BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **247,** 7–11 (1998)
ARTICLE NO. RC988657

# BC1 RNA Protein Particles in Mouse Brain Contain Two Y-,H-Element-Binding Proteins, Translin and a 37 kDa Protein

Takahiro Muramatsu, Akiko Ohmae, and Kaijiro Anzai[1]
*Division of Molecular Cell Biology, College of Pharmacy, Nihon University, Narashinodai, Funabashi, Chiba 274-8555, Japan*

Received April 9, 1998

**Neural BC1 RNA is distributed in dendrites in the form of ribonucleoprotein particles (RNP). Recently, Han *et al.* reported that testis-brain RNA-binding protein (the mouse homologue of Translin), known to be a translational repressor, links a subset of mRNAs to microtubules (MTs) through binding to their y-,h-sequence elements. We found that similar elements are also present in BC1 RNA. Therefore, it is possible that they also act as *cis*-elements, together with their trans-acting proteins, and control the transport of BC1 RNA along dendritic MTs. In this study, we demonstrated that two y-,h-element-binding proteins copurified with BC1 RNP. One was identified as mouse Translin and the other was a 37 kDa protein. This macromolecular assembly may constitute a transport particle for BC1 RNA. It is also possible that BC1 RNP or Translin itself may play a regulatory role(s) in the translation of mRNAs within dendrites.** 

Neural BC1 RNA is expressed preferentially in the brain (1, 2) and is distributed in neuronal dendrites in the form of ribonucleoprotein particles (RNP) (3-5). In addition to this RNP, several mRNAs have also been identified in dendrites (6-11). It has been suggested that local translation of these mRNAs near spine synapses may be necessary for maintaining and modulating the activity of individual synapses (12, 13). Recently, several investigators have reported that protein synthesis in dendrites is implicated in neurotrophin-induced synaptic plasticity (14) and dendritic growth cone function (15). Furthermore, Martin *et al.* (16) demonstrated that local protein synthesis is required for synapse-specific long term facilitation of sensory to motor synapses. However, as BC1 RNA is a non-protein coding RNA, it has been suggested, together with its associated proteins, to be involved in the translational processes operating in dendrites. In this context, Chicurel *et al.* (13) reported that BC1 RNA was particularly concentrated in the hippocampal synaptodendrosomal fraction.

Muslimov *et al.* (17) reported that the dendritic targeting signal of BC1 RNA was contained within its 5′-segment. As similar sequence motifs are also present in MAP2 a/b mRNAs, but not in nondendritic MAP2 c mRNA, they were suggested to be *cis*-elements common to both BC1 RNA and dendritic mRNAs and may direct the dendritic delivery of these RNAs. However, to the best of our knowledge, no information about their corresponding transacting factors has been reported. Han *et al.* (18) reported that testis-brain RNA-binding protein (TB-RBP) links mRNAs to microtubules (MTs) *in vitro* via their y-,h-sequence elements. TB-RBP is a phosphoprotein able to repress temporally mRNA translation (19). Furthermore, recently, Wu *et al.* (20) reported that TB-RBP is the mouse homologue of Translin protein (m-Translin). Translin was originally identified by Aoki *et al.* (21) as a recombination hot spot-binding protein associated with chromosomal translocation. We found that the 5′-segment of BC1 RNA also contains y-,h-homologous elements, which, unlike the motifs reported by Muslimov *et al.* (17), coincide with the 3′-side internal control region, the B-box, for RNA polymerase III. These observations suggest that this *cis*-element in BC1 RNA also interacts with m-Translin.

In this study, we demonstrated that BC1 RNP contained two y-,h-element-binding proteins, m-Translin and a 37 kDa protein.

## MATERIALS AND METHODS

### *Preparation of RNA Probes*

Subfragments of BC1 RNA (BC1-yh) and mouse protamine 2 mRNA (Mp2-yh) were prepared by *in vitro* transcription of the corresponding DNA segments cloned in the *Kpn* I/*Eco*RI site of the KS$^+$

[1] To whom correspondence should be addressed.



0006-291X/98 $25.00

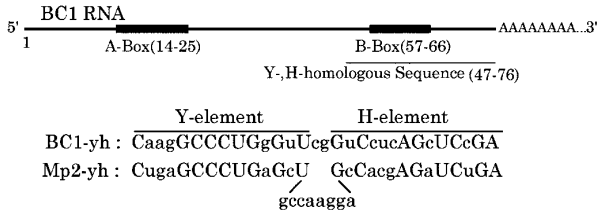


**FIG. 1.** Structures of y-,h-element homologous sequences in BC1 RNA. The location of y-,h-homologous sequence is indicated by a thin line, the numbers in parentheses indicate the distances, in nucleotide numbers, from the 5′-end of BC1 RNA, according to ref. 27 and identical nucleotide residues in BC1-yh and Mp2-yh are indicated by upper case letters. These two RNA probes also contained a $KS^+$ vector-derived 16-nucleotide extension at their 5′-ends.

vector DNA using the transcription kit (Stratagene). Both DNA constructs were linearized with *Eco*RI and then transcribed with T3 RNA polymerase. All the RNA probes synthesized were extracted once with phenol/chloroform, followed by electrophoretic purification on a polyacrylamide gel containing 4 M urea.

### *Electrophoretic Gel Mobility Shift Assays*

Electrophoretic gel mobility shift assays were performed essentially according to the method of Han *et al.* (18). For the supershift experiment using anti-Translin IgG (21), the sample proteins were preincubated with 1 $\mu$ 1 (550 ng) anti-Translin or control rabbit (Sigma) IgG solution in the assay mixture containing all the components except the required $^{32}$P-RNA probes. After incubation overnight on ice, the probe was added and the complexes generated were analyzed.

### *Estimation of the Molecular Masses of RNA-Binding Proteins*

The $^{32}$P-probe was incubated with BC1 RNP purified by a combination of DE 52 column chromatography, hydroxylapatite column chromatography and glycerol gradient centrifugation as described previously (23). After incubation, the mixture was irradiated on ice for 5 min using a germicidal ultraviolet (UV) lamp and subsequently separated electrophoretically under the gel shift assay conditions. Then, the gel was immersed in a buffer comprising 375 mM Tris-HCl, pH 8.8 and 0.1 % SDS and the UV-induced RNA-protein complexes were electroblotted onto Immobilon P membranes (Millipore). The C1 complexes eluted from the membranes were digested with 1 mg/ml RNaseA at 37 °C for 15 min, subjected to SDS-polyacrylamide gel electrophoresis and detected by autoradiography. Relative amounts of the protein bands were quantitated by scanning films in a microdensitometer.

### *Western Blotting*

Proteins separated on a 10% SDS-polyacrylamide gel were transferred to Immobilon-P membranes. The blot was reacted with anti-Translin IgG and detected using the ProtBlot Western Blot AP Systems (Promega).

## RESULTS AND DISCUSSION

Fig. 1 shows that y-,h-like elements were present in the distal part of the 5′-segment of BC1 RNA. The sequence homology of these elements with those in mouse protamine 2 mRNA (Mp2 mRNA) (22), which are the prototypes of this sequence family, was about 60%, although short stretches of identical nucleotide residues were found in both elements. Initially we synthesized $^{32}$P-labeled BC1 RNA and Mp2 mRNA fragments containing y-,h-elements (BC1-yh and Mp2-yh probes, respectively) and used them as probes to identify specific binding proteins. When brain cytosolic extracts were fractionated on a glycerol gradient as described previously (23) and then subjected to a gel mobility shift assay, one of the complexes detected (C1) cosedimented with BC1 RNP (Fig. 2a, b). We examined whether this C1 complex contained m-Translin, as m-Translin also cosedimented with the BC1 RNP (Fig. 2c). We did not characterize the C2 complex in more detail, which was distributed heterogenously through the gradient. As shown in Fig. 3a, the C1 complexes were supershifted with anti-Translin antibody. Such a supershift was not observed with control IgG. Further-

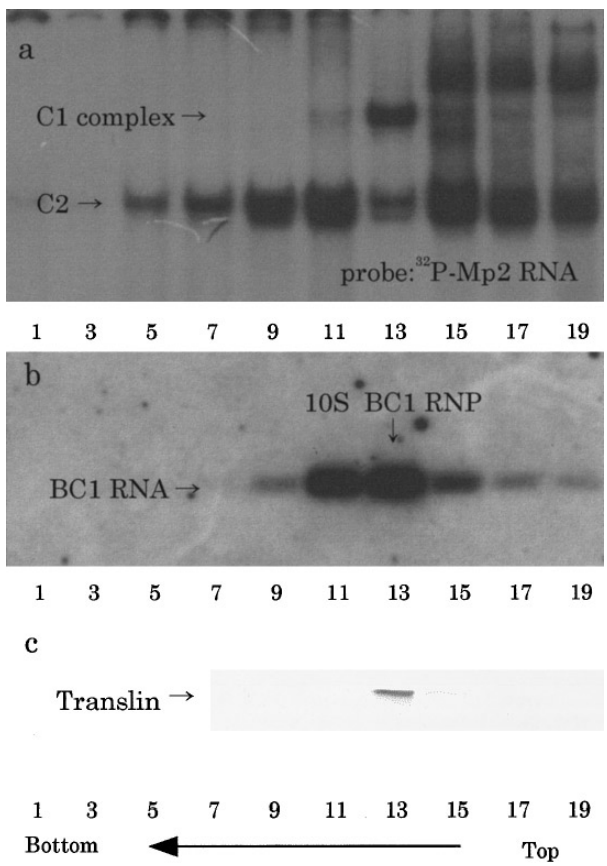


**FIG. 2.** Cosedimentation of m-Translin with BC1 RNP on a glycerol gradient. (a) The banding pattern of the C1 complex. A cytosolic fraction of brain homogenate was centrifuged on a glycerol gradient and an aliquot of each alternate fraction was subjected to a gel mobility shift assay using $^{32}$P-Mp2-yh as a probe. (b) The distribution of BC1 RNP was determined by Northern blot analysis of RNA in gradient fractions as described previously (23). (c) Distribution of m-Translin protein, determined by Western blot analysis using anti-Translin IgG as the primary antibody.

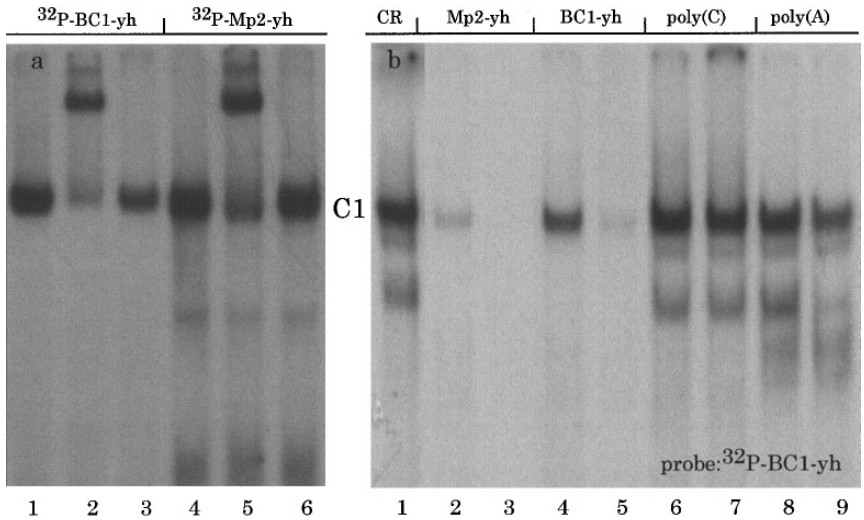


**FIG. 3.** Electrophoretic gel mobility shift analysis of the specificity of C1 complex. (a) The presence of m-Translin in the C1 complex. IgG (550 ng) used for pretreatment: lanes 1,4, no IgG added; lanes 2,5, Translin IgG; lanes 3,6, control rabbit IgG. Each lane contained 1.5 $\mu$ g proteins from the glycerol gradient fraction containing BC1 RNP. The $^{32}$P-labeled RNA probes used are indicated at the top of the panel. (b) The binding specificity of C1 protein. Competitor RNAs additionally included are indicated at the top of the panel. Amounts of competitors: 0 ng (lane 1), 5 ng (lanes 2, 4, 6, 8) and 50 ng (lanes 3, 5, 7, 9). All lanes contained $^{32}$P-BC1-yh (4000 cpm, ~10 pg).

more, the C1 complex formation was sequence-specific, as RNA fragments containing y-,h-elements competed for C1 proteins more effectively than did homopolymer RNAs (Fig.3b), indicating the specific interaction of m-Translin with BC1 RNA.

In order to determine whether m-Translin associated

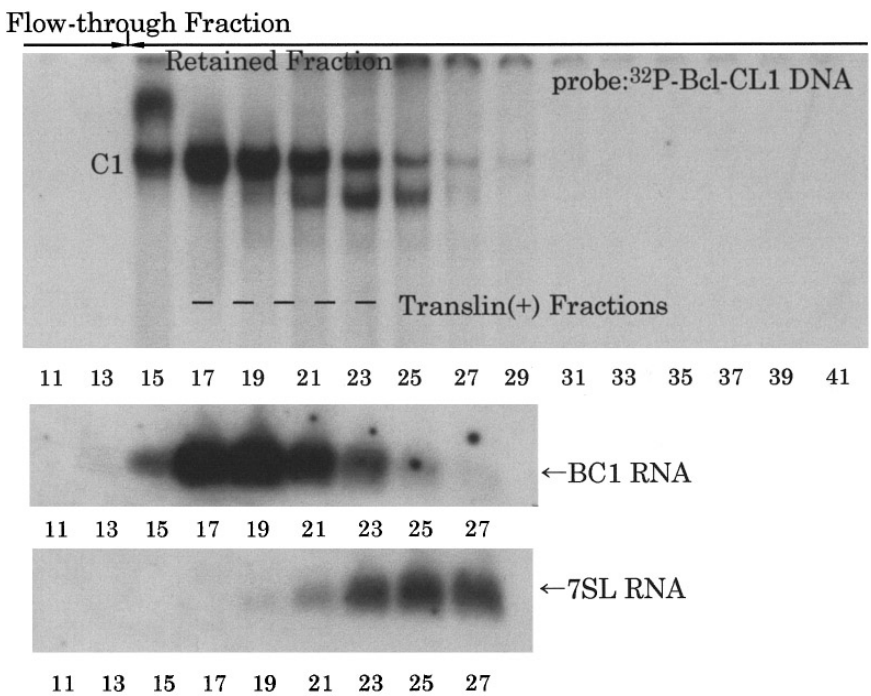


**FIG. 4.** Copurification of m-Translin with BC1 RNP by hydroxylapatite column chromatography. The retained materials were eluted from the column with a 25-500 mM sodium phosphate (pH 7.5) gradient (fractions 14-41) and the gradient fractions were subjected to a gel shift assay using $^{32}$P-ssDNA-Bcl-CL1 as a probe (top panel). Only the relevant fractions are shown. The elution profiles of BC1 RNP (middle panel) and signal recognition particle (bottom panel) were determined by Northern blot analysis of gradient fractions. The same blot was regenerated between hybridization. The DNA probe for 7SL RNA was prepared according to ref. 5.

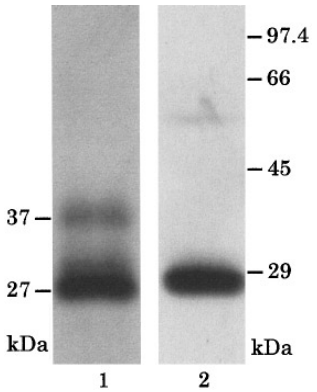


**FIG. 5.** Estimation of the molecular masses of the C1 proteins. UV-cross-linking adducts of C1 proteins (lane 1) and m-Translin (lane 2) to $^{32}$P-Mp2-yh. $^{32}$P-BC1-yh probe gave identical results (data not shown). Molecular masses of C1 proteins (37- and 27 kDa) are indicated at the left. Molecular mass standards are indicated at the right. Slightly higher molecular mass of the recombinant m-Translin than that of native m-Translin appear to be attributable to the six histidine tags added at its N-terminal (21).

with the BC1 RNP, we purified BC1 RNP by DE52 column chromatography, followed by hydroxylapatite column chromatography. An aliquot of each fraction eluted from the latter column was analyzed in a gel mobility shift assay using $^{32}$P-Bc1-Cl1 ssDNA as a probe, which contains the target sequences for m-Translin (21). Fig. 4 shows that the elution profiles of Bcl-CL1 binding proteins (C1; top panel) and BC1 RNP (middle panel) were identical and Western blot analysis demonstrated m-Translin in fractions 17-23 (data not shown). We also observed that this BC1 RNP copurified with m-Translin after subsequent purification by glycerol gradient centrifugation (our unpublished observations). Furthermore, Fig. 4 also shows that the association of m-Translin with BC1 RNA was specific, as signal recognition particles were eluted at higher phosphate concentrations (bottom panel). Therefore, it is suggested that BC1 RNA is the primary ligand for m-Translin in the cytoplasm of neuronal cells.

Next, we analyzed molecular composition of C1 complexes. UV-crosslinking adducts of $^{32}$P-Mp2-yh RNA to C1 proteins or recombinant m-Translin (24) were digested with RNase A and then subjected to SDS-polyacrylamide gel electrophoresis. Fig. 5 shows that the C1 complex consisted of 27- and 37 kDa proteins, while the recombinant m-Translin proteins formed only the 27 kDa adduct. These findings indicate that the C1 complexes contained m-Translin (27 kDa) and a 37 kDa protein. Fig.5 also shows that the RNA was cross-linked to these two proteins in a ratio of about 3.5:1 (m-Translin:37 kDa protein). This value appears to reflect the relative amounts of these two proteins present in BC1 RNP. Kasai *et al.* (25) reported that native Translin forms an octameric structure responsible for its single-stranded DNA-binding. Furthermore, Aoki *et al.* (26) recently isolated a 33 kDa Translin-like protein, Trax, from a human spleen cDNA library and showed that it was able to bind to Translin, suggesting its possible role in the selective nuclear transport of Translin. In this context, there is an interesting possibility that the 37 kDa protein may bind to m-Translin through protein-protein interactions and form a heteromeric structure.

The two y-,h-element-binding proteins associated with BC1 RNP appear to be relevant to the dendritic distribution of BC1 RNA in neuronal cells. Nevertheless, the presence of intrinsic dendritic targeting motifs in m-Translin seems unlikely, as they are also expressed in non-neuronal tissues (18, 21). Furthermore, Cheng *et al.* (5) have suggested that BC1 RNP contains proteins with a total mass of 138 kDa. Thus it is conceivable that one or more additional proteins with no RNA-binding activity are contained in the native BC1 RNP, and may also participate in the transport process. Finally, it is also possible that BC1 RNA is a molecular scaffold for the assembly of BC1 RNP, which is required for the dendritic delivery of Translin protein with the ability to repress mRNA translation (19). These transported Translin proteins in the form of RNP could play regulatory roles in the translation of mRNAs within dendrites, such as translational repression during transport and in the vicinity of spine synapses.

## ACKNOWLEDGMENTS

We thank Dr. M. Kasai of the National Institute of Infectious Diseases for the generous gifts of anti-Translin IgG and recombinant mouse Translin proteins. We also thank F. Misawa, C. Fujimoto for their excellent technical assistance. This work was supported by a General Individual Research Grant from Nihon University, Japan.

## REFERENCES

1. Sutcliffe, J. G., Milner, R. J., Bloom, F. E., and Lerner, R. A. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79,** 4942–4946.
2. Anzai, K., Kobayashi, S., Kitamura, N., Kanai, Y., Nakajima, H., Suehiro, Y., and Goto, S. (1986) *J. Neurochem.* **47,** 673–677.
3. Kobayashi, S., Goto, S., and Anzai, K. (1991) *J. Biol. Chem.* **266,** 4726–4730.
4. Tiedge, H., Fremeau, Jr., R. T., Weinstock, P. H., Arancio, O., and Brosius, J. (1991) *Proc. Natl. Acad. Sci. U.S.A.* **88,** 2093–2097.
5. Cheng, Jr-G., Tiedge, H., and Brosius, J. (1996) *DNA Cell Biol.* **15,** 549–559.
6. Steward, O., and Falk, M. (1986) *J. Neurosci.* **6,** 412–423.
7. Garner, C. C., Tucker, R. P., and Matus, A. (1988) *Nature* **336,** 674–677.
8. Burgin, K., Waxham, M., Rickling, S., Westgate, S., Mobley, W., and Kelly, P. (1990) *J. Neurosci.* **10,** 1788–1798.
9. Furuichi, T., Simon-Chazottes, D., Fujino, I., Yamada, N., Hasegawa, M., Miyawaki, A., Yoshikawa, S., Gueenet, J.-L., and Mikoshiba, K. (1993) *Receptors Channels* **1,** 11–24.
10. Miyashiro, K., Dichter, M., and Eberwine, J. (1994) *Proc. Natl. Acad. Sci. U.S.A.* **91,** 10800–10804.
11. Lyford, G., Yamagata, K., Kaufmann, W., Bames, C., Copeland,

N., Gilbert, D., Jenkins, N., Lanahan, A., and Worley, P. (1995) *Neuron* **14,** 433–445.

12. Steward, O., and Banker, G. A. (1992) *Trends Neurosci.* **15,** 180–186.
13. Chicurel, M. E., Terrian, D. M., and Potter, H. (1993) *J. Neurosci.* **13,** 4054–4063.
14. Kang, H., and Schuman, E. M. (1996) *Science* **273,** 1402–1406.
15. Crino, P. B., and Eberwine, J. (1996) *Neuron* **17,** 1173–1187.
16. Martin, K. C., Casadio, A., Zhu, H. E., Yaping, Rose, J. C., Chen, M., Bailey, C. H., and Kandel, E. R. (1997) *Cell* **91,** 927–938.
17. Muslimov, I. A., Santi, E., Homel, P., Perini, S., Higgins, D., and Tiedge, H. (1997) *J. Neurosci.* **17,** 4722–4733.
18. Han, J. R., Yiu, G. K., and Hecht, N. B. (1995) *Proc. Natl. Acad. Sci. U.S.A.* **92,** 9550–9554.
19. Kwon, Y. K., and Hecht, N. B. (1993) *Mol. Cell. Biol.* **13,** 6547–6557.
20. Wu, X.-Q., Gu, W., Meng, X. H., and Hecht, N. B. (1997) *Proc. Natl. Acad. Sci. U.S.A.* **94,** 5640–5645.
21. Aoki, K., Suzuki, K., Sugano, T., Tasaka, T., Nakahara, K., Kuge, O., Omori, A., and Kasai, M. (1995) *Nature Genet.* **10,** 167–174.
22. Kwon, K. Y. and Hecht, N. B. (1991) *Proc. Natl. Acad. Sci. U.S.A.* **88,** 3584–3588.
23. Kobayashi, S., Higashi, N., Suzuki, K., Goto, S., Yumoto, K., and Anzai, K. (1992) *J. Biol. Chem.* **267,** 18291–18297.
24. Aoki, K., Inazawa, J., Takahashi, T., Nakahara, K., and Kasai, M. (1997) *Genomics* **43,** 237–241.
25. Kasai, M., Matsuzaki, T., Katayanagi, K., Omori, A., Maziarz, R. T., Strominger, J. L., Aoki, K., and Suzuki, K. (1997) *J. Biol. Chem.* **272,** 11402–11407.
26. Aoki, K., Ishida, R., and Kasai, M. (1997) *FEBS Lett.* **401,** 109–112.
27. DeChiara, T. M., and Brosius, J. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84,** 2624–2628.