Association of the Breast Cancer Protein MLN51 with the Exon Junction Complex via Its Speckle Localizer and RNA Binding Module*

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MLN51 is a nucleocytoplasmic shuttling protein that is overexpressed in breast cancer. The function of MLN51 in mammals remains elusive. Its fly homolog, named barentsz, as well as the proteins mago nashi and tsunagi have been shown to be required for proper oskar mRNA localization to the posterior pole of the oocyte. Magoh and Y14, the human homologs of mago nashi and tsunagi, are core components of the exon junction complex (EJC). The EJC is assembled on spliced mRNAs and plays important roles in post-splicing events including mRNA export, nonsense-mediated mRNA decay, and translation. In the present study, we show that human MLN51 is an RNA-binding protein present in ribonucleoprotein complexes. By co-immunoprecipitation assays, endogenous MLN51 protein is found to be associated with EJC components, including Magoh, Y14, and NFX1/TAP, and subcellular localization studies indicate that MLN51 transiently co-localizes with Magoh in nuclear speckles. Moreover, we demonstrate that MLN51 specifically associates with spliced mRNAs in co-precipitation experiments, both in the nucleus and in the cytoplasm, at the position where the EJC is deposited. Most interesting, we have identified a region within MLN51 sufficient to bind RNA, to interact with Magoh and spliced mRNA, and to address the protein to nuclear speckles. This conserved region of MLN51 was therefore named SELOR for speckle localizer and RNA binding module. Altogether our data demonstrate that MLN51 associates with EJC in the nucleus and remains stably associated with mRNA in the cytoplasm, suggesting that its overexpression might alter mRNA metabolism in cancer.

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Human metastatic lymph node (MLN)§ 51 cDNA was identified from a breast cancer-derivated metastatic lymph node cDNA library by differential hybridization of malignant (metastatic lymph node) versus nonmalignant (breast fibroadenoma and normal lymph node) tissues (1). MLN51 presents a correlated pattern of gene amplification and transcript overexpression in breast cancers and cancer-derived cell lines (1–3). In addition, elevated quantities of MLN51 protein have been found in 30% of primary breast tumor samples tested, although no correlation between MLN51 overexpression and a specific histological tumor type or grade has been found (4). MLN51 is a nucleocytoplasmic protein containing, within its amino-terminal half, a coiled-coil domain followed by two nuclear localization signals responsible for its nuclear localization. Its carboxy-terminal half contains putative Src homology domain 2 and 3 binding sites and mediates its cytoplasmic retention (4). Finally, MLN51 is well conserved during evolution in mammals as well as in more distant species such as worm and fly. From these results, we proposed previously (4) that MLN51 might have a basal cellular function and that its overexpression in cancer cells may have deleterious effects.

The MLN51 counterpart in the fly, called Barentsz, has been isolated from a functional genetic screening, as a gene essential for oskar mRNA localization (5). Messenger RNA localization to discrete cellular regions is an important process well described during oogenesis in Drosophila melanogaster. For instance, localization of bicoid and oskar mRNAs to the anterior and posterior poles of the oocyte, respectively, defines the anterior-posterior axis of the embryo (6). Both mRNAs are synthesized in nurse cells and then routed to the oocyte at opposite poles where they remain (7, 8). Bicoid mRNA translation occurs at the anterior pole only after egg fertilization, whereas oskar mRNA translation is coupled to its localization at the posterior pole and starts at mid-oogenesis. The mechanisms that underlie oskar mRNA localization have been elucidated in part by the identification of mutants bearing oskar mRNA localization defects in D. melanogaster (9). To date, many mutants, including those with defects in the kinesin heavy chain, staufen, mago nashi, tsunagi, cytoplasmic tropomyosin II and barentsz genes, have been identified (10).

§ The abbreviations used are: MLN, metastatic lymph node; EJC, exon junction complex; PBS, phosphate-buffered saline; mRNP, mRNA protein particle; RT, reverse transcriptase; EYFP, enhanced yellow fluorescent protein; LMB, leptomycin B; NES, nuclear export signal; NLS, nuclear localization signal; GST, glutathione S-transferase; TAP, tandem affinity purification; EST, expressed sequence tag; oligo, oligonucleotides; AdML, adenovirus major late; PABP, poly (A)-binding protein.

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The trans-acting factors involved in oskar mRNA localization complex have diverse functions and are conserved in mammals. Kinesin heavy chain and tropomyosin II are related to cytoskeletal elements, whereas staufen, mago nashi, and tsunagi are RNA-binding proteins. Although the kinesin heavy chain and staufen mammalian orthologs have also been shown to be implicated in mRNA localization (11, 12), a similar role for the mago nashi and tsunagi human orthologs (Magoh and Y14) is not known. In contrast, the function of Magoh and Y14 in mRNA metabolism is well described. The stable Magoh/Y14 heterodimer is a core component of the exon junction complex (EJC) (13–16). This complex contains at least six proteins as follows: Aly/REF, SRm160, RNPS1, UAP56, Y14, and Magoh (13, 14, 16–19). As a consequence of splicing, EJC is assembled onto nascent mRNA in a sequence-independent manner at a defined position located 20–24 nucleotides upstream of the exon-exon junction (13). In addition, Y14 and Magoh remain stably associated with mRNA after export to the cytoplasm (15–17). Thus, the EJC provides a link between pre-mRNA splicing and downstream events, including mRNA nuclear export, nonsense-mediated mRNA decay (reviewed in Refs. 20 and 21), and translation (22, 23).

The functional characterization of barentsz, the fly orthology of MLN51, was especially supportive because it gave clues regarding MLN51 function. Barentsz was found to be in the oskar mRNA localization complex (5), indicating that this protein acts directly on the fate of some mRNAs. Introduction of the mouse Mfn51 gene into barentsz-deficient flies showed that MLN51 recapitulates some barentsz-specific features such as its subcellular localization, its interaction with staufen, and its incorporation into the oskar mRNA localization complex (24). However, MLN51 cannot rescue the barentsz-mutant phenotype, as oskar mRNA remains at the anterior pole of the oocyte in these mutant flies (24). Although MLN51 and barentsz have common structural elements, the low overall homology score between both proteins might underlie species-specific functions. In fly oocytes, barentsz subcellular localization is strongly altered in mago nashi mutants (5), suggesting a potential relationship between these proteins.

The role of human MLN51 remains elusive. However, as a core EJC component, we have investigated whether human MLN51 is functionally linked to Magoh in mammalian cells by interacting with EJC components and/or as part of the EJC.

**EXPERIMENTAL PROCEDURES**

**Probes, cDNA Library Screening, cDNA Cloning, and Sequence Analysis**—For cDNA library screening, 100,000 plaque-forming units were plated on LB agar, and the nylon filter replicas (Hybond N, Amersham Biosciences) were hybridized at 42 °C in 50% formamide, 5·SSC, 0.4% Ficoll, 0.4% polyvinylpyrrolidone, 20 mmol/liter sodium phosphate, pH 6.5, 0.5% SDS, 10% dextran sulfate, and 100 μg/ml denatured salmon sperm DNA for 36–48 h with 32P-labeled MLN51-specific mouse and fish probes diluted to 1 × 10⁶ cpm/ml. Stringent washes were performed at 60 °C in 0.1× SSC and 0.1% SDS. Filters were autoradiographed at −80 °C for 24–72 h. Plaques that gave a signal were subjected to a secondary screening using the same conditions. Pure plaques were directly recovered as bacterial colonies using the pBluescript/KS-ZAPII in vivo excision system (Stratagene, La Jolla, CA).

The mouse MLN51 cDNA was isolated in two steps. First, by using two overlapping mouse ESTs (GenBank accession numbers AI842301 and AI867671), we designed two oligonucleotides primers and amplified an 800-bp internal cDNA fragment from mouse stomach mRNA by RT-PCR. Second, this fragment was used as a probe to screen a mouse stomach cDNA library, from which the four independent clones that were isolated, the complete mouse MLN51 cDNA sequence was established (GenBank accession number A3292072). The fish MLN51 counterpart (GenBank accession number AJ655546) was isolated by using a similar strategy. A zebrafish MLN51-specific probe was obtained similarly by RT-PCR using 14-h post-fertilization fish mRNA as template and the two following synthetic oligonucleotides: 5′ primer ACT132 (5′-GAGCACAGTGAGAGAGGCCAGG-3′) and reverse primer ACT133 (5′-GGCTGTCTGACCTGGAACCCGG-3′). This probe was used to screen an 18–40-h post-fertilization zebrafish cDNA library. Inserts contained in the mouse- and zebrafish-positive clones were sequenced on both strands, and a consensus cDNA nucleotide sequence was established from the different clones using the Assembler software (Applied Biosystem, Foster City, CA). To construct the frog MLN51 cDNA sequences, we aligned overlapping ESTs from either Silurana tropicalis or Xenopus laevis, and we established the complete MLN51 sequences from both frog species (GenBank accession numbers BN000153 and BN000154). We then identified and defined the MLN51 protein family members, we used PipeAlign (25). PipeAlign is a protein family analysis tool that integrates a five-step process ranging from the search for sequence homologs in protein sequence and three-dimensional structure data bases to the definition of the hierarchical relationship between and within subfamilies. Research for other MLN51 homologous sequences in all available data bases, at both the nucleotide and protein levels, were performed using BLAST software (26). Multiple alignments and phylogenetic trees were built using ClustalW and Phylowin (Genetics Computer Group, Wisconsin package version 10), respectively. The ESPript program was used for multiple alignment representation (27). The Protparam and Profilescan softwares were used to determine the molecular weight and the pHi. Prosite (28), PSORTII (29), and Coils (30) software allowed the finding of putative motifs for post-translational modifications, the prediction of the subcellular localization, and the identification of coiled-coil domains in all MLN51 counterparts, respectively. These software packages are available from the Exasy Molecu- lar Biology Server.

**Cloning and Constructs**—Poly(A)+ mRNAs from HeLa cells were subjected to first strand cDNA synthesis using oligo(dT) or random primers and Expand reverse transcriptase (Roche Diagnostics). The complete open reading frames of Magoh (GenBank accession number NM_006362) (31), Y14/RNA-binding motif protein 8 (RBMS) (GenBank accession number NM_005105) (14), and nuclear RNA export factor 1 (NXF1/TAP) (32) (GenBank accession number NM_006362) were obtained by RT-PCR using HeLa cells single strand cDNA as template and the following synthetic oligonucleotides: 5′ primer AEI184 (5′-CTCTCTAGAAGTTACGATGGAAACACGAGATG-3′) and reverse primer AEI183 (5′-CACTATACGATGCTGACCGGAGG-3′), and primer AEI185 (5′-GTTACCGATTAACGAGCTAC-3′) and reverse primer AEI186 (5′-GTTATCGATATGGAAGG-3′), and reverse primer AEI188 (5′-GTTAATCCCGGATTCTGCTG-3′), respectively. To generate FLAG-tagged fusion proteins, synthetic oligonucleotides containing the nucleotide sequence encoding the FLAG peptide in-frame with 5′ end of each cDNA of interest were used as 5′ PCR primers. After amplification, the purified products (Nucleospin, Macherey Nagel, Duren, Germany) were directly cloned into the pcR3.1 expression vector (BD Biosciences, Palo Alto, CA), generating pCR3.1-FLAG-magoh, pCR3.1-FLAG-Y14, and pCR3.1-FLAG-TAP. The vectors encoding MLN51 and truncated version of the protein fused to the enhanced yellow fluorescent protein (EYFP) were constructed by PCR (using pCR3.1-MLN51 as template). PCR fragments corresponding to MLN51/1–703 and 1–383 were obtained using the following oligonucleotides: AE200 (5′-GAAACATTTGCGTTCCTCGAGTTAGGACGCACG-3′) as forward primer, AGF274 (5′-GGCTCTCCTCTTGGGATGCGTGGTCT-3′) as reverse primer, AEI207 (5′-GTCCTTCGCTGGCTGCTTTTCGCT-3′) and reverse primer AEI210 (5′-GTAATCCCTCTCCCTCCTC-3′), respectively. To generate TAG-Flag fusion proteins, synthetic oligonucleotides containing the nucleotide sequence encoding the FLAG peptide in-frame with 5′ end of each cDNA of interest were used as 5′ PCR primers. After amplification, the purified products (Nucleospin, Macherey Nagel, Duren, Germany) were directly cloned into the pcR3.1 expression vector (BD Biosciences, Palo Alto, CA), generating pCR3.1-FLAG-magoh, pCR3.1-FLAG-Y14, and pCR3.1-FLAG-TAP.
Purification of Recombinant Proteins in E. coli—Plasmids allowing the synthesis of full-length (1–703) or truncated (1–351, 352–703, and 137–283) proteins from Escherichia coli were generated as described above. The coding region was inserted in fusion with the tandem affinity purification (TAP) protein (Euroscarf, Frankfurt, Germany) and the polyhistidine tag at the amino- and carboxyl-terminal ends, respectively. All vectors were verified by sequencing from both strands. The pCR3.1-MLN51, pEGFP RevNES, and pSG5-Lasp-1 expression vectors have been described before (4, 33, 34).

Molecular Probes (Eugene, OR), respectively. Ziemiecki, respectively. Cy3- or Cy5-conjugated affinity-purified goat anti-rabbit or goat anti-mouse at 1/10000, respectively. After washing, the blots were incubated with AffiniPure donkey anti-rabbit or goat anti-mouse at 1/10000 (Jackson Immunoresearch, West Grove, PA), Amersham Biosciences, and Molecular Probes (Eugene, OR), respectively. Detection on normal phase NP20 ProteinChip Arrays (Ciphergen Biosystems, Fremont, CA) and by Coomassie Blue staining after SDS-PAGE. Finally, recombinant proteins were diazylated against 1.5× PBS containing 10% glycerol.

MLN51 and Lasp-1 were transcribed-translated using the TNT rabbit reticulocyte lysate or wheat germ systems (Promega, Madison, WI) from pCR3.1-MLN51 and pSG5-Lasp-1 vectors according to the manufacturer’s conditions. For RNA homopolymers binding assays, RNA homopolymer-conjugated agarose beads poly(A), poly(G), poly(C), and poly(U) (Sigma) were mixed with the in vitro transcribed-translated MLN51 and Lasp-1 proteins in RNA binding buffer (10 mM Tris-HCl, pH 8, 2.5 mM MgCl2, 0.5% Triton X-100, 150 mM NaCl) for 30 min at 20 °C. After three washes in RNA binding buffer, bound proteins were eluted with SDS sample buffer and analyzed by Western blot.

Oligo(dT) Chromatography, RNA Homopolymers Binding Assay, and Northwestern Analysis—Purification of poly(A)-containing mRNPs present in polyribosomes was done as described by Lindberg and Sundquist (36), with minor modifications. Briefly, fractions from sucrose gradient containing L7a were pooled, dialyzed, concentrated, and incubated in 1 ml of binding buffer containing 100 mM KCl, 25 mM Tris-HCl, pH 7.4, and 25 mM EDTA in the presence of 20 mg of oligo(dT)-cellulose (type 7, Amersham Biosciences) for 30 min at room temperature. After 2 washes with 1 ml of binding buffer, elution of the adsorbed mRNPs complexes was done in elution buffer containing 25 mM Tris-HCl, pH 7.4, and 25% formamide. Fractions corresponding to input, unbound, and bound RNA were analyzed by Western blot as described above. Briefly, recombinant control and MLN51-derived proteins were quantified, separated on SDS-PAGE, and electroblotted onto nitrocellulose membranes. After overnight renaturation, the blots were incubated for 1 h at room temperature with a 32P-labeled riboprobe, washed, and autoradiographed at −80 °C for 4 h.

Immunocytochemistry and LMB Treatment—Five thousand to ten thousand HeLa cells were plated on glass coverslips in 24-well plates and transfected using JetPEITM transfection reagent (Polyplus transfection, Illkirch, France) with a total of 10 μg of DNA containing various expression plasmids. After 24 h, cells were washed twice with medium and incubated for 24 h in demembranated conditions. Rabbit anti-PABP, anti-FLAG, and anti-9G8 antibodies were a kind gift of N. Sonenberg, B. Bardoni, R. Gattoni, and A. Ziemiecki, respectively. Cy3- or Cy5-conjugated affinity-purified goat anti-mouse IgG and Alexa Fluor 488-conjugated goat anti-Rabbit IgG were purchased from Jackson ImmunoResearch (West Grove, PA). Anti-FLAGM2 and rabbit anti-MLN51 antibodies for 1 h at room temperature with a 32P-labeled riboprobe, washed, and autoradiographed at −80 °C for 4 h.

Cell Fractionation Using Sucrose Gradient Sedimentation—Confluent 15-cm dishes of HeLa or MCF7 cells were washed twice in cold PBS, and cells were collected by gentle scraping in PBS. After centrifugation at 1500 rpm for 5 min, cells were resuspended in 1 ml of lysis buffer (25 mM Hepes, pH 7.5, 0.5 mM KCl, 1 mM MgCl2, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, and 1% protease inhibitor mixture). Cell lysates were then homogenized with 15 strokes in a 5-ml Dounce homogenizer and centrifuged at 10,000 × g for 15 min at 4 °C. The supernatant was collected and centrifuged again. The supernatant was aliquoted, frozen in liquid N2, and stored at −80 °C. For cell fractionation under EDTA, gradient containing lysis buffer with 10 mM EDTA and 10% glycerol was used.
RESULTS

MLN51 Is Associated with RNA- and Protein-containing Complexes—We first determined whether MLN51 is found in association with RNA-containing complexes in human epithelial cells, using fractionation of HeLa cytoplasmic lysates by sedimentation on linear sucrose gradients under native conditions or after RNA degradation. By using immunoblotting, we compared the sedimentation profile of MLN51 with the sedimentation profiles of three control proteins, the poly(A)-binding protein PABP (39), the ribosomal L7a protein (40), and the soluble cytoplasmic Lasp-1 protein (34). In parallel, the RNAs contained in the collected fractions were analyzed by denaturing agarose gel electrophoresis (bottom panel). Approximate sizes of the detected proteins are indicated on the left as kilodalton values. Total RNA is stained using ethidium bromide (EtBr), and RNA positions are indicated on the left.

We first determined whether MLN51 is found in human cells as part of large RNA-protein particles (RNPs). These particles are most probably enriched in mRNAs, because the sedimentation profile of MLN51 is very similar to the profile of the mRNA-binding protein PABP.

MLN51 Is Associated with mRNPs in Vivo—In order to confirm that MLN51 is associated with mRNPs (mRNA-protein particles), we performed oligo(dT)-cellulose chromatography on HeLa cell fractions enriched in ribosomes. After sedimentation on linear sucrose gradient, fractions containing the ribosomal L7a protein were pooled and incubated in the presence of EDTA with oligo(dT)-cellulose. The unadsorbed material was washed off, and the adsorbed material was recovered by elution with a buffer containing 25% formamide. Detection of MLN51, PABP, L7a, and FMRP (as positive control) were performed on each sample by Western blotting. The ribosomal L7a protein, used here as a negative control, does not bind to the oligo(dT)-cellulose (41), indicating that ribosomes are no longer associated with mRNAs (Fig. 2A). Conversely, both PABP (39) and FMRP (41), bound to oligo(dT)-cellulose, were released by formamide containing elution buffer (Fig. 2A, lane 4). Similarly, MLN51 was bound to oligo(dT)-cellulose and released by formamide containing elution buffer (Fig. 2A, lane 4). This result confirms that MLN51 is present in mRNP complexes.

MLN51 Is an RNA-binding Protein—We next examined whether MLN51 could directly interact with RNA. To do so, we first tested the ability of in vitro translated MLN51 to bind agarose beads on which the ribonucleotide homopolymers poly(A), poly(C), poly(G), and poly(U) were separately immobilized. Following binding of unpurified in vitro translated products to the column, bound proteins were eluted and detected by Western blot. Under these conditions, MLN51 produced in reticulocyte or wheat germ extracts did bind to both poly(G) and poly(U) RNA homopolymers with a slight preference for poly(G) (Fig. 2B). In contrast, we observed that in vitro translated Lasp-1 protein, which was used as a negative control, did not bind to ribonucleotide homopolymers (Fig. 2B). MLN51 binding to poly(G) and poly(U) was weak because it was not resistant to washes in the presence of 250 and 150 mM NaCl, respectively (not shown).

To assess whether part of MLN51 is associated with ribosomes, HeLa cell cytoplasmic extract treated with EDTA was similarly fractionated on linear sucrose gradients. In the presence of EDTA, polyribosomes and ribosomes were dissociated into subunits. Accordingly, the L7a distribution was altered, because the protein became mainly detected in fractions containing the large ribosome subunits (Fig. 1C, lanes 7–9), as indicated by the presence of the 28 S rRNA (Fig. 1C, bottom panel). In comparison, the sedimentation profiles of both PABP and MLN51 were not as severely modified by EDTA treatment. Nevertheless, both proteins were depleted from the last three fractions of the gradient (Fig. 1C, lanes 12–14), suggesting that a small proportion of MLN51 was associated to polysomes. The sedimentation profiles of cytoplasmic soluble protein Lasp-1, used as a negative control, was not affected by RNase nor by EDTA treatment (Fig. 1).

Taken together, these data show that MLN51 is mainly found in human cells as part of large RNA-protein particles (RNPs). These particles are most probably enriched in mRNAs, because the sedimentation profile of MLN51 is very similar to the profile of the mRNA-binding protein PABP.

MLN51 Is Involved in mRNA Metabolism

For protein injections, 25 nl of recombinant TAP-MLN51 at 0.5 μg/μl in 1.5× PBS, 10% glycerol were injected into oocyte cytoplasm 2 h before RNA mixture injection into oocyte nuclei.
Fig. 2. MLN51 is included in mRNP complexes and binds to RNA. A, oligo(dT)-cellulose purification of mRNP complexes from HeLa cells. After sucrose gradient sedimentations, fractions containing ribosomes (fractions 7–14 from Fig. 1A) were pooled, dialyzed, concentrated, and resuspended in oligo(dT)-binding buffer containing EDTA. 600 µg of pooled proteins (input, lane 1) were incubated with oligo(dT)-cellulose, and the supernatant was collected as the unadsorbed material (lane 2). After washing (lane 3), bound proteins were recovered using formamide containing elution buffer (lane 4). Proteins present in every collected fraction were analyzed by Western blot using anti-MLN51Ct, anti-PABP, anti-L7a, and anti-FMRP antibodies. 1/50 (lanes 1–3) and 1/5 (lane 4) of the total collected volumes were loaded on the blot. The proteins detected using the various antibodies and their respective sizes are indicated on the right and on the left, respectively. B, RNA homopolymer binding assay. Binding to agarose beads coupled to poly(A), poly(G), poly(C), and poly(U) RNA homopolymers is shown for in vitro translated MLN51 protein using either rabbit reticulocyte (left panel, lanes 1–5) or wheat germ (middle panel, lanes 6–10) extracts. As negative control, the Lasp-1 protein translated in vitro by using rabbit reticulocyte extracts (right panel, lanes 11–15) was incubated with RNA homopolymers in the same conditions. 1/4 of the initial translation reactions and 1/5 of the bound proteins were analyzed by immunoblotting using anti-MLN51Ct (lanes 1–10) and anti-Lasp-1 (lanes 11–15) antibodies. The protein molecular size markers are indicated on the left, and the positions of MLN51 and Lasp-1 are indicated by an arrowhead and an arrow, respectively. C, Northwestern analysis of MLN51 interaction to RNA. One microgram of purified recombinant proteins, MLN51/1–703 (lanes 2 and 7), MLN1/1–351 (lanes 3 and 8), MLN51/352–703 (lanes 4 and 9), and MLN51/352–703 (lanes 5 and 10), and 1 µg of bovine serum albumin (BSA, lanes 1 and 6) were separated on SDS-PAGE, electrophoretically transferred onto nitrocellulose membrane, and renatured. The Ponceau S-stained membrane is shown on the left. Binding assay was performed using a 32P-labeled 240-nucleotide riboprobe transcribed from PvuII-linearized pBluescript. After washing, the membrane has been subjected to autoradiography (right panel). The protein molecular size markers are indicated on the left.
a chromosome region maintenance 1 (CRM1)-specific inhibitor. The protein CRM1 is implicated in the nuclear export of NES-containing proteins (43). Indeed, a fusion protein between the enhanced green fluorescent protein and the NES of the Rev protein efficiently accumulated in the nucleus upon LMB treatment (33) (Fig. 3s–x). Similarly, the full-length MLN51 protein (MLN51/1–703) accumulated in the nucleus upon LMB treatment (Fig. 3a–f), as does its carboxyl-terminal half that contains the NES (MLN51/352–703, Fig. 3b, m–r). Although the carboxyl-terminal half of MLN51 is devoid of NLS, we noticed that under LMB conditions the protein entered in the nucleus, potentially through passive diffusion. Most interesting, we noted that after LMB treatment MLN51 appeared as labeled dots in the nucleus (Fig. 3g–l). To determine which region of the protein is responsible for the punctate subnuclear localization, we generated additional truncated MLN51 proteins fused to the EYFP. Cells transfected with constructs bearing the NES were treated with LMB to prevent their nuclear egress, whereas the constructs devoid of NES were not treated. A minimal region of 147 amino acids spanning residues 137–283 was sufficient to address the fusion protein to these subnuclear structures (Fig. 4A, m–o). In agreement with this, only constructs bearing this region showed a punctate nuclear staining (Fig. 4A, a, d, g, j, and m).

To identify the subnuclear structures where MLN51 resides, HeLa cells transfected with EYFP-fused constructs (including EYFP-MLN51/137–283) were labeled with antibodies directed against the known nuclear resident proteins 9G8, promyelocytic leukemia, or fibrillarin, three resident proteins of speckles (44), promyelocytic leukemia bodies (45), and nucleoli (46), respectively. Among these three proteins, only 9G8 staining overlapped with EYFP-MLN51 staining (Fig. 4B and data not shown). Most interesting, when sections containing the 9G8 signal were examined using confocal microscopy, MLN51 appeared as a ring around the 9G8 protein (Fig. 4B).

As Magoh is mostly addressed to nuclear speckles (17), we next examined MLN51 and Magoh co-localization. Full-length MLN51 and FLAG-tagged Magoh were transiently expressed in HeLa cells in the absence or in presence of LMB (Fig. 4C). In the absence of LMB, MLN51 was detected in the cytoplasm, and Magoh was detected in the nucleus (Fig. 4C, a–d). In contrast, in the presence of LMB both proteins co-localized and appeared as ring-shaped structures in the nucleus (Fig. 4C, e–h). We then confirmed that MLN51 and Magoh were present in nuclear speckles in cells overexpressing both EYFP-MLN51/137–283 and FLAG-tagged Magoh. The localization of Magoh and nuclear speckles was detected using anti-FLAG and anti-9G8 antibodies, respectively. Then the signals corresponding to the three proteins, MLN51, Magoh, and 9G8, were examined in 0.2-μm sections obtained by confocal microscopy. When using regular microscopy the MLN51/137–283 or FLAG-Magoh appeared as dots (not shown), using fine sections (Fig. 4D); MLN51 and Magoh co-localized in a ring at the periphery of 9G8.

These results confirm that MLN51 is shuttling between the...
cytoplasm and the nucleus (4) and indicate that in the nucleus MLN51 co-localizes with Magoh in speckles where SR proteins such as 9G8 are present. Moreover, we have defined a region of 147 amino acids within the MLN51 protein that is sufficient to address the protein to these subnuclear structures.

MLN51 Contains a Novel Conserved Protein Module Called the SELOR Module

MLN51 was originally identified as a gene overexpressed in breast cancer. At that time, no functional indication could be deduced from the MLN51 primary sequence because no homology to known proteins was found (1). To identify functional motifs within this protein, we have cloned MLN51 counterpart sequences from the following species: mouse (Mm MLN51), frog (Xl MLN51, St. MLN51), and fish (Dr MLN51). A multialignment of MLN51 protein sequences from man to fish showed a high homology score ranging from 55 to 91% identity (Fig. 5, A and B). A region spanning amino acids 168–246 of the human sequence was found to be more conserved than the rest of the protein in all the MLN51 ortholog sequences available (24, 42). To better define this region, we performed multialignment by using relevant sequences coming from every available species, allowing the definition of the signature of a novel 80-amino acid-long conserved protein region (Fig. 5, C and D). Because this region is only present among MLN51 orthologs, it cannot be considered as a novel protein domain, and for that reason we refer to it as a protein module. This module shares 100% identity among human, mouse, and rat orthologs and exhibits an average score of 95% similarity among fish and frogs, 65% similarity among insects, worms, and sea squirt, and 39% similarity in plant (Fig. 5, C and D). In insects, an insertion of 15 and 33 amino acids splits this region into two halves in mosquito (Ag MLN51) and fly (D. melanogaster MLN51/barentsz and Dp MLN51).
FIG. 5. MLN51 contains a conserved region named the SELOR module. A. multialignment of the complete MLN51 proteins from Homo sapiens (HsMLN51, GenBank™ accession number CAC27699), Mus musculus (MmMLN51, CAC27775), Rattus norvegicus (RnMLN51, NP_671485), X. laevis (XlMLN51, BN000152), S. tropicalis (StMLN51, BN000153) and Danio rerio (DrMLN5, AJ555546). Identical and similar amino acids are printed in boldface letters and boxed in black and white, respectively. B. phylogenetic tree built from the multialignment shown in A. The percentages of similarity and identity are indicated on the right. C. multialignment of the SELOR module using sequences from H. sapiens (Hs, CAC27699, from amino acids 170–245), R. norvegicus (Rn, NP_671485, from amino acids 167–242), M. musculus (Mm, CAC27775, from amino acids 167–242), Canis familiaris (Cf, CF412023, translated EST from nucleotide 263–490), D. rerio (Dr, AJ555546, from amino acids 170–244), Tetraodon nigroviridis (Tn, AL290552, translated genomic sequence from nucleotide 379–672), Fugu rubripes (Fr, CAAB01000412, translated genomic sequence from nucleotide 3519–3820), X. laevis (Xl, BN000152, from amino acids 161–237), S. tropicalis (St, BN000153, from amino acids 169–239),Apis mellifera (Am, AADG02002121, translated genomic sequence from nucleotide 8501–8749), Anopheles gambiæ (Ag, EAA121971, from amino acids 30–120), Drosophila pseudoobscura (Dp, AADE10000028, translated genomic sequence from nucleotide 73540–73887), Drosophila melanogaster (Dm, AAN14126, from amino acids 129–240), Ciona intestinalis (Ci, AV965432, translated EST from nucleotide 269–350), MalVIDIA arenaria (Ma, BIT46382, translated EST from nucleotide 96–382), Haemonchus contortus (He, BM139141, translated EST from nucleotide 271–483), Caenorhabditis elegans (Ce, NF_493346, from amino acids 81–162), Caenorhabditis briggsae (Cb, CAE73026, from amino acids 82–159), Schistosoma mansoni (Sm, CD179665, translated EST from nucleotide 340–349), and Arabidopsis thaliana (At, AAD55461, from amino acids 121–199). Identical and similar amino acids are printed in bold characters and boxed in black and white, respectively. The consensus sequence is indicated, identical and conserved amino acids in more than half of the aligned sequence are printed in capital and lowercase letters, respectively. D. phylogenetic tree built from the multialignment shown in C. The percentages of similarity and identity are indicated on the right.
orthologs, respectively (Fig. 5C). Because this module contained the RNA-binding and speckles-addressing region of MLN51, as shown above, we named it SELOR as an acronym for speckle localizer and RNA binding module.

MLN51 Interacts in Vivo with Components of the EJC—Although predominantly localized in the nucleus, Magoh and Y14 are accompanying mRNAs in the cytoplasm where they act as a mark to influence mRNA metabolism (15, 17, 47). Conversely, MLN51 is mainly located in the cytoplasm. However, as reported above, the protein is transiently present in nuclear speckles where Magoh resides. To investigate if MLN51 could interact with EJC components such as Magoh (17), Y14 (47), and NXF1/TAP (15), we used an immunoprecipitation assay (Fig. 6). FLAG-tagged Magoh, Y14, or NXF1/TAP proteins were transiently expressed in human HeLa cells (Fig. 6A). FLAG-tagged complexes were precipitated with an anti-FLAG antibody affinity resin and analyzed by immunoblotting using the anti-MLN51 antibody. This analysis showed that the endogenous MLN51 protein was co-immunoprecipitated with FLAG-tagged Magoh, FLAG-Y14, and FLAG-NXF1/TAP (Fig. 6A). To determine whether these interactions are RNA-dependent, the same experiment was performed after RNase treatment of the cell extracts. RNase treatment did not impair immunoprecipitation of the FLAG-tagged fusion proteins (Fig. 6A, lanes 17–22). Nevertheless, RNA degradation prevented FLAG-Y14 co-immunoprecipitation with MLN51, respectively (Fig. 6A). In contrast, MLN51 co-immunoprecipitation with FLAG-Magoh was only slightly reduced after RNase treatment (Fig. 6A), thus suggesting that a portion of the cellular pool of MLN51 interacts with Magoh in an RNA-independent manner.

To map the domain required to interact with Magoh within the MLN51 protein, we performed co-immunoprecipitation experiments from HeLa cell extracts. Cells were transiently
transfected using FLAG-Magoh and full-length or truncated MLN51 proteins fused to EYFP. Immunoprecipitates revealed with an anti-EYFP antibody showed that full-length EYFP-MLN51 was able to interact with FLAG-Magoh, indicating that the presence of the EYFP does not impair MLN51 incorporation into this complex (Fig. 6B, lane 7). By using co-transfection of truncated EYFP-MLN51 and FLAG-Magoh constructs, we observed that all expressed fusion proteins containing the region spanning amino acids 137–283, including the SELOR module, were able to interact with Magoh (Fig. 6B, lanes 7–9). In contrast, the EYFP protein alone or the construct devoid of this region did not interact (Fig. 6B, lanes 6 and 10 and data not shown). To assess if this interaction was RNA-dependent, the protein extracts were treated with RNase before immunoprecipitation. Following RNase treatment, full-length MLN51 and both truncated proteins containing the SELOR module (MLN51/137–383 and MLN51/137–277) were efficiently co-immunoprecipitated with Magoh (Fig. 6B, lanes 12–14) in an RNase-insensitive manner, suggesting that this interaction does not depend on RNA. These results indicate that the MLN51 region containing the SELOR module mediates the interaction between MLN51 and Magoh.

To study whether MLN51 interacts directly with Magoh, we produced Magoh fused to glutathione S-transferase (GST) and GST-Magoh/His-tagged Y14 heterodimers in E. coli. We then performed GST-pulldown analysis with recombinant MLN51 protein. GST-Magoh pulled down His-tagged Y14 (31) but failed to pull down recombinant MLN51 (not shown), thus suggesting that the interaction between MLN51 and Magoh is indirect. Taken together these results indicate that MLN51 is able to interact indirectly with Magoh. Given that Magoh is a core component of the EJC, it is temptng to speculate that MLN51 associates with mRNAs via EJCs.

MLN51 Specifically Associates with EJC Assembled in Vitro—To test this hypothesis, we next examined whether MLN51 interacts with the EJC assembled in vitro. To do so, the radiolabeled pre-mRNA AdML (derived from the adenovirus major late transcription unit) was incubated under splicing conditions in the presence of HeLa cell nuclear extracts to generate spliced mRNAs on which the EJC is assembled (13). The splicing reactions were supplemented with recombinant TAP-tagged MLN51 proteins. After splicing, these reactions were subjected to affinity precipitation with IgG-Sepharose beads, and co-precipitated RNAs were analyzed by denaturing PAGE (Fig. 7A). In splicing reactions supplemented with TAP-MLN51/1–703, AdML spliced mRNAs were specifically and efficiently precipitated (Fig. 7A, lanes 2 and 3), in contrast to RNA molecules not associated to the EJC such as the
pre-mRNA or the splicing intermediate (free exon 1 and lariat intermediate). Identical results were obtained when splicing reactions were supplemented with the truncated proteins TAP-MLN51/1–351 (Fig. 7A, lanes 4 and 5) and TAP-MLN51/137–283 (lanes 8 and 9), but no specific precipitations were observed with the truncated protein TAP-MLN51/352–703 (lanes 6 and 7) or the TAP tag alone (lanes 10 and 11). In parallel, we performed the similar experiments with the AdML control mRNA that was transcribed from cDNA and on which the EJC is not assembled under splicing conditions (Fig. 7A, lanes 12–21). We observed that the control mRNA was not co-precipitated when the reaction was supplemented with the full-length recombinant protein TAP-MLN51/1–703 or one of its truncated versions. Thus, these results indicate that recombinant MLN51 proteins containing the SELOR module are specifically incorporated in AdML mRNP complex generated by splicing.

We next examined whether recombinant MLN51 binds to spliced mRNA at the position where the EJC is assembled, 20–24 nucleotides upstream of the exon-exon junction (13). To do so, AdML mRNAs spliced in vitro in the presence of TAP-MLN51 were digested by RNase H with two short cDNA oligos centered at positions −48 and +12 (relative to the exon-exon junction defined as position 0). This digestion led to mRNA cleavage into three fragments named 5′, m, and 3′. The fragment “5′” contained the exon 1 portion up to position −48. The middle fragment “m” corresponding to the mRNA portion from −48 to +12 contained the region where the EJC is assembled. The fragment “3′” contained the exon 2 portion from +12 to the end. Reactions were then subjected to affinity precipitation, and co-precipitated RNA fragments were analyzed by denaturing PAGE (Fig. 7B). We observed that the middle fragment m was selectively co-precipitated when TAP-MLN51 proteins containing the SELOR module (TAP-MLN51/1–703, TAP-MLN51/1–351, or TAP-MLN51/137–283) were present in splicing reactions. In contrast, none of the mRNA fragments were precipitated when splicing reactions were supplemented with TAP-MLN51/352–703 (devoid of the SELOR module) or with the control protein TAP. To test the generality of the results obtained with AdML mRNA, we also performed affinity co-precipitation of in vitro spliced β-globin derived mRNAs, β/38 and β/17 (Fig. 7C). Because the EJC is assembled more than 20 nucleotides upstream of the exon-exon junction, spliced β/17 mRNA, which contained a 17-nucleotide-long 5′ exon, did not carry an EJC (15). In contrast, the EJC was efficiently assembled on the slightly longer mRNA β/38 containing a 38-nucleotide-long 5′ exon (15). A mixture of β/38 and β/17 pre-mRNAs was incubated under splicing conditions in the presence of TAP-MLN51/1–703, TAP-MLN51/1–351, or TAP-MLN51/352–703, and reactions were then subjected to affinity precipitation. As before, we observed that MLN51/1–703 and TAP-MLN51/1–351 associate specifically with spliced β/38 mRNAs carrying the EJC but not TAP-MLN51/352–703. Taken together, these results showed that recombinant MLN51, via its conserved SELOR module, specifically associates with mRNAs as a consequence of splicing and that this association is spatially restricted to the mRNA fragment on which the EJC is assembled.

**MLN51 Associates with Spliced mRNAs Both in the Nucleus and the Cytoplasm—As shown previously, whereas MLN51 is mainly localized in the cytoplasm, it has the ability to shuttle between the nucleus and the cytoplasm. Given that MLN51 binds spliced mRNA in vitro, we next examined in *X. laevis* oocytes whether MLN51 is specifically found in association with spliced mRNAs both in the nucleus and in the cytoplasm in vivo (Fig. 8). To do so, TAP-MLN51/1–703 and TAP-MLN51/1–351 were separately injected into oocyte cytoplasm. Oocytes were then incubated for 2 h to allow the recombinant proteins to be imported in the nucleus. Subsequently, body-labeled β-globin pre-mRNA was injected into oocyte nuclei along with two RNAs serving as controls for nuclear export. U6Δ5ss was not exported to the cytoplasm, whereas the human initiator methionyl-tRNA was rapidly exported. All RNAs were nuclear immediately after injection (Fig. 8, lanes 1 and 2). After a 2-h incubation, all U6Δ5ss RNA was still nuclear, and the control tRNA was exclusively cytoplasmic. During the same period, the
pre-mRNA was efficiently spliced, and more than half of the mRNA was exported to the cytoplasm (Fig. 8, lanes 3 and 4). Nuclear and cytoplasmic fractions of injected oocytes were then subjected to affinity precipitation with IgG-Sepharose beads, and co-precipitated RNAs were analyzed in parallel. When oocytes were pre-injected with either TAP-MLN51/1–703 or TAP-MLN51/1–351, we observed that spliced β-globin mRNAs were the only molecules efficiently co-precipitated both in the nucleus and the cytoplasm. Indeed, the RNA molecules that did not carry the EJC, such as the pre-mRNA, the intron, and the control RNAs U6 and 18S, were not significantly precipitated. This observation was not restricted to β-globin mRNA because identical results were obtained when similar experiments were performed with the Fushi Tarazu (Ftz) pre-mRNA (Fig. 8, lanes 11–20). We confirmed that TAP-MLN51 binding to β-globin and Ftz mRNAs in oocytes was restricted to mRNAs generated by splicing in analogy in parallel the β-globin and Ftz control mRNAs that were transcribed from the corresponding cDNAs (Fig. 8, lanes 21–30). Indeed, in comparison to in vivo spliced β-globin and Ftz mRNAs, the corresponding control mRNAs were not efficiently co-precipitated.

Therefore, these results clearly indicate that the recombinant MLN51 protein, when injected in the cytoplasm of oocytes, has the ability to be imported in the nucleus where it is incorporated in mRNP complexes generated by splicing. Because recombinant MLN51 also associates specifically with spliced mRNAs in the cytoplasm, MLN51 most probably accompanies spliced mRNA after their transport from the nucleus to the cytoplasm. However, we cannot exclude the possibility that a proportion of the injected recombinant MLN51 present in the cytoplasm also interacts with the mRNAs carrying an EJC, after export of these mRNAs to the cytoplasm.

**DISCUSSION**

The goal of this study was to progress further in the understanding of the function of human MLN51 in epithelial cells from which breast cancer arises. MLN51 is overexpressed in 10–30% of human breast cancers as a result of its gene amplification (2–4, 48). Moreover, MLN51 is amplified in a subset of cancers associated with a poor clinical outcome (4). Therefore, the constitutive action of this protein might contribute to the deleterious progression of breast cancer. Determination of its normal function is a prerequisite to understanding its potential role in cancer. We show by oligo d(T) chromatography that MLN51 is incorporated into an mRNP complex in human cells. Moreover, sucrose velocity gradient ultracentrifugation demonstrates that MLN51 is present with RNA in a high molecular weight complex and that part of it might be associated with polysomes. Nevertheless, because RNAse treatment was not sufficient to free MLN51, we can postulate that either MLN51 remains in an oligomerized form after RNA degradation via its functional coiled-coil domain (4) or MLN51 is engaged into a multiprotein complex resistant to RNase treatment.

Although no evidence for a direct interaction with RNA had been reported for barentsz and mouse MLN51 (5, 24), we show that MLN51 is an RNA-binding protein. By using different deleted MLN51 constructs, we isolated the protein portion, from amino acids 137 to 283, able to bind RNA. When compared with the full-length protein, this region binds RNA less efficiently, suggesting that different regions of MLN51 might cooperate for binding RNA, as is the case for other RNA-binding proteins such as DAX-1 (49). This region shares no homology with previously known RNA binding domains, and computer analysis failed to predict secondary structural elements within this region. For example, structural analysis of the most common RNA recognition motif from several different proteins showed that these domains are characterized by a four-stranded anti-parallel β-sheet packed against two α-helices arranged in a β/α/β/α/β/α topology (50). We propose that the interaction between the MLN51 RNA-binding region and specific partners, such as RNA and/or proteins, might stabilize and give structure to this part of the protein. Determination of the three-dimensional structure of this region would be of interest to understand this interaction.

MLN51 is a nucleocytoplasmic protein. It contains two NLSs in the amino-terminal half that mediate its nuclear import into the nucleus, whereas its carboxyl-terminal half, containing a NES, maintains the protein in the cytoplasm (4, 24). Computer analysis indicates that the presence of NLSs and NES is conserved from man to fly in MLN51 proteins, suggesting that MLN51 is nucleocytoplasmic in these species. Most interesting, the worm MLN51 counterpart is devoid of potential NES, and the protein is mainly found in the nucleus in all cell types and at all developmental stages (www.wormbase.org/db/gene/gene/?name = W08E3.2), indicating that in the worm, MLN51 has a nuclear function. We have further investigated the nucleocytoplasmic shuttling of MLN51 by using LMB, an inhibitor of CRM1-dependent nuclear export (43). Under these conditions, we observed that MLN51 is present around subnuclear structures identified as nuclear speckles positives for the SR protein 9G8. Speckles are subnuclear regions, containing little or no DNA, thought to be storage compartments for pre-mRNA splicing factors (51). By immunofluorescence microscopy, splicing factors (such as 9G8) show a punctate nuclear localization of variable size and irregular shapes corresponding to nuclear speckles. To date, several domains have been implicated in targeting proteins to nuclear speckles, such as the RNA recognition motif of PTB-associated splicing factor (52), the serine/arginine-rich domain of SR proteins (53), or two adjacent regions of the EJC protein SRm160 (54). We show here that the MLN51 portion necessary for speckle localization corresponds to the RNA-binding portion of the protein. Multialignment of MLN51 protein orthologs in this part of the protein underlined a conserved region 80 amino acids long. Indeed, the homology score varies between 100 and 57% similarity from mammal to worm, highlighting the functional relevance of this region. MLN51 is present in the human genome as a single gene without paralog, and the conserved region of the protein is only present among MLN51 orthologs. Therefore, we consider this region as a protein module rather than a domain. As this module does not share homology to known speckle localization domains or RNA-binding domains, we named it “SELOR.”

In mago nashi mutant flies, the normal cellular localization of barentsz is affected, not only at the posterior pole during development but also in the perinuclear region of nurse cell cytoplasm, further suggesting that mago nashi and barentsz are in close functional association. In human cells, we show that MLN51 and Magoh co-localize around nuclear speckles revealed by the SR protein 9G8. Most interesting, a similar distribution at the periphery of speckles was observed for the EJC component SRm160 (54). Speckles are dynamic structures that are not delimited by a membrane; however, the core of the speckle is likely a storage compartment for pre-mRNA processing factors such as splicing factors (51). Moreover, nuclear speckles are often observed close to highly active transcription sites, indicating that they have an important function for coupling transcription to pre-mRNA processing (51). The finding of several EJC components at the periphery of these nuclear structures suggests that they represent sites where the EJC is assembled and may recruit proteins important for further steps of mRNA metabolism.

We further investigated whether MLN51 associates with Magoh. By using transfection experiments, we show that
FLAG-tagged Magoh is able to co-precipitate endogenous MLN51 and that this association is mostly resistant to RNase treatment. Most interesting, FLAG-Y14 also co-precipitates endogenous MLN51, but this association is mainly RNase-sensitive. These results suggest that a privileged interaction exists between Magoh and MLN51. Nevertheless, RNase treatment dissociates partially the interaction between MLN51 and Magoh. We can speculate that in addition to their RNA-independent interaction, MLN51 and/or Magoh associate with general or specific cellular mRNAs independently of EJC, leading to a RNA-dependent association of these proteins. The region of MLN51 involved in the association with Magoh was delineated by using co-transfection experiments and again coincides with the portion of the protein that includes the SELOR module. These data favor a direct link between MLN51 and the EJC containing the Magoh/Y14 heterodimer. We first demonstrated this link in vitro by showing that recombinant MLN51 specifically associates with different mRNAs carrying an EJC. In addition we show that this association is spatially restricted to the mRNA region where the EJC is deposit and mediated by the SELOR module of MLN51. Subsequently, we used X. laevis oocytes to confirm that this specific association with the EJC also occurs in vivo. Accordingly, MLN51 injected in the cytoplasm is imported in the nucleus where it is incorporated into mRNP complexes generated by splicing and still interacts with spliced mRNA after their transport to the cytoplasm.

Our findings are in agreement with recent reports. Palacios and colleagues (55) identified eIF4A3, a member of the eukaryotic translation initiation factor 4 family of RNA helicases, as a new trans-acting factor involved in oskar mRNA localization. They showed that recombinant eIF4A3 interacts simultaneously with MLN51 and with the heterodimer Magoh/Y14, thus providing a physical link between MLN51 and Magoh/Y14. This position of eIF4A3 is probably responsible for the absence of direct interaction between purified recombinant MLN51 and Magoh/Y14 observed here. In addition, two independent studies reported that eIF4A3 is a core component of the EJC (56, 57). Together with these data, our results clearly indicate that MLN51 associates with spliced mRNAs both in the nucleus and in the cytoplasm via its specific interaction with the EJC. It would be of particular interest to know whether the RNA binding activity of MLN51 also contributes to its association with spliced mRNA carrying an EJC.

In Drosophila, mago nashi, tsunagi, Barentsz, eIF4A3, and staufen are clearly involved in the process of mRNA localization during early development. In mammals, Magoh/Y14, eIF4A3, and MLN51 are bona fide components of the EJC. If the EJC is involved in post-splicing events including mRNA export, nonsense-mediated mRNA decay, and translation, there is no evidence for a direct role of the EJC in mRNA localization, and there is no evidence for EJC assembly on spliced mRNAs in Drosophila. Most interesting, murine MLN51 was shown to localize in dendrites together with RNP granules containing murine Staufen1 and specific mRNA including brain cytoplasmic 1 transcript (24). Taken together, these data suggest that the role of MLN51 in mRNA localization is conserved from fly to mammals and reinforce the potential link between EJC and mRNA localization in mammals. Besides a role in mRNA localization, MLN51 is likely to be involved in some of the cellular functions associated with the EJC, such as nonsense-mediated mRNA decay, as recently suggested (55), mRNA nuclear export, and translation. Future studies should explore how MLN51 over-expression might participate in cancer progression.

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Association of the Breast Cancer Protein MLN51 with the Exon Junction Complex via Its Speckle Localizer and RNA Binding Module
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