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Testis/brain RNA-binding protein attaches translationally repressed and transported mRNAs to microtubules

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ABSTRACT **We have previously identified a testicular phosphoprotein that binds to highly conserved sequences (*Y* and *H* elements) in the 3′ untranslated regions (UTRs) of testicular mRNAs and suppresses *in vitro* translation of mRNA constructs that contain these sequences. This protein, testis/brain RNA-binding protein (TB-RBP) also is abundant in brain and binds to brain mRNAs whose 3′ UTRs contain similar sequences. Here we show that TB-RBP binds specific mRNAs to microtubules (MTs) *in vitro*. When TB-RBP is added to MTs reassembled from either crude brain extracts or from purified tubulin, most of the TB-RBP binds to MTs. The association of TB-RBP with MTs requires the assembly of MTs and is diminished by colcemid, cytochalasin D, and high levels of salt. Transcripts from the 3′ UTRs of three mRNAs that contain the conserved sequence elements (transcripts for protamine 2, tau protein, and myelin basic protein) are linked by TB-RBP to MTs, whereas transcripts that lack the conserved sequences do not bind TB-RBP. We conclude that TB-RBP serves as an attachment protein for the MT association of specific mRNAs. Considering its ability to arrest translation *in vitro*, we propose that TB-RBP functions in the storage and transportation of mRNAs to specific intracellular sites where they are translated.**

The polarized distribution of mRNAs within cells greatly facilitates the distribution of proteins to specific sites within cells (reviewed in refs. 1–4). During oogenesis, numerous mRNAs, including Vgl, a homologue of transforming growth factor β (TGF-β) that induces mesoderm formation, are selectively distributed to the animal or vegetal poles and subsequently partitioned to a subset of cells during early embryonic development (5–8). In *Drosophila*, mRNAs encoding the proteins bicoid and nanos are transferred from nurse cells to the oocyte and then localized to the anterior and posterior poles, respectively. Bicoid mRNA encodes a homeodomain protein that initiates a series of transcriptional events leading to the formation of the anterior body segment (9). Nanos mRNA encodes a RNA-binding protein that promotes the formation of the posterior body plan by blocking the translation of hunchback mRNA, which encodes a transcription factor induced by the bicoid cascade (10, 11).

Many mRNAs appear to be transported in cells in association with the cytoskeleton. Tau mRNA, an mRNA selectively distributed in the proximal axon of neurons, has been found to be associated with microtubules (MTs) and is believed to employ MT tracks for its polarized transport (12). Injected myelin basic protein (MBP) mRNAs are incorporated into "particles" that undergo unidirectional movement in oligodendrocytes (13). Such observations suggest that there is an ordered pathway for mRNA localization consisting of ribonucleoprotein particle formation, anchoring and translocation of the particle, and ultimately translation of the localized mRNA. This mechanism suggests the existence of proteins that anchor mRNAs to cytoskeletal structures and are capable of suppressing translation of the mRNAs during their transport.

The 3′ untranslated regions (UTRs) of mRNAs play important roles in cell growth and differentiation (14), localization of mRNAs (15), and cytoskeletal association (16). We have previously identified a RNA-binding phosphoprotein in the testis that suppresses cell-free translation of mRNA constructs containing specific 3′ UTR sequence elements (17, 18). This protein, testis/brain RNA-binding protein (TB-RBP), is present in both testis and brain (19). Here we demonstrate that in addition to binding to 3′ UTR sequences in translationally regulated testis mRNAs, it also binds to similar sequences in the 3′ UTRs of the tau and MBP mRNAs and facilitates attachment of mRNAs to MTs. We propose that TB-RBP is a component in the directional transport of cytoplasmic mRNAs to specific intracellular locations in the brain and is a suppressor of translation.

MATERIALS AND METHODS

Preparation and Fractionation of Tissue Extracts. Cytoplasmic extracts were prepared and fractionated by DEAE-Sepharose chromatography from tissues of adult male CD-1 mice as described (17, 19). The 300 mM KCl eluant, containing most of the TB-RBP, was treated with 40–50% ammonium sulfate, precipitating "enriched TB-RBP."

Gel Retardation Binding Assays. The following RNA probes were prepared: (*i*) transcript c, 42 nucleotides (nt) of the 3′ UTR of mP2 in pGEM; (*ii*) transcript se, 462 nt of the 3′ UTR of rat tau mRNA (containing one *Y* element) in pBluescript (20); (*iii*) pKA, 13, 284 nt of the 3′ UTR of rat MBP mRNA (containing one *H* element) in pBluescript (13); and (*iv*) a 170-nt transcript of pGEM. Labeled transcripts were synthesized with SP6, T7, or T3 RNA polymerase with 50 μCi (1 μCi = 37 kBq) of [^{32}P]CTP, and RNA-binding assays were performed as described (18).

MT Reassembly Conditions. The brains of two mice were homogenized on ice in Mes buffer (100 mM Mes, pH 6.4/0.5 mM $MgCl_2$/0.1 mM EDTA/1 mM EGTA/1 mM dithiothreitol/0.5 μg of leupeptin per ml/2 μg of aprotinin per ml/1 mM phenylmethylsulfonyl fluoride/0.75 μg of pepstatin per ml). After centrifugation at 1000 × *g*, the supernatant (S100) was centrifuged at 100,000 × *g* at 4°C for 30 min and used for MT "*in vitro*" reassembly studies (21). Aliquots (100 μl) of S100 were incubated in 0.5 mM dithiothreitol/2.0 mM GTP at 37°C for 30 min and then centrifuged at 200,000 × *g* at 4°C for 30 min. After centrifugation, protein in the redissolved pellets and in the supernatant (S200) was quantitated.

For MT reassembly with purified tubulin, tubulin (50–100 μg) in 0.7 M glutamate was incubated with 2.0 mM GTP, 1.0 mM dithiothreitol, 30 μg of enriched TB-RBP, and 10^6 cpm of radiolabeled transcript for 30 min at 37°C and then was

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Abbreviations: MT, microtubule; TB-RBP, testis/brain RNA-binding protein; UTR, untranslated region; MBP, myelin basic protein; BSA, bovine serum albumin; nt, nucleotide unit(s).

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centrifuged for 30 min as described above. The presence of 0.7 M glutamate does not interfere with the binding between RNA and TB-RBP (data not shown).

RESULTS

TB-RBP from Mouse Brain Binds to a Macromolecule Whose Assembly Requires GTP and Incubation at 37°C and Is Inhibited by Ca^{2+}. To determine whether TB-RBP could serve as a linker protein to bind specific mRNAs to MTs, enriched TB-RBP was added to MTs that were assembled *in vitro* from mouse brain extracts. MTs were pelleted from extracts containing Mg^{2+} and GTP after incubation at 37°C, and the distribution of TB-RBP in the bound and unbound fractions was determined by gel retardation assays (17, 18) (Fig. 1). Most of the TB-RBP and the MTs were found in the pellets (Fig. 1*A*, compare lanes 3 and 4). In contrast, TB-RBP did not sediment under identical centrifugation conditions when MTs were not formed, such as when the incubation and ultracentrifugation were performed at 4°C (Fig. 1*A*, compare lanes 5 and 6) or in the presence of Ca^{2+} (Fig. 1*A*, compare lanes 7 and 8), colcemid, (lanes 9 and 10), or cytochalasin D (lanes 11 and 12).

Additional evidence that MT assembly was required for the pelleting of TB-RBP was obtained when GTP was omitted from the incubation and the identical incubation and centrifugation conditions employed in Fig. 1*A* were followed. Most of the TB-RBP remained in the supernatant (Fig. 1*B*, compare lanes 3 and 4 and lanes 7 and 8 with the control lanes 1 and 2). Similarly, when no brain extract was added, the enriched TB-RBP did not pellet under the identical incubation and centrifugation conditions (data not shown). Although less RNA–TB-RBP complex is seen when Mg^{2+} is absent, the majority of TB-RBP detected was associated with the MT pellet when GTP was present (Fig. 1*B*, lanes 5 and 6). We conclude from the requirement for GTP, the inhibition by Ca^{2+}, the need to incubate the extracts at 37°C, and the colcemid and cytochalasin D effects that TB-RBP is pelleting with a macromolecule that has assembly requirements similar to those of MTs.

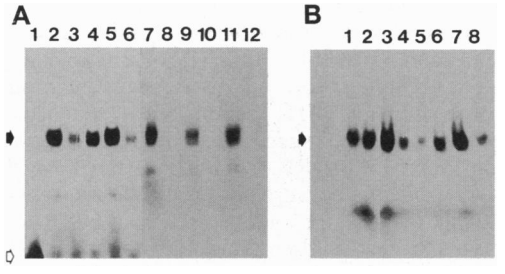

FIG. 1. Gel retardation assays of supernatant and pellet fractions of brain microtubules assembled *in vitro* in the presence of TB-RBP. (*A*) Brain extracts were incubated at 37°C for 30 min and centrifuged at 26°C for 30 min at 200,000 × *g* with 0.5 mM Mg^{2+} and 2 mM GTP unless indicated otherwise. ^{32}P-labeled transcript c and enriched TB-RBP were incubated with equal volumes of supernatant (S) and pellet (P), digested with RNase T1 (0.8 unit/20 μl) for 10 min at 25°C, and assayed (1). Solid arrow, RNA–TB-RBP complexes; open arrow, unbound transcript c. Lanes: 1, transcript c; 2, extract assayed before incubation; 3 and 4, S and P after centrifugation; 5 and 6, S and P after incubation and centrifugation at 4°C; 7 and 8, S and P after incubation with 1 mM Ca^{2+}; 9 and 10, S and P after incubation with 2 mM colcemid; and 11 and 12, S and P after incubation with cytochalasin D (60 ng/ml). (*B*) Brain extracts after dialysis against 20 mM Hepes, pH 7.4/40 mM KCl/1 mM dithiothreitol/2 mM $MgCl_2$ were incubated at 37°C with or without 2 mM GTP followed by centrifugation. Lanes: 1 and 2, S and P after incubation with 0.5 mM Mg^{2+} and 2 mM GTP; 3 and 4, S and P after incubation with 0.5 mM Mg^{2+}; 5 and 6, S and P after incubation with 2 mM GTP; and 7 and 8, S and P after incubation in the absence of Mg^{2+} and GTP.

TB-RBP from Mouse Brain Cosediments with MTs Reassembled from Purified Bovine Brain Tubulin. To more precisely demonstrate that TB-RBP binds to reassembled MTs, MTs were assembled "*in vitro*" from enriched TB-RBP and purified tubulin. When tubulin was deleted, TB-RBP did not pellet after incubation and centrifugation (Fig. 2*A*, compare lane 1 to lane 2). When TB-RBP was omitted, the added radiolabeled transcript c also did not pellet with the MTs or bind protein and was digested by RNase T1 (Fig. 2*A*, lanes 3 and 4). However, when TB-RBP, transcript c, and tubulin were incubated together, most of the transcript c–TB-RBP complex was found in the pellet (Fig. 2*A*, compare lanes 7 and 8). A similar RNA–TB-RBP–MT complex was seen when transcript se, a subclone of the 3′ UTR of tau mRNA containing the *Y* element, was used (Fig. 2*A*, lanes 11 and 12). When BSA was substituted for TB-RBP, no RNA–protein complexes were detected for transcripts c or se in either the supernatants or pellets (Fig. 2*A*, lanes 5 and 6 for transcript c; Fig. 2*A*, lanes 9 and 10 for transcript se). This suggests that the enriched TB-RBP fraction is needed to bind mRNAs to assembled MTs.

To confirm that MTs were assembled and pelleted under our experimental conditions, proteins assayed for RNA binding in Fig. 2*A* were analyzed by SDS/PAGE (Fig. 2*B*). Since we detected most of the tubulin (denoted by arrows) in the pellets, we conclude that MTs are reassembled under the conditions used (Fig. 2*B*). When BSA was substituted for the enriched TB-RBP, the BSA (denoted by arrowheads) remained in the supernatants (Fig. 2*B*, lanes 3 and 7). These data establish that MTs are formed under the conditions we employ and that the association of the RNA–TB-RBP complex with MTs is dependent upon TB-RBP.

To further prove that the pelleting of TB-RBP is dependent upon its association with assembled MTs, TB-RBP and tubulin

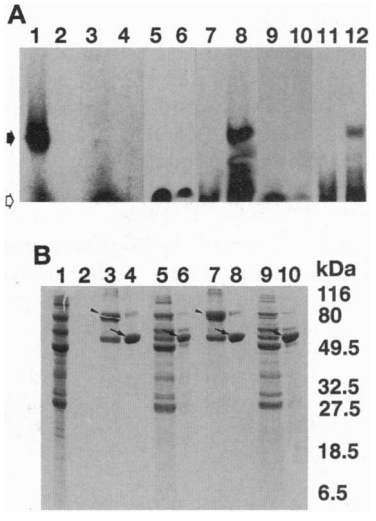

FIG. 2. TB-RBP associates with MTs assembled *in vitro* from purified bovine brain tubulin. Tubulin (100 μg) was incubated with 30 μg of bovine serum albumin (BSA) or enriched TB-RBP and ^{32}P-labeled transcripts c or se under MT reassembly conditions. MTs were pelleted at 200,000 × *g* for 30 min, and equal aliquots of supernatant (S) and pellet (P) were digested with RNase T1 and electrophoresed on native gels. (*A*) Lanes: 1 and 2, S and P of TB-RBP and transcript c, but no tubulin; 3 and 4, S and P of tubulin and transcript c, but no TB-RBP; 5 and 6, S and P of tubulin, BSA, and transcript c; 7 and 8, S and P of tubulin, TB-RBP, and transcript c; 9 and 10, S and P of tubulin, BSA, and transcript se; and 11 and 12, S and P of tubulin, TB-RBP, and transcript se. Solid arrow, RNA–TB-RBP complex. (*B*) SDS/PAGE of 50 μg of protein from *A* stained with Coomassie blue. Lanes: 1 and 2, aliquots of lanes 1 and 2; 3 and 4, aliquots of lanes 5 and 6; 5 and 6, aliquots of lanes 7 and 8; 7 and 8, aliquots of lanes 9 and 10; 9 and 10, aliquots of lanes 11 and 12. Arrowheads, BSA; arrows, tubulin.

were incubated together with increasing amounts of colcemid. After centrifugation, aliquots of the redissolved pellets were analyzed for tubulin distribution by SDS/PAGE (Fig. 3 *Upper*) and for TB-RBP by gel retardation assays (Fig. 3 *Lower*). When the amount of tubulin in the pellet fraction was used as a measure of MT formation, the amount of tubulin detected decreased as the colcemid concentration increased (Fig. 3 *Upper Left*, lanes 3–6). A parallel decrease in the amount of TB-RBP in the pellets was seen as the colcemid level increased (Fig. 3 *Lower Left*, lanes 3–6). We conclude that the pelleting of TB-RBP is dependent on the formation of MTs.

Specific Interactions Bind TB-RBP to MTs. To demonstrate that the association of TB-RBP to MTs is not due to a physical entrapment, a high-salt experiment was performed (Fig. 3 *Right*). Increasing amounts of KCl were added to the MT reassembly mixtures, and the relative amounts of TB-RBP and tubulin that pelleted with the MTs were determined by gel retardation assays (Fig. 3 *Lower*) and by SDS/PAGE (Fig. 3 *Upper*). Concentrations of KCl ranging from 150 to 1300 mM KCl did not affect the formation of MTs under our *in vitro* reassembly conditions (compare the amounts of tubulin in lanes 1–5 of Fig. 3 *Upper Right*). However, as the salt concentration was raised, the amount of TB-RBP recovered in the pellets decreased (compare the amounts of bound transcript c in lanes 1–5 of Fig. 3 *Lower Right*). This indicates that TB-RBP associates with MTs through a salt-sensitive interaction and therefore that the pelleting of TB-RBP with MTs is not due to a physical entrapment.

TB-RBP Binds to mRNAs Containing Conserved Sequence Elements. To demonstrate that TB-RBP facilitates specific RNA binding to MTs, four different transcripts were incubated with TB-RBP and purified tubulin under conditions that allow reassembly of MTs. Complexes of RNA and TB-RBP were formed when transcripts from protamine 2 (transcript c), tau (transcript se), and myelin basic protein (transcript pKA 13) were used (Fig. 4, lanes 2, 4, and 6). In contrast, pGem RNA did not bind to TB-RBP and was digested by T1 RNase (Fig. 4, lanes 7 and 8). This demonstrates that transcripts containing one or both of the conserved elements present in the 3′ UTR of protamine 2 specifically bind to MTs. Of all our tested subclones, transcript c produced the strongest RNA–protein complex signal.

Many Brain and Testis mRNAs Contain Putative Recognition Sequences for TB-RBP. Analyzing the GenBank data base for RNAs that contain the *Y* and *H* elements, we have found a large number of additional testicular and brain mRNAs that

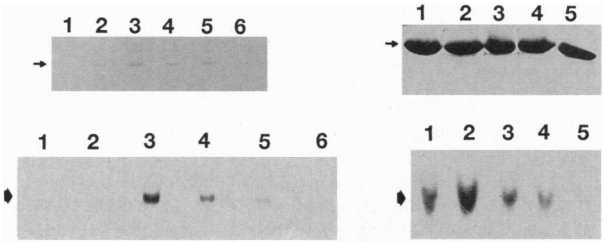

FIG. 3. Colcemid and salt reduce the binding of TB-RBP to assembled MTs. (*Left*) After incubation and centrifugation of MTs reassembled from purified tubulin and enriched TB-RBP, SDS/PAGE of MT pellets (*Upper*) and gel retardation assays (*Lower*) with radiolabeled transcript c were performed. Lanes: 1, TB-RBP alone; 2, tubulin alone; 3–6, incubation with TB-RBP, tubulin, and colcemid (0, 50, 500, and 2000 μM, respectively). Fat arrow, RNA–TB-RBP complexes; thin arrow, tubulin. (*Right*) As in *Left* except that KCl was substituted for colcemid. Lanes: 1–5, incubation with TB-RBP, tubulin, and KCl (150, 700, 850, 1000, and 1300 mM, respectively). Thin arrow, tubulin; fat arrow, RNA–TB-RBP complexes. The differences in protein staining in *Left* and *Right* reflect differences in the amount of protein loaded.

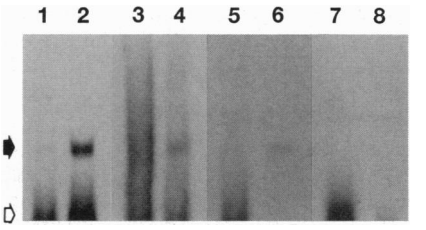

FIG. 4. Specificity of RNA binding to TB-RBP and assembled MTs. MTs were assembled "*in vitro*" as done in Fig. 2. Lanes: 1 and 2, supernatant (S) and pellet (P) of incubation with transcript c; 3 and 4, S and P of incubation with transcript se; 5 and 6, S and P of incubation with transcript pKA 13; and 7 and 8, S and P of incubation with pGem RNA. Solid arrow, RNA–TB-RBP complexes; open arrow, free RNA. For identical amounts of protein, RNA binding is routinely greater with transcript c than with the tau and MBP transcripts.

contain putative TB-RBP binding sites. Their conserved sequences and sites of homology are listed in Table 1.

DISCUSSION

Interactions between mRNAs and the cytoskeleton play a crucial role in the regulation of translation and mRNA cellular localization (22). Many mRNAs undergoing translation are attached to microfilaments, but not to MTs or intermediate filaments (23, 24), whereas untranslated mRNAs are often bound to intermediate filaments (25). Other mRNAs, such as Vgl from *Xenopus* oocytes, appear to associate with both types of cytoskeletal structures (26, 27). The MTs and intermediate filaments of the cytoskeleton appear to be linked, since MT depolymerization by colchicine also causes collapse of the intermediate filament network (28). Although interactions between the intermediate filament network and untranslated mRNAs appear to play an important role in mRNA transport and sequestration of specific mRNAs to specific locations within cells (3), the factors that bind untranslated mRNAs to the cytoskeletal network remain to be defined.

We have reported that a testicular phosphoprotein recognizes specific sequence elements in the 3′ UTRs of several testicular mRNAs and suppresses mRNA translation "*in vitro*" (17, 18). By numerous chromatographic and assay criteria, an identical protein is present in brain (19). Here we demonstrate that this RNA-binding protein, TB-RBP, can bind brain RNAs that contain similar 3′ UTR sequences and also attach these mRNAs to MTs, thereby suggesting that TB-RBP serves as an anchoring molecule for RNA binding. We believe that this interaction is specific because (*i*) when MTs are reassembled from brain extracts, exogenous TB-RBP, but not BSA, pellets with the MTs; (*ii*) in the absence of tubulin or under incubation conditions (low temperature, in the presence of Ca^{2+}, or in the absence of GTP) that prevent MT reassembly, TB-RBP does not pellet; (*iii*) colcemid inhibits both the reassembly of MTs and the pelleting of TB-RBP when MTs are reassembled from either extracts or purified tubulin; and (*iv*) an enriched fraction of brain or testis TB-RBP only binds specific, conserved RNA sequences to MTs. Although these experiments do not allow us to demonstrate direct binding of TB-RBP, we can conclude that TB-RBP associates with MTs either directly or indirectly in association with other proteins. However, gel mobility-shift assays, protein blots probed with radiolabeled transcript c (Northwestern blots), and UV crosslinking indicate that TB-RBP is the sole RNA-binding protein in the crude and enriched fractions that specifically recognize transcript c.

We believe that TB-RBP is needed for specific RNA binding to MTs since neither transcript c nor se binds to MTs in the absence of TB-RBP. In contrast, RNA is not required for MT binding of TB-RBP, since TB-RBP associates with MTs when they are assembled from extracts pretreated with RNase T1 or

Table 1. The Y and H elements are present in many mRNAs expressed in the testis and brain

Gene	*Y* element		*H* element			Region of homology
M protamine 2	442	TCTGAGCCCTGAGCTGCCAAGGAGCCACGAGATCTGAGT	477			3′ UTR
M protamine 1	52	G--TC-----CT--	65			5′ UTR
			54	-T-C-CT-C------	68	5′ UTR
M transition protein 1	185	A-----T---CC--	198			3′ UTR
			252	C-A-A---------A	266	3′ UTR
M tau			1466	---------G--AGT	1480	3′ UTR
R tau protein kinase 1			2	----A----A-GA--	16	5′ UTR
			808	-G---C---G----T	822	CR
M microtubule-associated glycoprotein	469	--------C-----	482			CR
	652	---C-G--A-----	665			CR
	1639	-A-----TC-----	1652			CR
			473	---C----C-GG---	487	CR
			654	--GG-C---G-----	668	CR
M MAP1B	29	--C--G-T-----A	42			5′ UTR
	411	G-C---A-----G-	424			CR
	5934	-G--C------AG-	5947			CR
	7988	--C--A--AC----	8001			3′ UTR
			1710	----GT-A-----T-	1724	CR
M MAP2	968	-A------CC----	981			CR
			3343	--A----A-G-----	3357	CR
M MAP4	4077	TC--------G---	4090			CR
R MAP kinase			1111	AT--A---------T	1125	CR
			1773	----T--T-G---G-	1787	3′ UTR
M myelin basic protein	735	--C--------CT-	748			3′ UTR
H acrosin	1308	G--------TCA--	1321			3′ UTR
H smooth muscle gamma actin	786	AA----TA------	799			CR
R smooth muscle gamma actin	186	-GA-----A----A	199			CR
R androgen binding protein	1164	-AA----------A	1177			CR
			1181	----G--C-----GA	1195	CR
X sperm-specific basic nuclear protein 4	340	A------A----AC	353			3′ UTR
			245	----GA--------T	259	CR
R cAMP-protein kinase subunit	224	-A--C----T----	237			CR
H sex hormone-binding related protein	719	-A--------G--C	732			CR
			922	----T--------GA	936	3′ UTR
H glutathione transferase	1044	--C----------	1057			3′ UTR
			724	----G---G-GG---	738	3′ UTR
H sperm surface protein PH-20			571	-----T----A----	585	CR
M M_r 80,000 fibrous sheath component	1504	-A-----T----T-	1517			CR
			2125	----A----C----A	2139	CR
M c-abl	3878	-C---G----T---	3891			3′ UTR
	4354	--C-----A-----	4367			3′ UTR
M testis-specific glyceraldehyde 3-P dehydrogenase S			10	----T-------T--	24	5′ UTR
M calmegin 1	57	-G--CA--------	70			5′ UTR
M meiosis-specific nuclear structural protein 1			1706	-----T----G-C--	1720	3′ UTR
M sulfated glycoprotein 2	1055	---C---AG-----	1068			CR
R heat-shock protein 27	16	----------C-A	29			5′ UTR
R metalloendopeptidase	132	---G-A-------G	145			CR
R testicular dynamin			423	----------GA--	437	CR
R sorbitol dehydrogenase	258	-CA-T---------	271			CR
H protocadherin 42			3422	T-----G-C------	3436	CR
H cadherin 5	3116	------A----A-C	3129			3′ UTR
H glutathione *S*-transferase 5	1017	----T-T-------	1030			3′ UTR
H protein phosphatase 2A regulatory subunit	3746	---------C--AC	3759			CR
M G-protein β-subunit	226	G----T-G------	239			CR
M neural cell adhesion molecule L1	2437	G----------A--	2450			CR
	2944	--C-A------T--	2957			CR
	3175	G----------T-A	3188			CR
M Ca-activated K channel	2419	-AT-----A-----	2432			CR
M neurofilament protein 68			660	--T----A-G-----	674	CR
M neurofibromin 1	10955	-----G--G----C	10968			3′ UTR
R brain-specific neuronal polypeptide 1B236	567	-A-----TC-----	580			CR
R brain creatine kinase	19	G--C----G-----	32			5′ UTR
	195	----C---C-----	208			CR
	954	---G--A---G---	967			CR
R 12-lipoxygenase	1057	---G-T--G-----	1070			CR
	1125	------T--TT---	1138			CR
R opioid receptor B	224	-A--C----T----	237			CR
R glycogen synthase kinase-3α			310	---T----C----G-	324	CR

Variations in nucleotide sequence from the *Y* or *H* elements of mouse protamine 2 mRNA are indicated. M, mouse; R, rat; H, human; *X*, *Xenopus*. The numbers indicate the GenBank nucleotide number. CR, coding region. Wherever possible, the mouse sequence is shown.

micrococcal nuclease (data not shown). We propose that TB-RBP binds to a subpopulation of more slowly sedimenting MTs, since we must centrifuge the reassembled MTs at 200,000 $\times g$ to maximally pellet TB-RBP and the MTs. TB-RBP alone does not pellet under these centrifugation conditions. At lower centrifugation speeds, significant amounts of TB-RBP remain in the supernatant. Several reports have suggested the presence of novel tubulins and unique MT structures in male germ cells (29, 30), variations in concentrations of MT-associated proteins have been reported to alter properties of brain MTs (31), and different neuronal populations show different susceptibilities to colchicine (32).

Numerous mRNAs bind to cytoskeleton structures (22), leading to their transport and localization to specific sites in cells before translation (1). The mRNAs of tau, a MT-associated protein (12), and MBP (13) have been shown to associate and be transported along MTs. Our data suggest that transcripts from subclones of the 3′ UTRs of tau and MBP mRNAs bind TB-RBP and are linked to MTs, as seen with other MT-associated ribonucleoproteins (1, 4). In this way, the tau and MBP mRNAs can be directed to the cytoplasmic locations where their translated products are required. The enhanced amount of RNA–protein complex seen with transcript c compared with the complexes formed with tau and MBP transcripts is reproducible (Figs. 3 and 4) and may reflect the presence of the two protein-binding elements in transcript c compared with the single protein-binding elements in the tau and MBP mRNAs. Preliminary data indicate that transcript c microinjected into primary cultures of oligodendrocytes is transported in a similar manner to that reported for MBP (13) (J. Carson, personal communication). These mRNA–MT interactions are not restricted to mammalian brain and testis, since specific mRNAs are enriched in reconstituted MT preparations from sea urchin embryos (33), and in *Drosophila*, sequences in the 3′ UTR of bicoid mRNA bind to staufen protein-forming particles that also show MT-dependent localization (34).

Although two conserved RNA-binding elements are present in the 3′ UTRs of transition protein 1 and protamine 1 and 2 (17), the fact that the binding elements of the 3′ UTRs of tau and MBP contain either the *Y* or *H* elements suggests that both sequences are not necessary for RNA–protein interactions (Table 1). A detailed molecular analysis of additional known transported mRNAs listed in Table 1 will be informative. We speculate that in addition to transporting and localizing mRNAs, TB-RBP also represses translation of mRNAs until the complexes arrive at their proper cellular sites. The translation of the mRNAs could be mediated by additional factors or by posttranslational changes such as dephosphorylation, which dissociates TB-RBP from RNA "*in vitro*" (17).

Multiple cytoskeletal components are likely to be involved in TB-RBP binding. TB-RBP is bound to MTs assembled from either crude extracts (Fig. 1) or purified tubulin (Fig. 2), suggesting interaction with MTs. The release of TB-RBP by cytochalasin D treatment suggests that, in addition to binding to MTs, TB-RBP also associates with microfilaments or a RNA actin complex is formed that itself is localized by MTs. We do not know whether TB-RBP binds RNA to both structures simultaneously. Cytochalasin D releases about 80% of the mRNA from the somatic cell matrix (35) and also releases Vgl mRNA in oocytes (16). Our data suggest an involvement of both MTs and microfilaments in the translation suppression/mRNA localization functions of this class of MT-associated RNA-binding protein. Future studies will help to define the precise role that TB-RBP plays in brain and testis mRNA storage, movement, and translation.

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1. Wilhelm, J. E. & Vale, R. D. (1993) *J. Cell Biol.* **123,** 269–274.
2. Macdonald, P. M. (1992) *Semin. Dev. Biol.* **3,** 413–424.
3. Singer, R. H. (1992) *Curr. Opin. Cell Biol.* **4,** 15–19.
4. Steward, O. & Banker, G. A. (1992) *Trends Neurosci.* **15,** 180–186.
5. Frigerio, G., Burri, M., Bopp, D., Baumgartner, S. & Noll, M. (1986) *Cell* **47,** 735–746.
6. Macdonald, P. M. & Struhl, G. (1988) *Nature (London)* **336,** 595–598.
7. Mowry, K. & Melton, D. (1992) *Science* **255,** 991–994.
8. Thompsen, G. H. & Melton, D. A. (1993) *Cell* **74,** 433–441.
9. Dreiver, W. & Nusslein-Volhard, C. (1988) *Cell* **54,** 83–93.
10. Gavis, E. R. & Lehman, R. (1992) *Cell* **71,** 301–313.
11. Wharton, R. P. & Struhl, G. (1991) *Cell* **65,** 955–967.
12. Litman, P., Barg, J., Rindzoonki, L. & Ginzburg, I. (1993) *Neuron* **10,** 627–638.
13. Ainger, K., Avossa, D., Morgan, F., Hill, S. J., Barry, C., Barbarese, E. & Carson, J. H. (1993) *J. Cell Biol.* **123,** 431–441.
14. Rastinejad, F. & Blau, H. M. (1993) *Cell* **72,** 903–917.
15. Jackson, R. J. (1993) *Cell* **74,** 9–14.
16. Schwartz, S. P., Aisenthal, L., Elisha, Z., Oberman, F. & Yisraeli, J. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 11895–11899.
17. Kwon, Y. K. & Hecht, N. B. (1993) *Mol. Cell. Biol.* **13,** 6547–6557.
18. Kwon, Y. K. & Hecht, N. B. (1991) *Proc. Natl. Acad. Sci. USA* **83,** 3584–3588.
19. Han, J., Gu, W. & Hecht, N. B. (1995) *Biol. Reprod.* **53,** 705–715.
20. Sador, E., Marx, R., Barg, J., Behar, L. & Ginzburg, I. (1994) *J. Mol. Biol.* **241,** 325–331.
21. Shelanski, M. L., Gaskin, F. & Cantor, C. R. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 765–768.
22. Hesketh, J., Campbell, G., Piechaczyk, M. & Blanchard, J.-M. (1994) *Biochem. J.* **298,** 143–148.
23. Fulton, A. B., Wan, K. W. & Penman, S. (1980) *Cell* **20,** 849–857.
24. Vedeler, A., Pryme, I. F. & Hesketh, J. E. (1991) *Mol. Cell. Biochem.* **100,** 183–193.
25. Grossi de La, M.-F., Martin de Sa, C., Harper, F., Olink-Coux, M., Huesca, M. & Scherrer, K. (1988) *J. Cell Biol.* **107,** 1517–1530.
26. Pondel, M. D. & King, M. L. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 7612–7616.
27. Yisraeli, J. K., Sokol, S. & Melton, D. A. (1990) *Development (Cambridge, U.K.)* **108,** 289–298.
28. Hynes, R. O. & Destree, A. S. (1978) *Cell* **13,** 151–163.
29. Simerly, C. R., Hecht, N. B., Goldberg, E. & Schatten, G. (1993) *Dev. Biol.* **158,** 536–548.
30. Ashman, J. B., Hall, E. S., Eveleth, J. & Boekelheide, K. (1992) *Biol. Reprod.* **46,** 120–129.
31. Ferreira, A., Busciglio, J. & Caceres, A. (1989) *Dev. Brain Res.* **49,** 215–228.
32. Goldschmidt, R. B. & Steward, O. (1982) *Neuroscience (Oxford)* **7,** 695–714.
33. Hamill, D., David, J., Drawbridge, J. & Suprenant, K. A. (1994) *J. Cell Biol.* **127,** 973–984.
34. Ferrandon, D., Elphick, L., Nüsslein-Volhard, C. & St. Johnson, D. (1994) *Cell* **79,** 1221–1232.
35. Ornelles, D. A., Fey, E. G. & Penman, S. (1986) *Mol. Cell. Biol.* **6,** 1650–1662.