Identification of Novel Nuclear Protein Interactions with the N-Terminal Part of Filamin A

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Since certain missense mutations in the N-terminal part of filamin A (FLNA) cause inherited skeletal malformation, we screened for proteins that bind to this part of FLNA. We identified two nuclear proteins that are specifically associated with the N-terminal region of FLNA. This suggests more extensive nuclear function of filamin than expected.

Key words: cytoskeleton; filamin; OPD-spectrum disorders

Cytoskeletal structures affect various forms of intra-cellular signal transduction associated with cellular adhesion, migration, and differentiation, and thus their reorganization is essential to the development of multicellular organisms. Filamin A (FLNA) is a large actin-binding protein required to cross-link cortical actin filaments into a three-dimensional meshwork. FLNA protein has an actin-binding domain (ABD) at its N-terminus, followed by a rod-like domain consisting of 24 filamin repeats, and the C-terminal 24th repeat allows homodimerization, which is important to its actin cross-linking activity (Fig. 1). The rod domain is interrupted by two short loops that are thought to form flexible hinge structures (H1 and H2).

FLNA protein is also known to interact with more than 30 cellular proteins of great functional diversity. For example, filamin-binding proteins include small GTPases Rac1 and RaLa, cell-adhesion receptor integrin β1, protein phosphatase SHIP2, and cytokine signal mediator TRAF2. A certain fraction of FLNA protein enters the cell nucleus and associates with transcription factors such as androgen receptor and FOXC1. This suggests that FLNA serves as a signal transduction scaffold in diverse cellular processes in the cytosol and, presumably, in the nucleus.

Certain missense mutations in the FLNA gene have been reported to cause a group of human inherited disorders including otopalatodigital syndrome (OPD) types 1 and 2, frontometaphyseal dysplasia, and Melnick-Needles syndrome. These OPD-spectrum disorders are characterized primarily by malformations of skeletal and craniofacial structures. This indicates that proper cytoskeletal structures are required for developmental morphogenesis, but it remains unknown how FLNA mutations cause these morphogenic anomalies.

Most of the filamin-binding proteins identified thus far interact with FLNA in the region between H1 and the C-terminus. Only a few proteins are known to associate with the N-terminal half of FLNA. On the other hand, the majority of the known missense mutations related to OPD-spectrum disorders reside between the N-terminus and H1 of FLNA, especially in the ABD and the 10th filamin repeat (Fig. 1). This suggests that unknown filamin-binding proteins may associate with the N-terminal half of FLNA and that changes in their binding states due to FLNA mutations may be involved in the pathogenesis of OPD-spectrum disorders.

To gain insight into such potential protein interactions, we screened a mouse embryonic cDNA library (BD Matchmaker Pretransformed Library, 11 day mEmbryo in Yeast, BD Biosciences, Palo Alto, CA) for proteins that bind to the N-terminal half of FLNA by a yeast two-hybrid system (Matchmaker GAL4 System 3, BD Biosciences). Four fragments of mouse FLNA (R1-7, R6-H1, R15-24 and R1-24) were used as bait (Fig. 1). Fragments R1-24 and R15-24 showed interaction with known filamin-binding proteins (cvHSP, Migfilin, Glycoprotein Ibα, and FILIP), confirming the validity of the screening (Table 1). The N-terminal fragments of FLNA, R1-7 and R6-H1, showed interaction with clone no. 188 and clone no. 429 and no. 444 respectively. Clone no. 188 contained the entire coding sequence for TAF1B, a subunit of the TIF-I β transcription initiation factor for RNA polymerase I. Both clone no. 429 and no. 444 coded the N-terminal part (amino acids 179–360) of mKIAA1903, a homolog of human M18BP1, which is required for CENP-A localization to the centromeres.

Direct binding of these proteins to the filamin fragments was confirmed by pull-down assays with in vitro translated proteins (Fig. 2A, B). They showed little or no binding to the R15-24 fragment, indicating their specific association with the N-terminal parts of FLNA. Two-hybrid analysis further confirmed specific interaction of TAF1B with the R1-7 fragment (Fig. 2C). Only the combination of the R1-7 bait and TAF1B (no. 188) target plasmids supported the growth of yeast on the selective media.

Since previous studies of the mutant FLNA genes associated with OPD-spectrum disorders revealed mutation hot spots in the 10th filamin repeat, we examined the interaction of mKIAA1903 (clone no. 444) with...
mutant R6-H1 fragments carrying D1159A, A1188T, and S1199L substitutions that have been recurrently observed in OPD-spectrum disorder patients. 3) Yeast two-hybrid assays showed no difference in mKIAA1903 interaction with the mutant and the wildtype fragments (data not shown).

TAF1B is a nuclear protein which, together with other RNA polymerase I-specific TAF proteins, is associated with the TATA-box binding protein to form the TIF-IB complex, which is required for transcription of the rRNA genes. Interestingly, a significant fraction of FLNA protein is reported to reside in the nucleus and to regulate the activities of certain transcription factors. For example, the H1 region of FLNA is cleaved by calpain, and the resulting C-terminal fragment is transported into the nucleus, binding to the androgen receptor and regulating its activity. 4) Full-length FLNA can also enter the nucleus and affect the activity of FOXC1 transcription factor. 5) The association of FLNA with TAF1B suggests that it may affect rRNA production and general protein translation activity.

mKIAA1903 is a protein of unknown function that has been assumed to be a transcription factor, because it has DNA-binding domains that are shared by some transcription repressors. Recently, its human homolog, M18BP1, was reported to form a complex with hMis18/C11/C12 and to accumulate at telophase-G1 centromeres. This process was required for the subsequent accumulation of CENP-A. 6) This suggests that the interaction of FLNA with mKIAA1903/M18BP1 may have a role in the organization of centromeres.

The mKIAA1903 cDNAs obtained by our screening contained exons 1–5, but lacked exon 2, in which the assumed initiation codon resides, according to the NCBI consensus CDS database. Multiple EST sequences of this form of mKIAA1903 mRNA have been reported, suggesting that this form of mRNA is expressed in

Table 1. Summary of Two-Hybrid Screening for FLNA-Binding Proteins

<table>
<thead>
<tr>
<th>Bait</th>
<th>R1-24</th>
<th>R15-24</th>
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<tbody>
<tr>
<td>cvHSP* (3)</td>
<td></td>
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</tr>
<tr>
<td>Migfilin* (3)</td>
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</tr>
<tr>
<td>FILIP* (1)</td>
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<tr>
<td>TAF1B (1)</td>
<td></td>
<td></td>
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<tr>
<td>mKIAA1903 (2)</td>
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</tbody>
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Asterisks indicate previously reported filamin-binding proteins.

Numbers of clones obtained are indicated in parentheses.

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**Fig. 1.** Schematic Diagrams of FLNA Protein and Its Fragments Used in the Screening Experiments. Boxes indicate the actin-binding domain and the 24 filamin repeats. Examples of the reported interaction regions are shown for some of the known FLNA-binding proteins. The arrowheads indicate amino acid substitutions associated with OPD-spectrum disorders.

**Fig. 2.** In Vitro Association of TAF1B and mKIAA1903 Proteins with FLNA Fragments.

A, TAF1B and mKIAA1903 proteins were synthesized in vitro from clone no. 188 and no. 444 respectively by the TnT Coupled Reticulocyte Lysate System (Promega, Madison, WI) in the presence of l-[35S]methionine, and were incubated with the GST-fused fragments of FLNA protein in phosphate-buffered saline. The FLNA fragments were recovered by glutathione-sepharose, and co-precipitated proteins were detected by autoradiography. B, GST-fused filamin fragments were separated by SDS–PAGE and stained by Coomasie Brilliant Blue. C, Specific interaction of TAF1B and the R1-7 fragment of FLNA was confirmed by two-hybrid assay. Yeast AH109 was transformed with pGADT7-TAF1B (clone no. 188) and pGBKTK7-R1-7, -R6-H1, or -R15-24. The transformants were isolated and grown on quadruple (left panel) or double (right panel) dropout media.
mouse tissues. Since the transcriptional start site of the mKIAA1903 gene has not been identified and there is no in-frame initiation or termination codon in the exon 1 sequence determined to date, the cDNAs we obtained may represent an alternatively spliced variant of mKIAA1903 that is translated from an initiation codon in an unidentified upstream exon. The relationship between the splicing variation and the interaction with FLNA remains to be determined.

Since interaction between mKIAA1903 and FLNA was not affected by the mutations in the 10th filamin repeat, the relation between mKIAA1903 interaction and OPD-spectrum disorders is unclear. However, identification of two nuclear proteins as novel filamin-binding partners suggests that FLNA has more extensive functions in nucleus than expected. Detailed studies of the nuclear translocation of FLNA are needed to understand its nuclear function and its relation to these inherited disorders.

References