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Demonstration of L1-related mRNAs in rat brain using DNA oligonucleotide probes

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Only one copy of the cell adhesion molecule L1 gene is present in the mouse genome, and only one mRNA of 6 kilobases (kb) is expressed in mouse brain [1987, Neurosci. Lett. 82, 89–94]. We have constructed 5 synthetic oligonucleotide probes covering different parts of the published mouse L1 cDNA sequence. Using these probes 3 distinct mRNAs of 9.0, 7.0 and 6.0 kb in rat brain could be demonstrated. Hybridizations performed at different stringency conditions indicated that the 9.0 and 7.0 kb mRNAs were highly related to the L1 mRNA of 6.0 kb expressed in rat brain. The 7.0 kb mRNA is possibly coding for a rat homologue of chicken Nr-CAM, whereas the 9.0 kb mRNA may code for a new member of the L1 family.

L1; Adhesion molecule; Northern blotting; Brain; Rat

1. INTRODUCTION

The neural cell adhesion molecule, L1, is an integral membrane glycoprotein of ca. 200 kDa expressed in both the CNS and PNS [2]. The mouse cDNA sequence indicates that L1 is a member of the immunoglobulin (Ig) superfamily [3]. It contains six C2 Ig homology domains and five fibronectin type III cell binding domains [3].

Several molecules found in other species than mouse have been suggested to be identical or closely related to L1 as judged from the cDNA sequences. These include the proposed L1 homologues rat NILE glycoprotein [4], human 5G3 antigen [5] and neuroglian from *Drosophila melanogaster* [6], and the highly L1-related molecules, chicken Ng-CAM [5] and Nr-CAM [6]. The relationship between L1 in mouse and these identical or related molecules in other species still remains to be established in detail. Another subgroup of L1-related molecules with somewhat less homology to L1 consists of the fasciculation molecules TAG-1 in rat and F3 in mouse [9,10].

Mouse L1 is encoded by a single gene which is transcribed to a 6 kb mRNA [1]. However, neuroglian has been demonstrated to be expressed as two isoforms by alternative RNA splicing [11]. Other genes encoding Ig superfamily cell adhesion molecules, such as NCAM and MAG, also generate multiple isoforms as a result of differential splicing [12,13].

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We therefore decided to investigate whether alternatively spliced L1 mRNA or L1-related mRNA molecules exist in rat brain. Since various mRNA species often have portions with similar sequences, cDNA probes may lead to ambiguous results. This ambiguity was partly circumvented by the use of oligonucleotide probes. We designed five oligonucleotide probes covering different parts of the mouse L1 cDNA sequence and used these to investigate the expression of L1 mRNA by Northern blotting analysis. By means of these probes we demonstrate the presence of L1-related mRNAs of 9.0 and 7.0 kb in rat brain.

2. MATERIALS AND METHODS

Five oligonucleotides were constructed using the published mouse L1 cDNA sequence [3]. The L1-1dom probe was derived from the first extracellular C2 domain (covering nucleotides (nt.) 175–210). The L1-4dom probe was derived from the fourth extracellular C2 domain (covering nt. 1116–1145). The L1-extra probe was derived from the extracellular part between the second and third fibronectin type III domains (covering nt. 2995–3024). The L1-cyt.1 and L1-cyt.2 probes were derived from the cytoplasmic part of the L1 molecule (covering nt. 3499–3537 and nt. 3574–3603, respectively). The five oligonucleotides were synthesized on a Biosearch 8750 DNA synthesizer and labelled with [α-^{32}P]dATP with a specific activity of 6000 Ci/mmol (New England Nuclear, USA) using a DNA tailing kit from Boehringer-Mannheim (Germany). The theoretically calculated dissociation temperatures (T_D) for use in filter hybridization of the oligonucleotide probes were determined using the computer program OLIGO (National Biosciences). Optimum hybridization temperatures are 5–10°C below the T_D. Homology percentages between the cDNA sequences of known L1-related molecules and the L1 DNA oligonucleotide probes were determined using GAP from the GCG package [14].

Northern blotting analysis was performed as described by Andersson et al. [12].

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3. RESULTS

The specificity of the five oligonucleotide probes was tested by Northern blotting using mRNA isolated from rat brain at different stages of early postnatal development. mRNA from rat liver was used as a negative control. All probes were tested using hybridization temperatures of both 50 and 65°C on duplicate blots, and for some probes at 60 or 70°C as well.

When blots were hybridized at 50°C more than one band appeared. The L1-1dom probe hybridized to the major mRNA band of 6.0 kb and occasionally to a weak band of 7.0 kb (Fig. 1a, lane 1). The L1-4dom probe hybridized to three mRNAs of 9.0, 7.0 and 6.0 kb, with the 6.0 kb mRNA as the main band, and occasionally to mRNA material in the region of 2–4 kb (Fig. 1a, lane 2). The L1-extra probe hybridized to mRNA of 6.0 kb and, after prolonged exposures, to a 7.0 kb band and some mRNA material in the area between 2–4 kb (Fig. 1a, lane 3). The L1-cyt.1 probe hybridized to the 7.0 and 6.0 kb mRNAs, with the 6.0 kb mRNA as the major band, and after prolonged exposures, to the 9.0 kb mRNA as well as mRNA material of 2–4 kb (Fig. 1a, lane 4). The L1-cyt.2 probe showed the same hybridization pattern as the L1-extra probe (Fig. 1a, lane 5). The results are summarized in Table I.

In an attempt to clarify whether the distinct mRNA bands of 9.0 and 7.0 kb were alternatively spliced L1 mRNAs or L1-related species we performed hybridizations at temperatures higher than 50°C. At 60°C the L1-4dom probe detects the 6.0 kb mRNA clearly and the 7.0 kb weakly (Fig. 1b, lane 1). When the L1-4dom probe was hybridized at 70°C the 6.0 kb L1 mRNA band was only observed after prolonged exposure times (not shown). We therefore conclude that the T_D for the L1-4dom probe is ca. 70°C. When all probes were hybridized at 65°C only the mRNA of 6.0 kb could be detected clearly on all blots. In Fig. 1b, lane 2, the result of hybridization at 65°C with the L1-cyt.1 probe is shown. These results indicated that L1 mRNA is contained in the 6.0 kb mRNA but not in the 9.0 or 7.0 kb mRNAs, which therefore are related mRNA species.

Table II

Homology percentages between the cDNA sequences of known L1-related molecules and L1 DNA oligonucleotide probes

	L1-1dom	L1-4dom	L1-extra	L1-cyt.1	L1-cyt.2
TAG-1	58.3	56.7	56.7	56.7	53.3
F3	50.0	56.7	56.7	51.3	53.3
Nr-CAM	58.3	60.0	56.7	66.7	56.3
Ng-CAM	63.9	66.7	56.7	87.2	56.7

The percentages have been calculated by dividing the number of base matches between the actual cDNA sequence and the aligned L1-probe (only the best fit is shown) with the length in base pairs of the same L1-probe, and multiplying by 100.

Table I

Results of hybridization with the indicated probes to L1 or L1-related mRNAs in rat brain

Kb	L1-1dom	L1-4dom	L1-extra	L1-cyt.1	L1-cyt.2
9.0	–	+	–	(+)	–
7.0	(+)	+	(+)	+	(+)
6.0	+	+	+	+	+
2-4	–	(+)	(+)	(+)	(+)

+ indicates reaction in all experiments performed

(+) indicates a weak reaction not always observed in individual experiments and usually observed after prolonged exposures

4. DISCUSSION

The five L1 probes used in this study all recognized a single distinct mRNA band of 6.0 kb at hybridization temperatures of 65°C. We therefore conclude that this band contains the L1 mRNA in rat brain. Since the 9.0 and 7.0 kb mRNAs are observed at hybridizations of 50°C (see Table I), but not at 65°C, we conclude that these mRNAs are not alternatively spliced L1 mRNA forms but rather L1-related mRNA species.

The overall identity at the amino acid level between L1 in mouse and the related molecules TAG-1 in rat [9] and F3 in mouse [10] is between 28–29% [5]. We aligned our five L1 probes against the cDNA sequences of TAG-1 and F3 (Table II). T_D for the L1-4dom probe was experimentally determined to 70°C. According to the computer program OLIGO (see Materials and Methods) this allows the probe at 50°C to hybridize to complementary sequences with only five or less base mismatches. This means that the actual sequence re-

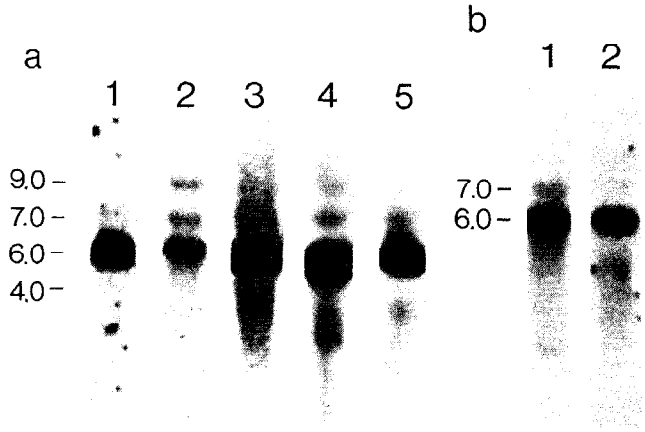

Fig. 1. Northern blotting analysis of rat brain using L1 oligonucleotide probes. The sizes of the detected mRNA bands are shown in kilo bases (kb) at the left side of the figure. All lanes contain 10 μg mRNA from early postnatal rat brain. (a) The following probes were used at hybridization temperatures of 50°C: (lane 1) L1-1dom; (lane 2) L1-4dom; (lane 3) L1-extra; (lane 4) L1-cyt.1; (lane 5) L1-cyt.2. (b) the following probes were used as hybridization temperatures of 60°C (lane 1) and 65°C (lane 2), respectively: (lane 1) L1-4dom; (lane 2) L1-cyt.1. For probe design, see Materials and Methods.

cognized by the L1-4dom probe under these conditions must be more than 83% identical. TAG-1 mRNA exists as 9.5, 7.5 and 5.3 kb mRNA forms due to differential polyadenylation [9]. F3 mRNA exists as a 6.3 kb mRNA and some 3.3–3.9 kb mRNA material [10]. Some of these mRNA sizes are comparable in size to the distinct 9.0, 7.0 and 6.0 kb mRNAs and the 2–4 kb mRNA material described. However, the homologies of the L1-4dom probe, which recognizes the 9.0, 7.0 and 6.0 kb mRNAs most strongly, to both TAG-1 and F3 is 56.7% (see Table II). This percentage of identity is much lower than the calculated necessary 83% and therefore cannot explain the hybridization of this probe to the described mRNAs. The highest homology between the L1 probes and TAG-1 or F3 is 58.3%. We therefore conclude that none of the L1 probes recognize TAG-1 or F3 mRNA sequences under the chosen experimental conditions.

The amino acid sequences of L1 in mouse [3], Nr-CAM and Ng-CAM in chicken [7,8] resemble each other more closely than they resemble any other known molecules. The overall identity between these molecules is 40% [8]. However, rodent homologues of Nr-CAM and Ng-CAM have not been described yet. We therefore aligned our five mouse L1 probes against the cDNA sequences of chicken Ng-CAM and Nr-CAM (Table II). The homologies between the five L1 probes and Nr-CAM or Ng-CAM sequences are 56.7–66.7% and 56.7–87.2%, respectively. According to the above mentioned mismatch considerations, the possibility exists that our probes recognize mRNAs of Nr-CAM and Ng-CAM rat homologues. The L1-cyt.1 probe shows 87.2% identity with Ng-CAM, and mRNA for a rat Ng-CAM would probably be detected by this probe at hybridizations at 50°C. Northern blotting analysis of Nr-CAM mRNA expression in chicken brain demonstrates the presence of an mRNA of ca. 7 kb [8], whereas Ng-CAM mRNA in chicken brain is expressed as a single mRNA of ca. 6 kb [7]. If Nr-CAM and Ng-CAM mRNAs are expressed in rat with sizes comparable to those in chicken, the 7.0 kb mRNA detected in this study may be rat Nr-CAM mRNA. Also, the rat Ng-CAM mRNA may co-localize to the 6 kb area together with L1 mRNA.

The L1-4dom probe recognizes the 7.0 kb mRNA but not the 9.0 kb mRNA at hybridizations of 60°C, indicating that these two mRNA are different molecules. We therefore suggest that the 9.0 kb mRNA described here encodes an as yet unsequenced protein, which is highly related to the L1 molecule and probably is a new member of the L1 subgroup of Ig superfamily molecules.

The L1 oligonucleotide probes described here may be used for isolation of cDNA sequences corresponding to the 9.0 and 7.0 kb mRNAs. Translation of such isolated cDNA sequences would establish the relationship between the L1 protein and the proteins encoded by these mRNAs. Our findings do not exclude the possibility that alternatively spliced L1 mRNA forms do indeed exist. Minor size differences of alternatively spliced L1 mRNA forms not detectable within the limits of the sensitivity of the Northern blotting analysis technique may exist. In fact, during the preparation of this manuscript, it was published that alternatively spliced forms of L1 in rat exist [15]. The four amino acids RSLE are shown to be present in the cytoplasmic region of some but not all L1 isoforms in rat brain [15].

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