we performed FRAP experiments on live salivary glands of third instar larvae and found that during interphase there is no significant exchange between the cytoplasmic and the nuclear Moesin pools. Nevertheless, if we treated the glands with stimuli which trigger nuclear processes such as chromatin remodeling or transcription, Moesin accumulated in the nucleus. These data suggest that under normal conditions there is no significant nuclear transport of Moesin, but certain stimuli can induce the regulated nuclear import of the protein.

3, Interestingly, if the fluorescent signal of Moesin-GFP was bleached in a small area of the nucleus, the fluorescence was rapidly lost in the entire nucleus. This means that all the protein inside the nucleus exchanges within seconds, so while the nuclear transport activity of Moesin is low during interphase, its binding is very dynamic in the nucleus.

4, To gain more insight into the nuclear transport mechanisms of Moesin, we carried out an RNA interference screen on S2R+ cultured cells. We found that, upon silencing the *nup98* (Nucleoporin98) or its interaction partner *rae1* (RNA export 1) genes, Moesin strongly accumulated in the nucleus. This means that either these proteins are involved in the active nuclear export of Moesin, or they all share a common function.

5, Next, we identified a functional, conserved nuclear localization signal (NLS) sequence within the Moesin protein which is essential for active nuclear import.

6, To explore the nuclear function of Moesin first we analyzed its chromosomal localization in detail by immunostaining giant chromosomes. Moesin exhibited a staining fully complementary with DAPI, thus Moesin associates with the loosely packed chromosome regions, the sites of active transcription. We also observed that Moesin accumulated in the

chromosome puffs, which are special euchromatic regions with extreme high level of transcription, suggesting that it may be involved in transcription. Since we detected Moesin also at active transcriptional sites devoid of the puff structure, we could exclude the possibility that the protein is involved in the organization of the chromatin structure.

7, In the next set of experiments we compared the chromosomal localizations of Moesin and the transcription initiation- and elongation-specific forms of RNA-polymerase II, and concluded that Moesin is not involved in the initiation phase of RNA transcription.

8, The analysis of Moesin's recruitment to elongating or terminating gene loci revealed an accumulation of the protein during the elongation phase of transcription and a loss of Moesin signal as the transcription terminated. These results together suggest that Moesin is recruited to the actively transcribed genes and its function is coupled to the elongation phase of transcription or to elongation-related processes such as RNA splicing or export.

9, To distinguish between these possible functions, first we compared the nuclear distribution of Moesin and the sites of mRNA splicing by using the SC35 marker, but found no colocalization between them indicating that Moesin is not involved in splicing.

10, Next, we compared the distribution pattern of Moesin to the localization of proteins known to function in mRNA export: the Poly(A)-binding protein (PABP) and Rae1. As Moesin colocalized with both export factors, we concluded that Moesin is a new member of the mRNP particles.

11, To confirm Moesin's role in mRNA export, we performed protein co-immunoprecipitation experiments, in which we demonstrated that Moesin physically interacts with the mRNA export factors Nup98, Rae1 and PCID2.

12, Since both the FERM and the actin-binding domains alone localized to the nucleus and to the chromosomes in a pattern identical to that of the full-length protein, we hypothesize that similarly to its function in the cytoplasm, nuclear Moesin anchors proteins, for example mRNA export factors, to actin.

Summary

Our data presented in the thesis suggest that Moesin's biological function is not restricted to the cytoplasm, but the protein is an active component of the cell nucleus as well. Moesin is transported into the nucleus by an active, regulated, NLS-dependent mechanism, where it is a possible new component of the mRNP particles. In the mRNP particles Moesin directly or indirectly interacts with the Nup98, Rae1 and PCID2 proteins, thereby most likely participates in the nuclear export of mRNA molecules. In summary, our results uncover a novel nuclear function for the ERM protein, Moesin, thereby reveal a new aspect for the field.

Publications

MTMT number: 10047471

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Publications related to the thesis:

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. doi: 10.1007/s00418-015-1400-9. Epub 2016 Feb 4.

PMID: 26847179, **IF: 3,054 (2015)**

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PMID: 27006187 **IF: 1,933 (2015)**

Other publications:

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Conference lectures

In Hungarian

Az Eip74EF és Eip75B ekdizon indukált gének szabályozásának vizsgálata

Bodai László, Zsindely Nóra, **Kristó Ildikó**, Gáspár Renáta, Boros Imre

XII. Kolozsvári Biológus Napok, 2011. Április 8–10., Kolozsvár, Romania

Az aktinkötő Moesin fehérje sejtmagi funkciójának vizsgálata

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Egy aktinkötő, citoszkéletális fehérje funkciója a sejtmagban

Kristó Ildikó, Bajusz Csaba, Umesh Kumar Gautam, Maria Vartiainen, Vilmos Péter

XIV. Genetikai Műhelyek Magyarországon Minikonferencia, 2015. Szeptember 4., Szeged, Hungary

In English

Nuclear function for a cytoskeletal actin binding protein

Ildikó Kristó, Péter Vilmos and Miklós Erdélyi

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Nuclear function for the actin binding cytoskeletal protein, Moesin

Ildikó Kristó, Csaba Bajusz, Umesh Kumar Gautam, Joseph Dopie, Maria Vartiainen, Péter Vilmos

24th Wilhelm Bernhard Workshop on the Cell Nucleus, 2015. August 17-22., Vienna, Austria