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Heterodimer SRP9/14 is an integral part of the neural BC200 RNP in primate brain

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Abstract

BC200 RNA is a brain-specific, small non-messenger RNA with a somatodendritic localization in primate neurons and a constituent of a ribonucleoprotein (RNP) complex. The primary and secondary structure of the 5′ domain of BC200 RNA resembles that of the Alu domain of 7SL RNA, which is an integral part of the signal recognition particle (SRP). This would predict that similar proteins bind to this defined domain of both RNA species in vitro and in vivo. The data presented in this paper reveal that a protein that binds BC200 RNA in vivo is immunoreactive with antibodies against SRP9. This further supports the notion that the 5′ domain of the BC200 RNA can fold into structures similar to the SRP Alu domain and, as a result, bind identical or similar proteins in vivo. The SRP9 protein binds only as dimer with SRP14 protein to the Alu domain of 7SL RNA to form a subdomain that, in SRP, is functional in translation arrest. Therefore, our data also indicate that the neuronal BC200 RNP is a candidate for regulating decentralized protein biosynthesis in dendrites, possibly with a mechanism that resembles translation arrest of the SRP. © 1998 Elsevier Science Ireland Ltd.

Keywords: BC200 ribonucleoprotein; Exaptation; Dendritic translation; SRP9/14; Elongation arrest

BC200 RNA is a 200 nucleotide (nt) long cytoplasmic non-messenger RNA. Unlike the majority of small RNAs that are expressed in every cell-type, BC200 RNA can be found almost exclusively in the nervous system of humans and simians [19,21]. In neurons its location is not only restricted to cell bodies, but can also be detected in dendritic processes [19]. Like its analog in rodents, the 152 nt BC1 RNA [6,9], BC200 RNA is part of a ribonucleoprotein particle [7]. The BC200 gene was exapted [4] from a monomeric Alu repetitive element [12]. The progenitor of this sequence family was identified as the 7SL RNA, the RNA component of the signal recognition particle SRP [20] hence also termed SRP RNA. BC200 RNA contains three different segments: a 5′ domain with a high sequence similarity to the Alu domain of the SRP RNA; a central A-rich domain and a 3′ terminal segment with a sequence unique for BC200 RNA [19]. Recent data showed that the Alu domain of SRP RNA as well as the 5′ segment of BC200 RNA form a very similar secondary stem-loop structure [2,11,22]. The Alu domain of SRP RNA binds the SRP9/14 heterodimer and mediates elongation arrest of SRP-attached mRNAs [2]. Binding of SRP9 to SRP RNA in vitro is dependent on the formation of a heterodimer with SRP14 [1]. Because of the structural similarity between the Alu domain of SRP RNA and the 5′ segment of BC200 RNA, we analyzed in vitro the binding of SRP9/14 to BC200 RNA. For in vivo binding studies immunoprecipitation experiments were done using polyclonal anti-SRP9 antiserum and cytosolic brain

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extracts. The binding of BC200 RNA to SRP9/14 is not a temporary feature (in evolutionary terms), but is conserved for at least 35–55 million years, since the association could not only be detected in brain extracts from humans but also from old world and new world monkeys.

RNA binding to canine SRP9/14 in vitro was measured by filter binding experiments whereby radiolabeled RNA is incubated with protein. The mixture is applied to a nitrocellulose filter where free RNA passes through and RNA bound to protein is retained. Assays were carried out essentially as described [18], except that RNAs were labeled in vitro by transcription with T7 RNA polymerase in the presence of ^{32}P nucleotide triphosphate. Ten fmol of RNA were used per assay with a 100-fold excess of SRP9/14 or BSA. SRP9/14 was purified from dissociated SRP [15].

SRP RNA was derived from p75swt linearized with *Xba*I [18], yeast U4 RNA from pSP6-U4 (a kind gift of B. Schwer and C. Guthrie, UCSF) linearized with *Sty*I. BC200 RNA was transcribed from PCR-amplified templates, amplified with 5′ primers containing the T7 promoter sequence [16]. RNAs were labeled in vitro in the presence of α-^{32}P GTP by T7-RNA polymerase transcription [13].

Human brain material was provided from Dr. Mehrain at the 'Brain Bank' of the Institute for Neuropathology, Ludwig Maximilian University of Munich (Germany). Cynomolgus monkey (*Macaca fascicularis*) brain material was provided by Drs. G. Weinbauer and E. Nieschlag at the Institute of Reproductive Medicine, University of Münster (Germany), and cortex from owl monkey (*Aotus trivirgatus*) was obtained from the New England Regional Primate Center at Harvard Medical School via M. O'Connel and from Drs. T. Preuss and J. Kaas at Vanderbilt University (Nashville, TN, USA). Brain tissue was homogenized in extraction buffer (10 mM Tris–HCl, pH 7.5; 100 mM KCl; 15 mM $MgCl_2$; 0.25 M sucrose; 1 mM dithiothreitol; protease inhibitor mix 'complete' (one tablet per 50 ml extract; Boehringer Mannheim). After differential centrifugation [6,7] the S100 supernatant was stored until further use at –80°C. For the isolation of total RNA, the TRIZOL purification protocol was used (Gibco-BRL).

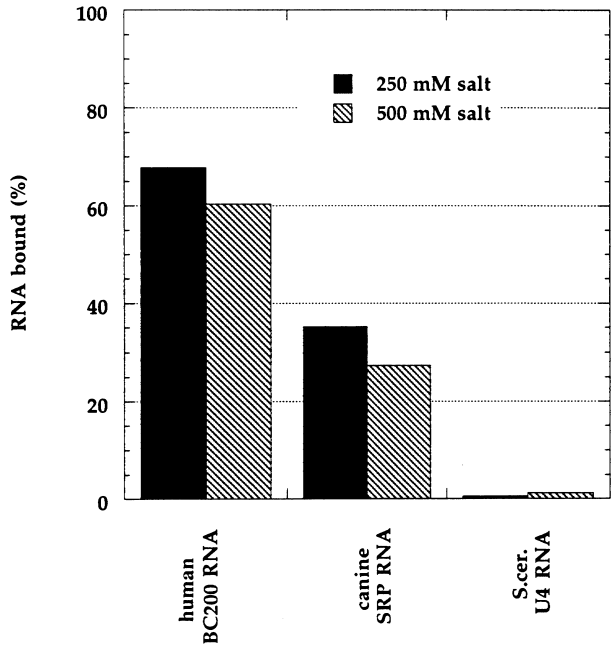

Fig. 1. Binding of canine SRP9/14 to BC200 RNA in vitro. Radiolabeled RNAs were mixed with a 100 molar excess of canine SRP9/14 and incubated in SRP-buffer containing 250 or 500 mM potassium acetate (see text for details). Binding reactions were filtered through nitrocellulose and the amounts of RNA retained were determined by scintillation counting. Relative amounts of bound RNAs are plotted after subtraction of the background values obtained with BSA. Yeast U4 RNA was used as negative and SRP RNA as positive control.

Human cDNA of SRP9 was overexpressed as a glutathione-S-transferase (GST)-SRP9 fusion protein from the vector pGEX-4T-2 [10]. After purification using glutathione affinity chromatography (Pharmacia), the SRP9 polypeptide was released from GST by thrombin cleavage and was used to immunize rabbits. Crude anti-SRP9 antisera was stored at –20°C. An aliquot was affinity-purified using recombinant SRP9 protein coupled to activated Sepharose 4B (Pharmacia). Western blot analysis revealed no cross-reactivity of the SRP9 antiserum with other cytosolic proteins from brain extracts (data not shown).

For immunoprecipitations affinity-purified anti-SRP9 antibodies were first adsorbed onto protein A Sepharose beads (Pharmacia). The antibody-loaded beads were washed three times with NET-2 buffer (150 mM NaCl, 50 mM Tris–HCl, pH 7.5, 0.05% NP40) and then incubated with brain extracts for 90 min at 4°C. After washing four times with NET-2 buffer, RNA was purified from the isolated complexes by phenol/chloroform extraction and ethanol precipitation [8].

Isolated RNA was electrophoresed on a 1% agarose/formaldehyde gel and transferred onto a Quiabrane membrane (Quiagen, Germany) using capillary blotting [14]. After UV-crosslinking the membrane was stored at 4°C. Blots were hybridized with ^{32}P-labeled oligonucleotides complementary to the central non-Alu sequence of SRP RNA (7SL36B: 5′ CACUAAGUUCGGCAUCAAUAUGGUGACCUC 3′) or the unique region of BC200 RNA from human and *Aotus trivirgatus* (BC207: 5′ cTTGTTGCTTTGAGGGAAGTTACGCTTATTTggtac 3′) or *Macaca fascicularis* (BC218: 5′ cGGGTTGTTGCTTTAAGGGGAGTTGCGCTTATTTTTggtac 3′) as described earlier [19]. Lower case letters refer to nucleotides added for cloning purposes and are not derived from BC200 RNA. Membranes were exposed to X-Ray films (BioMax-MS, Kodak) for 6 h to 7 days at –80°C using intensifying screens.

BC200 RNA is an RNA polymerase III transcript with a conserved expression pattern in human as well as old world and new world monkey brains and a low level expression in testes (Skryabin et al., [17]). Human BC200 RNA has recently been shown to be complexed with proteins as an 11.4S ribonucleoprotein particle [7]. By native gel electrophoresis we determined that BC200 RNPs are also present

in cytosolic brain extracts from old world (*Macaca fascicularis*) and new world (*Aotus trivirgatus*) monkeys [17]). The overall size of the RNP complexes appeared to be similar, indicating that the protein components assembled into the BC200 RNP may not have changed over a period of 35–55 million years [17]. The structure of the Alu domains of SRP RNA and BC200 RNA (5′ domain) are very similar and we therefore tested whether SRP9/14 dimer which binds to the Alu domain of SRP RNA is also associated with BC200 RNA. Binding of BC200 RNA to SRP9/14 was determined in vitro using filter binding experiments. In vitro synthesized, radiolabeled RNAs were incubated with purified canine SRP9/14 proteins under conditions used for reconstitution of functional SRP. RNA/protein complexes were separated from unbound RNA by filtration through nitrocellulose. As shown in Fig. 1, BC200 bound to SRP9/14 at both salt conditions, similar to SRP RNA that was used as a positive control. No binding was observed for yeast U4 RNA, the negative control. Recently, Bovia et al. [3] showed that human and mouse heterodimer SRP9/14 bind with high affinity to BC200 RNA in vitro. In order to demonstrate whether this binding also occurs in the cell, we tested whether one of the proteins (SRP9) would also be associated with BC200 RNA in material isolated from primate brains. Indeed, affinity-purified polyclonal antibodies that were reactive against the SRP9 polypeptides from *Homo sapiens*, *Macaca fascicularis* and *Aotus trivirgatus* were capable to immunoprecipitate SRP RNA as well as BC200 RNA from cytosolic brain extracts (Fig. 2). Preimmune sera as a negative control did not immunoprecipitate material containing these RNAs. In HeLa cells, where BC200 RNA expression is deregulated as in many other cell lines [19,21] SRP9 was also found to be associated with BC200 RNA (K. Hsu and R.J. Maraia, unpublished data).

The gene encoding the neuron-specific BC200 RNA arose from a monomeric Alu element [12]. Unlike most Alu elements, BC200 RNA is not transcriptionally silent, but is expressed almost exclusively in neurons of primates. Because BC200 RNA expression is conserved in apes, old world (*Macaca fascicularis*) as well as new world (*Aotus trivirgatus*) monkeys [17], this exaptation [4] occurred prior to the diversification of anthropoidea. Due to the somatodendritic distribution in a subset of primate neurons [7,19], BC200 RNA has been proposed to be involved in mRNA targeting and/or regulation of localized translation [5,12, 19].

Our analysis revealed that the SRP9/14 heterodimer, which represents a well known protein component of the signal recognition particle, is associated with BC200 RNA in vitro and, importantly, also in vivo. Since we could show here that this association of SRP9/14 with BC200 RNA is not only present in humans but also conserved in old world and new world monkeys and therefore for a period of at least 35–55 million years, it is clear that there is selective pressure not only to conserve the primary structure of BC200 RNA but also secondary structure features that are involved in binding the protein heterodimer.

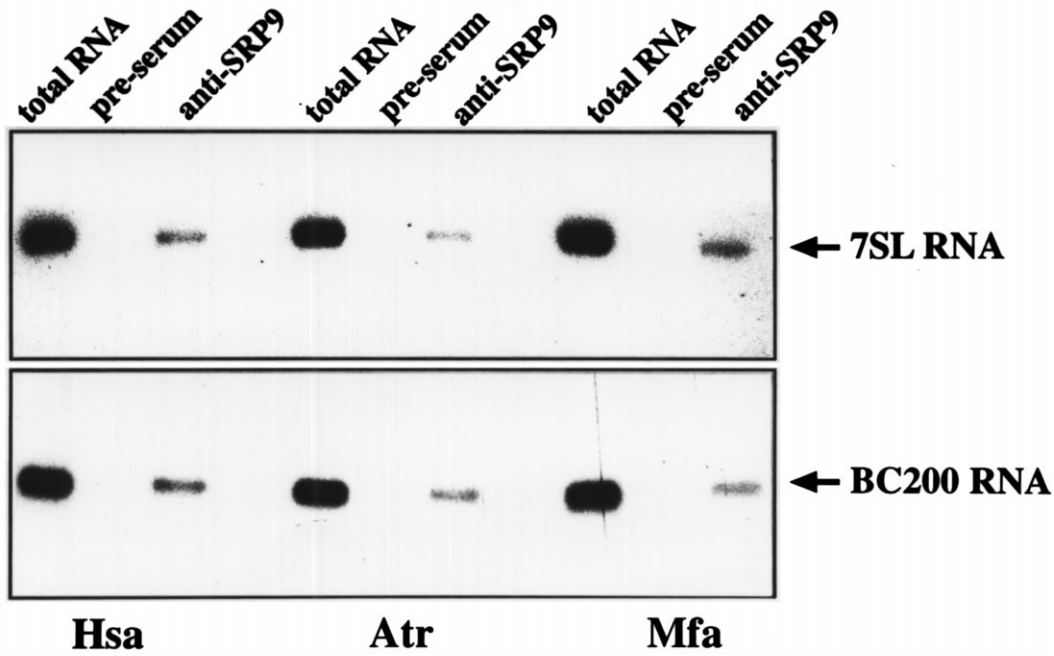

Fig. 2. Immunoprecipitation of SRP RNA and BC200 RNA from primate brain extracts. For immunoprecipitation experiments anti-SRP9 antibodies were first immobilized on protein A sepharose beads. Preimmune sera served as a negative control (C). After incubation of the beads with cytosolic brain extracts from *Homo sapiens* (Hsa), *Aotus trivirgatus* (Atr) and *Macaca fascicularis* (Mfa) RNA was isolated from the immunoprecipitates by phenol-chloroform extraction/ethanol precipitation and was subjected to RNA blot hybridization using radiolabeled SRP- or BC200 RNA-specific oligonucleotides (see text for details). Purified total RNA (total RNA, 3 μg per lane) from the respective primate brains served as controls.

As a component of SRP, the SRP9/14 dimer is involved in the elongation arrest of polypeptides translated from SRP-associated mRNAs. It is tempting to speculate that the 5′ domain of BC200 RNA and SRP9/14 display an analogous function in the BC200 RNP. This will be the topic of further investigations. The conserved association of the BC200 RNA and the SRP9/14 dimer in humans and simians stresses the important functional role of the BC200 RNP in primate brain evolution and makes it plausible that the particle is involved in regulation of decentralized protein biosynthesis in dendritic processes of neurons (as proposed before, see [12]) thus possibly modulating such fundamental processes as long-term memory and learning.

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