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The "Lamin B-fold"

ANTI-IDIOTYPIC ANTIBODIES REVEAL A STRUCTURAL COMPLEMENTARITY BETWEEN NUCLEAR LAMIN B AND CYTOPLASMIC INTERMEDIATE FILAMENT EPITOPES*

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Previous studies have shown that nuclear lamin B binds specifically to the C-terminal domains of type III intermediate filament (IF) proteins under *in vitro* conditions. To further explore such site-specific interactions, we have used a two-step anti-idiotypic antibody approach. First, a monoclonal antibody disrupting the cytoplasmic IF network organization of living cells (mAb7A3) (Matteoni, R., and Kreis, T. E. (1987) *J. Cell Biol.* 105, 1253–1265) was characterized. Epitope mapping demonstrated that this antibody recognized a site located in the C-terminal domains of vimentin and peripherin (type III IF proteins). mAb7A3 was able to inhibit more than 80% of the *in vitro* binding of nuclear lamin B to PI, a synthetic peptide modeled after the C-terminal domain of peripherin that comprises a lamin B-binding site (Djabali, K., Portier, M. M., Gros, F., Blobel, G., and Georgatos, S. D. (1991) *Cell* 64, 109–121). In a second step, animals were immunized with mAb7A3 and the resulting anti-idiotypic sera were screened. Two of these antisera reacted specifically with nuclear lamin B but not with type A lamins or cytoplasmic IF proteins. The anti-lamin B activity of one of the antisera was isolated by affinity chromatography using a lamin B-agarose matrix. The reaction of these affinity-purified antibodies with lamin B was inhibited by mAb7A3. Furthermore, the anti-lamin B antibodies reacted with Fab fragments of mAb7A3 and abolished binding of lamin B to PI. From these data we conclude that anti-idiotypic antibodies against the paratope of mAb7A3 recognize specific epitopes of the lamin B molecule that have shapes complementary to the one of the C-terminal domain of type III IF proteins. We speculate that these (regional) conformations, which we term the "lamin B-fold," may also occur in non-lamin proteins that mediate the anchorage of IFs to various membranous organelles.

Intermediate filaments (IFs)[1] are major components of the cytoskeleton of higher eukaryotic cells. They are organized as fibrillar networks that extend from the nucleus to the plasma membrane (1, 2). The composition of IFs varies depending on the cell type. The known IF protein subunits are classified into six categories (I–VI) on the basis of their sequence characteristics. All subunit species have a similar domain substructure. They possess an N-terminal (head) domain, a helical middle (rod) domain, and a C-terminal (tail) domain (for a recent review, see Ref. 2).

Although some aspects of the self-assembly of IF subunits are well understood, it remains unclear how IF *networks* are formed and how these filaments may associate with other cellular structures. Previous ultrastructural work has indicated that arrays of IFs converge around the nucleus. Specifically, direct and indirect connections between the nuclear pores and cytoplasmic IFs as well as spatial associations between these filaments and the nuclear lamin filaments have been reported (3–6). In addition, other studies have demonstrated lateral and "end-on" contacts of IFs to the plasma membrane (7, 8). On a molecular level, *in vitro* reconstitution experiments have shown that the IF proteins vimentin and desmin bind to the membrane skeletal polypeptides ankyrin (8, 9) and spectrin (10). Similar studies have yielded evidence for an association between type III subunits and nuclear lamin B (11–13). Further analysis has suggested that, whereas ankyrin binds at the N-terminal domain of vimentin and desmin, lamin B specifically interacts with a part of the C-terminal domain of these proteins (9, 11–13).

The *in vitro* binding of cytoplasmic IF proteins to nuclear lamin B has been puzzling, because the nuclear lamins are thought to be restricted to the nucleoplasmic face of the inner nuclear membrane (see Ref. 12 for a discussion). Although further ultrastructural work will be necessary to resolve this topological question, it should be noted that recent experiments implicate two "lamin B-like" proteins in the attachment of IFs to the desmosomal plaques and other specialized regions of the plasmalemma *in vivo* (14, 15). In support of this, another lamin B-like protein has been localized in the lens plasma membrane by immunoelectron microscopy (16). These new findings raise the possibility that nuclear lamin B and other non-lamin membrane proteins may share some common structural features that confer to them the ability to bind and nucleate cytoplasmic IFs (for discussion, see Refs. 14 and 15).

Identifying and fully characterizing such lamin B-like proteins (which may have only limited primary structure homologies or no homologies at all) would be a difficult task. To facilitate it, it may be necessary to develop a screening procedure that selects for a structural complementarity to the C-terminal domains of type III IF proteins. A method of choice toward this objective is provided by the anti-idiotypic antibody approach.

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[1] The abbreviations used are: IF, intermediate filaments; SDS, sodium dodecyl sulfate.

As shown in a number of cases, the ability to generate anti-idiotypic antibodies specifically recognizing a "receptor" protein of a given "ligand-receptor" system constitutes strong evidence for a structural complementarity between the corresponding components (17–22). Employing similar methods, we have recently shown that immunization of rabbits with a synthetic derivative of the tail domain of peripherin (a type III IF subunit) results in anti-peripherin and anti-lamin B antibodies that are found in the same serum and behave as a typical idiotypic/anti-idiotypic pair (23). Such anti-idiotypic antibodies most probably develop by an autoimmune response to the idiotypic antibodies (see also Ref. 19).

To proceed further, we decided to examine whether we can raise anti-idiotypic antibodies that recognize the IF-binding site of lamin B by immunizing animals with monoclonal antibodies recognizing the lamin B-binding site of type III IF subunits. To generate anti-lamin B antibodies in ways that do not rely on autoimmune responses (as in the previous case) would be of analytical significance. In addition, from a more practical point of view, such reagents would be easier to use for screening purposes (no need to separate the idiotypic from the anti-idiotypic antibodies) and could be characterized more rigorously.

EXPERIMENTAL PROCEDURES

Protein Chemical Procedures—Mouse vimentin, purified according to the method of Nelson and Traub (24), was digested with α-chymotrypsin and thrombin as specified previously (12, 13, 25, 26). Mouse peripherin was a gift from K. Djabali (College de France, Paris). Nuclear lamins from rat liver nuclei were isolated according to Georgatos and Blobel (12). Karyoskeletal protein extracts from turkey erythrocyte nuclei were obtained as previously described (11, 12). For typical SDS-polyacrylamide gel electrophoresis profiles of the purified proteins and the fragments used in this paper, consult Refs. 11–13 and 23. The synthetic peptides, PI and PII, corresponding to residues 405–434 and 432–461 of rat peripherin (27), were made at the Biopolymer Facility of Rockefeller University (New York).

Immunological and Immunochemical Methods—Antibodies against nuclear lamins A/B/C (aLI) were prepared, characterized, and affinity-purified as described in Ref. 23. The production of mAb7A3 has been published previously (28). Hybridoma cells were cultured in serum-free medium (Nutridoma SP, Boehringer Mannheim), and IgG was prepared from the culture supernatant by precipitation with 50% saturation of ammonium sulfate. Fab fragments were produced and purified from IgG preparations using papain beads and protein G affinity chromatography (39). To generate anti-idiotypic antibodies, the following scheme was followed. 200 μg of purified mAb7A3 IgG in complete Freund's adjuvant were injected in the thigh lymph nodes and subcutaneously in rabbits at day 0. Boosting with 200 μg of IgG, in incomplete adjuvant, was done by intramuscular injections at day 30, and blood was collected at day 37. A second boosting was performed at day 51, and blood was collected at day 58. Finally, the animals were killed, and their blood was collected at day 150. Affinity purification of the antibodies was executed exactly as described in Ref. 23, using a lamin B Affi-Gel 15 affinity matrix. Immunoblotting and dot-blotting were done as previously described (11, 12, 23). Nonimmune mouse and rabbit IgG were purchased from Sigma.

Other Methods—Polyacrylamide gel electrophoresis was performed as described by Laemmli (29). Protein concentrations were determined using a Bio-Rad kit.

RESULTS

Characterization of mAb7A3—In the course of previous experiments, an antibody (mAb7A3, an IgG1) was produced. This antibody reacts with the IF protein vimentin and has the ability to dramatically alter the vimentin filament organization in microinjected tissue culture cells (28). Because of this interesting *in vivo* effect, we attempted to map the mAb7A3 epitope within one of the three structural domains of vimentin. Immunoblotting assays were performed, testing thrombic fragments of mouse vimentin that lack the entire N-terminal (head) domain of the molecule (30) and chymotryptic digests of the same protein that contain the middle (rod) domain and the C-terminal (tail) domain (11, 25) (for details, see "Experimental Procedures"). As depicted in Fig. 1*A*, mAb7A3 reacted specifically with the C-terminal peptide of mouse vimentin and the thrombic fragments of mouse vimentin peptide but did not recognize the vimentin middle domain (rod fragment). To further localize the mAb7A3 epitope, we also tested other IF preparations. Fig. 1*B* shows that the antibody recognizes mouse neuroblastoma peripherin (a type III IF protein) (27, 31, 32) and avian vimentin but not rat liver nuclear lamins A, B, and C (type IV IF subunits).

To find out which part of the C-terminal domain of peripherin and vimentin is recognized by mAb7A3, we carried out additional experiments using two synthetic peptides of peripherin (23), PI (residues 405–434) and PII (residues 432–461). Fig. 1*C*, *upper panel*, shows that mAb7A3 recognizes the PI peptide but not the PII peptide. In addition, the PI peptide inhibits mAb7A3 binding to vimentin (Fig. 1*C*, *middle panel*), whereas the PII peptide does not (Fig. 1*C*, *lower panel*). Taken together, these data allow mapping of the mAb7A3 epitope within 30 residues of the C-terminal domain of peripherin and vimentin.

Previous studies have demonstrated a direct and specific *in vitro* binding of lamin B to peripherin and the PI peptide (23). Taking advantage of this, we performed binding experiments in the presence of increasing amounts of mAb7A3 IgG. Results shown in Fig. 2 (*upper panel*) indicate that the monoclonal antibody inhibits more than 80% of the *in vitro* binding of lamin B to the PI peptide in a concentration-dependent manner. Similar results are obtained when the PI peptide is substituted for intact neuroblastoma peripherin (data not shown). In contrast with this, control antibodies (purified nonimmune mouse IgG) do not inhibit this interaction (Fig. 2, *lower panel*).

Characterization of the Anti-idiotypic Antibodies—Based on the fact that mAb7A3 inhibits binding of lamin B to PI, we thought that this antibody may associate near or at the lamin B-binding site of PI. Therefore, to explore the anti-idiotypic antibody approach, rabbits were immunized with mAb7A3 IgG and the resulting immune sera were tested against various IF preparations. Fig. 3*A* shows that one serum (no. 24) gives a strong reaction with mammalian lamin B, a weak reaction with avian lamin B, and no reaction with mammalian or avian vimentin. A similar reaction was observed with another serum (no. 25) that was not further analyzed.

To examine the anti-idiotypic character of the no. 24 antibodies, we purified the anti-lamin B activity using a lamin B affinity column. Fig. 3*B* demonstrates that mAb7A3 inhibits the reaction of affinity-purified no. 24 with rat liver lamin B. Furthermore, the affinity-purified no. 24 antibodies bind directly to intact mAb7A3 but not to an unrelated mouse IgG1, and this reaction is not inhibited by an excess of purified mouse IgG (Fig. 3*C*, *upper panel*). The affinity-purified no. 24 antibodies, as well as the no. 24 immune serum, react strongly with mAb7A3 Fab fragments, whereas the preimmune no. 24 serum does not (Fig. 3*C*, *lower panel*). These data suggest that mAb7A3 and no. 24 constitute an idiotypic/anti-idiotypic antibody pair (21, 22) and that no. 24 most probably binds to the antigen-binding site of mAb7A3.

To complete our analysis, we also tested the ability of no. 24 antibodies to interfere with the binding of lamin B to PI. As seen in Fig. 4 (*upper panel*), no. 24 IgG inhibits the binding of lamin B to PI in a concentration-dependent fashion. A control experiment shows that equivalent concentrations of a nonimmune rabbit IgG do not affect the lamin B-PI interac-

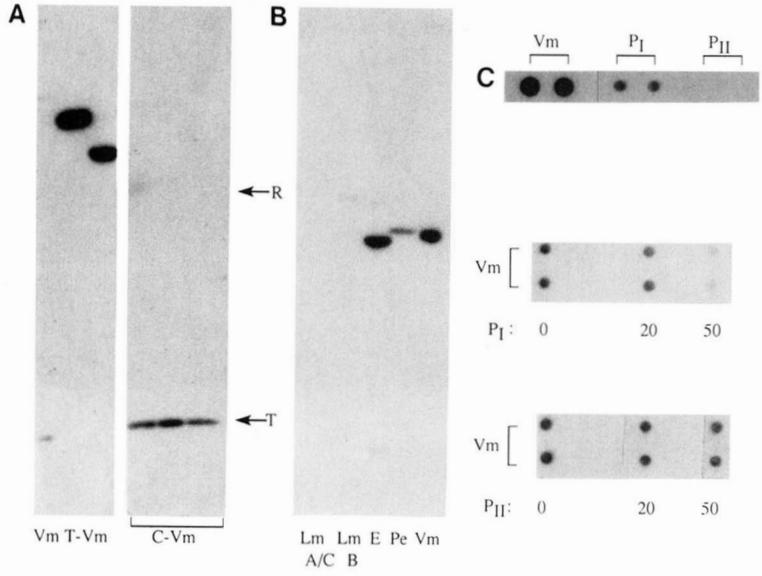

FIG. 1. **Identification of the mAb7A3 epitope.** *A* and *B*, mouse vimentin (*Vm*), a purified 48-kDa peptide of thrombin-digested vimentin (*T-Vm*), 5-, 10-, and 20-min (from *left* to *right*) chymotryptic digests of vimentin (*C-Vm*), purified rat liver lamins A/C (*Lm A/C*), purified rat liver lamin B (*Lm B*), 8 M urea extracts of turkey erythrocyte nuclear envelopes (*E*), and purified mouse neuroblastoma peripherin (*Pe*) were resolved either in a 12% gel that was ran for two-thirds of its length (*A*), or in a 10% SDS-polyacrylamide gel (*B*). After electrotransfer onto nitrocellulose filters, the strips were probed with 20.0 μg/ml purified mAb7A3 IgG. *R* and *T* point to the 38-kDa middle (rod) domain and the C-terminal (tail) peptide of vimentin, respectively. *C*, *upper panel*, ~1.0 μg of purified mouse vimentin and 0.6 μg of the synthetic peptides PI and PII were immobilized (in duplicates) onto nitrocellulose membranes and then probed with 20.0 μg/ml of mAb7A3 IgG. *C*, *middle* and *lower panels*, ~1.0 μg of vimentin was immobilized onto nitrocellulose membranes and then probed with 10 μg/ml mAb7A3 in the absence of additives (0), or in the presence of 20.0 μg/ml (20) and 50.0 μg/ml (50) of soluble peptides PI and PII, respectively, as indicated. The reactions were revealed by ^{125}I-goat anti-mouse IgG (Du Pont-New England Nuclear, diluted 1:500).

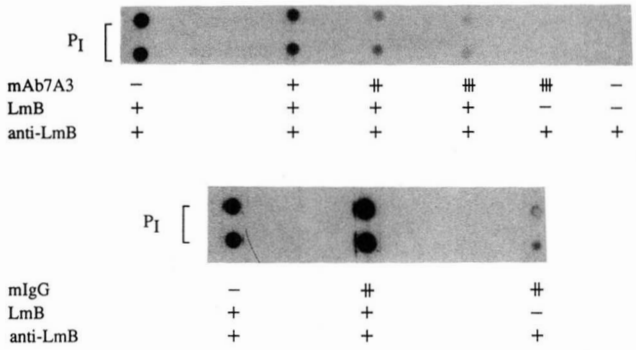

FIG. 2. **Inhibition of lamin B binding to PI by mAb7A3.** 0.6 μg of peptide PI was immobilized onto nitrocellulose filters. After blocking, the strips were incubated for 3 h at room temperature with none (−), 50.0 μg/ml (+), 100.0 μg/ml (++), and 300.0 μg/ml (+++) mAb7A3 (*upper panel*) or with 100.0 μg/ml (++) nonimmune mouse IgG (*lower panel*) both diluted in 0.9% (w/v) NaCl, 20 mM Tris-HCl, pH 7.3, 0.1% Tween 20 and 0.2% (w/v) cold water fish skin gelatin (gelatin buffer). After this incubation, buffer (−) or purified rat liver lamin B (to a final concentration of 8.0 μg/ml) (+) was added, and a further incubation for 15 h at room temperature ensued. After appropriate washings, the strips were probed with affinity-purified rabbit anti-lamin antibodies (aL1) and ^{125}I-protein A. Note the concentration-dependent inhibition of lamin B binding to PI by mAb7A3 and the lack of an effect with control mouse IgG.

tion (Fig. 4, *lower panel*). These data are consistent with the idea that the no. 24 antibodies specifically recognize the PI-binding site of lamin B.

DISCUSSION

By immunizing with a monoclonal antibody that recognizes the lamin B-binding site of vimentin and peripherin, we have obtained anti-idiotypic antibodies that specifically react with nuclear lamin B and inhibit its interaction with a synthetic derivative of peripherin *in vitro*. Taking into account the tenets of the network hypothesis (21, 22), it seems likely that the *in vitro* association between lamin B and type III IF proteins has been "reproduced" in the course of an immune response in specifically challenged animals.

One may argue that these results are hardly surprising, since the *in vitro* binding of lamin B to IF proteins already implies a substantial affinity between the two ligands. However, for a protein-protein interaction to be "replicated" in pairs of network antibodies, a series of very precise processes must take place. To copy the "image" of a receptor-binding site, the anti-idiotypic antibodies should satisfy strict spatial arrangements and complex bonding regimes. Recent studies reveal that it is rather rare to successfully replicate an idiotope that corresponds to the interacting site of a ligand in a second set of anti-idiotypic antibodies, although antibodies against irrelevant idiotopes can be easily elicited by immunizing animals with homologous or heterologous immunoglobulins (for a review, see Ref. 33). Because of these considerations, we feel that the anti-idiotypic antibody approach, when successful, provides evidence for a structurally meaningful interaction based on a very precise complementarity between two ligands. By comparison, the conventional *in vitro* binding assays, although useful as initial screening means, do not necessarily address "specificity" at the level of structural compatibility.

The physiological significance of the lamin B-IF interactions remains to be further examined. However, for the time being, we find it rather remarkable that anti-idiotypic reagents prepared in two different ways, *i.e.* by immunizing with the PI peptide (23) or by immunizing with an antibody-

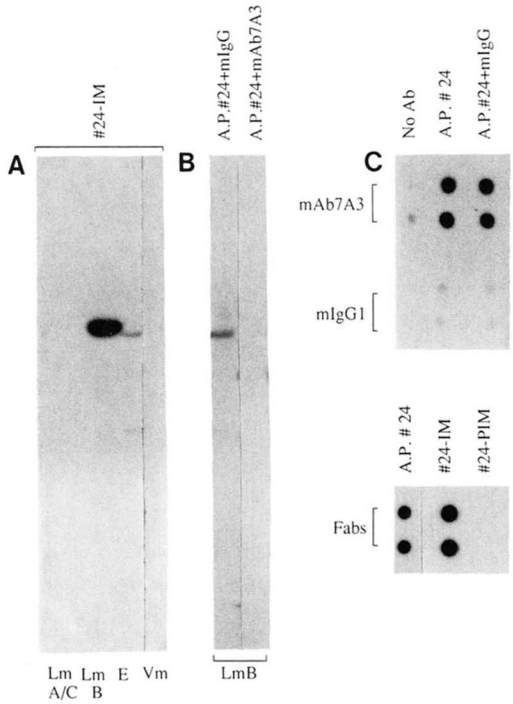

FIG. 3. **Characterization of anti-idiotypic antibodies.** *A*, equivalent amounts of purified rat liver lamins A/C (*Lm A/C*), purified rat liver lamin B (*Lm B*), 8 M urea extracts of turkey erythrocyte nuclear envelopes (*E*) and purified mouse vimentin (*Vm*) were resolved in 10% SDS-polyacrylamide gels. The proteins were transferred to nitrocellulose filters and probed with no. 24-immune serum (*#24-IM*) diluted 1:200. Note the strong reaction with mammalian lamin B (mainly B1 form) (37, 38) and the weaker reaction with avian lamin B (mainly lamin B2 form) (34, 35). *B*, no. 24-immune serum was passed through a lamin B Affi-Gel 15 column (see "Experimental Procedures"). The eluted antibodies (*A.P. #24*) at a concentration of 5.0 μg/ml were used to probe replica blots of purified rat liver lamin B in the presence of 200.0 μg/ml nonimmune mouse IgG (*A.P. #24 + mIgG*), or 200.0 μg/ml mAb7A3 (*A.P. #24 + mAb7A3*). The reactions were developed with ^{125}I-protein A. Note the specific inhibition of the binding of the no. 24 affinity-purified antibodies to lamin B by mA7A3. *C*, *upper panel*, 3.0 μg of purified mAb7A3 and 3.0 μg of an irrelevant mouse IgG1 (obtained from R. Duden, EMBL) were applied to nitrocellulose filters. *Panel A.P. #24* shows a blot incubated with the no. 24 affinity-purified antibody (*A.P. #24*), diluted 1:50 (approximate concentration 1 μg/ml). *Panel A.P. #24 + mIgG* shows blot incubated with a mixture of affinity-purified no. 24 antibodies (1.0 μg/ml) and nonimmune mouse IgG (50.0 μg/ml). *Panel No Ab* shows a blot done in the absence of antibodies. *Lower panel*, mAb7A3 Fab fragments were prepared as indicated under "Experimental Procedures." 2.0 μg of Fabs were applied to nitrocellulose filters and probed with the affinity-purified no. 24 antibodies (*A.P. #24, 2.5 μg/ml*), the no. 24 immune serum before affinity purification (*#24-IM, 1:200*), and the corresponding preimmune serum (*#24-PIM, 1:200*). The reactions were developed by ^{125}I-protein A.

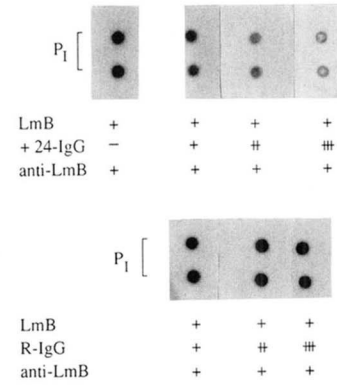

FIG. 4. **Inhibition of lamin B binding to PI by no. 24 antibodies.** 0.6 μg of PI was immobilized onto nitrocellulose filters. After blocking, the strips were incubated for 15 h at room temperature with none (−), 50.0 μg/ml (+), 200.0 μg/ml (++), and 300.0 μg/ml (+++) of purified no. 24 IgG (*upper panel*) or with the corresponding amounts of purified nonimmune rabbit IgG (*lower panel*) in a mixture with 8.0 μg/ml purified lamin B, as indicated. After appropriate washings, lamin B binding was detected by probing with affinity-purified anti-lamin antibodies (aLI) and ^{125}I-protein A. Note the gradual inhibition of lamin B binding by no. 24 antibodies and the lack of an effect with comparable concentrations of the nonimmune rabbit IgG.

recognizing PI (this report), recognize the nuclear lamin B. In particular, the ability of the anti-idiotypic antibodies to differentiate between type A and type B lamin proteins (which show considerable structural homology and share many solution properties), provides evidence for a specific association between vimentin/peripherin and lamin B. If this interaction does take place at interphase, one has to assume either passage of IFs through the nuclear pores, or a transient association of newly synthesized lamin B with cytoplasmic IFs (as the latter is being transported toward the nucleus). Alternatively, cytoplasmic IFs may transiently interact with lamin B during mitosis (when the lamins are available in the cytoplasm).

The structural complementarity between lamin B and type III IF proteins may be functionally exploited by lamin B-like factors as described in the Introduction (14, 15). These proteins may possess sites (not necessarily related to lamin B by primary structure criteria) with conformations similar to the PI-binding site of lamin B. Unlike nuclear lamin B, the postulated lamin B-like factors could be located in regions directly accessible to cytoplasmic IFs. We would term the regional conformation of such hypothetical sites the lamin B-fold to indicate that a variety of proteins may in fact interact with cytoplasmic IFs by virtue of a common complementary conformation without being necessarily homologous to nuclear lamin B. Apart from the two already characterized lamin B-like proteins (14, 15), reasonable candidates for such a function are also the widespread protein plectin (34), which binds both vimentin and lamin B *in vitro* (35), and the newly characterized protein filensin, a filament-forming component of the lens fiber cell membrane that associates with the C-terminal domain of vimentin *in vitro* (36).

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