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A tissue-specific change in repetitive DNA in rats

(brain DNA/LINE sequence/mammalian development)

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ABSTRACT **From a genomic library constructed from an *Eco*RI digest of Wistar rat brain DNA, we isolated a clone (BL-1) that gave a 0.6-kilobase restriction fragment only in brain (or lens) DNA upon Southern hybridization. The tissue-specific fragment was present in Wistar and other strains (Sprague–Dawley and Donryu) of rats regardless of their sex and age. Sequencing of the clone indicated that it is closely related to a part of the LINE 3 sequence, one of the highly repetitive sequences present throughout mammalian genomes. Polymerase chain reaction using primer sequences in the BL-1 clone indicated that it is derived from an amplified (rearranged) sequence, although other explanations are possible. These results suggest that there are tissue-specific changes in DNA primary structure during mammalian developmental processes.**

It has long been assumed that DNA sequences in various tissues and organs of individual mammals are identical except for the DNA rearrangement observed in immunoglobulin genes in lymphocytes (1). This suggests that most of the DNA sequences do not change during mammalian developmental processes, thus making it unlikely that changes in DNA structure such as DNA rearrangement or amplification play any crucial role in the regulation of development and differentiation. The nuclear transplantation experiments by Gurdon (2, 3), although performed in *Xenopus*, have lent strong support for this notion. Furthermore, recent mounting experimental evidence emphasizes the importance of specific protein–DNA interaction and possibly DNA methylation (or demethylation) in regulation. On the other hand, there are limited examples, mostly in non-mammals, in which specific DNA sequences are amplified at specific stages of developmental processes. A typical example of such amplification is that of the ribosomal genes which occurs in early oogenesis of amphibians and other species (4). In *Drosophila*, genes coding for major chorionic proteins are also amplified manyfold during the final stage of oogenesis (5). These amplifications are probably designed to provide sufficient quantities of templates for the bursts of gene transcription required at certain developmental stages. Aside from these examples, amplification of specific genes is also observed in certain drug-resistant mammalian cells (6), exemplified by amplification of the dihydrofolate reductase gene in methotrexate-resistant cells (7, 8) and of the c-*myc* gene in neuroblastomas (9, 10).

In this paper, we report that a clone isolated from a rat brain genomic library, when used as a probe, gave unique, apparently amplified or modified DNA hybridization patterns with restriction enzyme-digested DNA isolated from brain (or lens). The DNA sequence¶ was very similar to that of a part of LINE (long interspersed element), one of the highly repetitive DNA sequences found in mammals. Polymerase chain reaction (PCR) using DNA templates from different tissues suggested that the sequence is amplified (rearranged) in the specific tissues. The biological significance of this finding is discussed with respect to possible changes in DNA primary structure during mammalian developmental processes.

MATERIALS AND METHODS

Materials. Restriction enzymes, kinases, and *Thermus aquaticus (Taq)* DNA polymerase were purchased through Takara Shuzo (Kyoto, Japan). Proteinase K, RNase A, and Hoechst 33258 were supplied by Sigma. Radioisotopes were purchased from ICN ([α-^{32}P]dCTP) and Amersham ([γ-^{32}P]ATP). Random-priming kits were obtained from Boehringer Mannheim. All the agents used were reagent grade. The animals were obtained from Nippon Bio-Supply Center (Tokyo).

Isolation of Tissue DNA. Tissue DNA was isolated by the conventional phenol procedure (see below for details). Tissues and organs that had been removed from the animal after anesthesia with chloroform were homogenized with a Dounce homogenizer in 10 mM Tris·HCl, pH 8.0/10 mM EDTA/100 mM NaCl (10 ml per gram of tissue). SDS and proteinase K were then added at 0.5% and 0.2 mg/ml, respectively. The samples were incubated at 37°C for 18 hr and treated with an equal volume of phenol three times. The aqueous phase was dialyzed for 18 hr against 10 mM Tris·HCl, pH 8.0/1 mM EDTA and then treated with RNase A (2 μg/ml) for 1 hr at 37°C followed by proteinase K (100 μg/ml) in the presence of SDS (0.5%) for 18 hr at 37°C. The samples were again treated with phenol (three times) as before, and the aqueous phase was treated twice with chloroform/isoamyl alcohol (24:1, vol/vol) to remove residual phenol. DNA was then precipitated with 2.5 volumes of ethanol and dissolved in 10 mM Tris·HCl/1 mM EDTA. The DNA concentration of each sample was determined by a fluorometric method (11) that measures fluorescence developed in the presence of Hoechst 33258 (2-[2-(4-hydroxyphenyl)-6-benzimidazolyl]-6-(1-methyl-4-piperazyl)benzimidazole), using a Hitachi F2000 fluorescence spectrometer (excitation at 356 nm and emission at 458 nm). Calf thymus DNA (Sigma) was used as a standard DNA.

Cloning and Selection of BL-1. BL-1, used as a probe for Southern hybridization, was isolated from a pUC119 rat brain genomic library generated by in-gel competitive reassociation. In essence, before cloning into pUC119, *Eco*RI digests of brain DNA (from an 8-week-old female Wistar rat) were denatured and reassociated in gel after electrophoresis in the presence of a large excess of liver DNA (from the same rat)

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Abbreviation: PCR, polymerase chain reaction.

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¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. M29143).

that had been treated with *Eco*RI and alkaline phosphatase. The details of the in-gel competitive reassociation, which was designed for the cloning of DNA fragments that have different structures in two DNA preparations, and the analysis of the clones thus isolated will be published elsewhere (H.Y. and M.O.). More than 700 clones in the library were labeled in groups (5 clones each) and hybridized with *Eco*RI digests of DNA from various tissues (brain, liver, and heart) from the same rat after 1.4% agarose gel electrophoresis. BL-1 was originally selected as a clone from a group of probes that gave different hybridization patterns with brain and liver DNA. Subsequent experiments showed that three clones were identical with BL-1.

Southern Hybridization. Each purified DNA (20 μg) was incubated with 200 units of restriction enzyme (Takara Shuzo) for 16 hr at 37°C in the buffer recommended by the supplier. The digested DNA samples (20 μg each) were electrophoresed in 1.4% agarose gels, transferred to nylon membranes (Pall Biodyne), and hybridized with the ^{32}P-labeled BL-1 probe according to the procedure of Southern (12). We used a low probe concentration (≈3 ng/20 ml of hybridization buffer) and stringent washing conditions (see below) to contrast the bands specific to BL-1. After hybridization, the membranes were washed twice with 2× SSC/0.1% SDS, twice with 0.5× SSC/0.1% SDS, and twice with 0.1× SSC/0.1% SDS (65°C, 30 min per wash). (SSC is 0.15 M NaCl/0.015 M sodium citrate, pH 7.) The filters were then autoradiographed on Kodak XAR film for 18–36 hr at −80°C. The BL-1 probe was labeled [α-^{32}P]dCTP (ICN; 3000 Ci/mmol; 1 Ci = 37 GBq) by random priming (13).

Sequencing of BL-1. BL-1 was sequenced by the dideoxy method (14) after subcloning in M13 bacteriophage.

PCR. Oligodeoxynucleotides used as primers for PCR were synthesized by the solid-phase triester method with an Applied Biosystems model 380B synthesizer. PCR was performed as described (15). In brief, template DNA (10 ng and 100 ng) was mixed in a reaction mixture (98 μl; 50 mM KCl/10 mM Tris·HCl, pH 8.3/1.5 mM $MgCl_2$ with 100 μg of gelatin per ml) containing the four deoxynucleoside triphosphates (200 μM each) and two oligonucleotide primers of opposite direction (1.0 μM each). The mixture was incubated at 95°C for 5 min and 2 μl (2 units) of *Taq* DNA polymerase (Takara Shuzo) was then added. The mixture was covered with 100 μl of light mineral oil and PCR was performed in a DNA Thermal Cycler (Perkin–Elmer/Cetus) by 25 cycles of denaturation–annealing–polymerization, each cycle consisting of 1 min at 94°C, 2 min at 60°C, and 3 min at 72°C for each stage, respectively. After the reaction, DNA samples were subjected to 1.8% agarose gel electrophoresis and Southern blot hybridization.

RESULTS

A Band Specific to Brain and Lens DNA. To find a DNA sequence that changes in a tissue-specific manner in mammals, we screened more than 700 rat genomic DNA clones. These clones were isolated by an in-gel competitive reassociation procedure (H.Y. and M.O., unpublished work) for any DNA sequences exhibiting different hybridization patterns among restriction enzyme-digested DNA samples from various tissues of the same animal. One clone (BL-1), isolated from a genomic DNA library of *Eco*RI digests of rat brain DNA, was found to give a hybridization pattern unique to *Eco*RI digests of brain DNA. When BL-1 was used to probe *Eco*RI digests of brain, liver, and heart DNA from an 8-week-old female Wistar rat, an extra hybridized band (arrow) of ≈0.6 kilobase (kb) was revealed only with brain DNA (Fig. 1*A*). All the other hybridized bands seemed to be common to the three DNAs examined. From the intensities of the bands obtained with different batches of brain DNA,

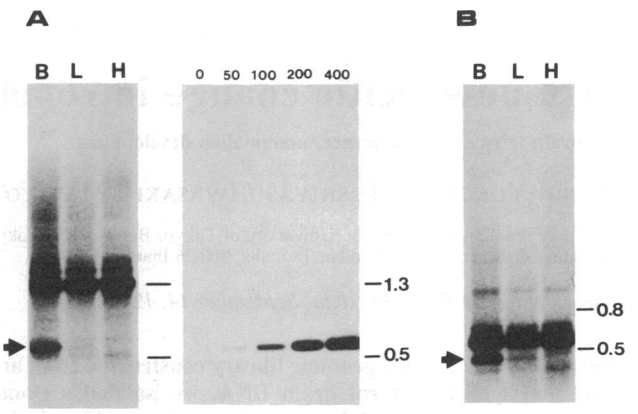

FIG. 1. Southern blot analysis of rat brain, liver, and heart DNA with the BL-1 probe. (*A*) DNA samples (20 μg each) isolated from brain (lane B), liver (lane L), and heart (lane H) of an 8-week-old female Wistar rat were digested with *Eco*RI and subjected to 1.4% agarose gel electrophoresis, along with various amounts (0–400 copies) of control (standard) BL-1 DNA. After transfer to a membrane, the DNA was hybridized with ^{32}P-labeled BL-1 and the filter was exposed to a x-ray photographic film. (*B*) Samples (20 μg) of the same brain, liver, and heart DNAs as used for *A*, but digested with *Eco*RI plus *Bgl* II, were subjected to Southern blot hybridization. Arrows indicate brain-specific bands hybridized with BL-1. Size markers indicate length of DNA in kilobases.

we estimated the 0.6-kb band to be equivalent to ≈100 copies of BL-1, provided that the efficiency of BL-1 hybridization to the genomic DNA was equal to that of hybridization to itself. In three experiments using DNA digested with five restriction enzymes, the band unique to brain was observed only with DNA digested with *Eco*RI or combinations of *Eco*RI with another enzyme, suggesting that most of the sequences in the 0.6-kb band were similar to the other major bands (see below). Another example of a band unique to brain DNA was a 0.5-kb band observed with *Eco*RI/*Bgl* II double-digested DNA (Fig. 1*B*).

When we examined *Eco*RI-digested DNA from an 8-week-old male Wistar rat, brain DNA gave the 0.6-kb band but neither liver, heart, nor sperm DNA (Fig. 2*A*) nor any of the other tissue DNAs examined (thymus, kidney, lung,

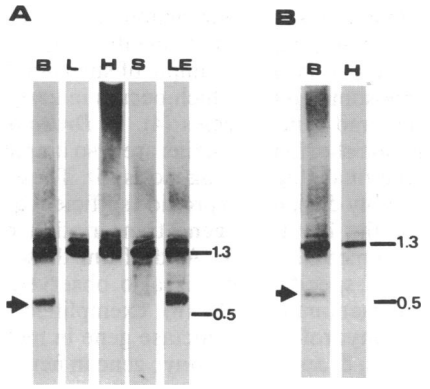

FIG. 2. Southern blot analysis of DNA from various rat tissues with the BL-1 probe. (*A*) DNA isolated from brain, liver, heart and sperm of an 8-week-old male Wistar rat and from the lenses of fifteen 8-week-old male Wistar rats were digested with *Eco*RI. The digested DNA samples (20 μg each) were subjected to Southern blot hybridization with ^{32}P-labeled BL-1 as a probe. Lanes: B, brain; L, liver; H, heart; S, sperm; LE, lens. (*B*) DNA (20 μg) isolated from an 8-week-old female Sprague–Dawley rat was digested with *Eco*RI and subjected to Southern blot hybridization with ^{32}P-labeled BL-1. Lanes: B, brain; H, heart. Arrows indicate brain-specific bands hybridized with BL-1. Numbers at right indicate length of DNA in kilobases.

spleen, and intestine; data not shown) yielded the band. There were no differences in the pattern among DNAs isolated from different regions (cerebellum, cerebrum, brainstem) of the same brain (data not shown). Since brain was the only tissue of ectodermal origin among those analyzed, we examined DNA from another tissue of the same origin, lens DNA (isolated from lenses of fifteen 8-week-old male Wistar rats). The lens DNA yielded the 0.6-kb band with an intensity even higher than that observed with brain DNA (Fig. 2*A*).

The 0.6-kb band was also seen with brain DNA from other strains of rat: Sprague–Dawley (Fig. 2*B*) and Donryu (data not shown). No significant changes in the pattern obtained with brain DNA were observed during advancement of age of female Wistar rats from birth up to 8 weeks (data not shown). These results indicate that the 0.6-kb band in brain (lens) exists regardless of strain, sex, and age of rats and suggest that either a portion of the repetitive DNA is modified in the brain at an *Eco*RI site so that it becomes sensitive to the enzyme or a specific DNA sequence with the *Eco*RI site is amplified in the brain (and lens).

Origin of the 0.6-kb Band. We sequenced BL-1, which consists of 621 base pairs (bp) (Fig. 3*A*) and found it to be a part of LINE, one of the highly repetitive mammalian sequences that exist at $>10^4$ copies per genome. The sequence homology of BL-1 to LINE 3 (16) was ≈97%. BL-1 corresponded to a portion of the distal putative open reading frame of LINE, with a possible reverse transcriptase function (17, 18). The clone covers bp 5141–5757 of the published LINE 3 sequence (16).

From the pool of the original DNA clones (from which BL-1 was derived), we isolated 12 other clones that hybridized with BL-1 and also gave the 0.6-kb band. Sequencing of a portion of all the 12 clones, however, indicated that only 3 of them showed exact sequence homology with BL-1 (data not shown), suggesting that the brain-specific 0.6-kb band consists of heterogenous species of LINE DNA and that BL-1 represents a fraction of them (see below).

Analysis by PCR. These are two likely possibilities, either one of which could account for the results described above. One is that the band present in brain (lens) DNA represents DNA sequences amplified (or rearranged) specifically in these tissues. Alternatively, it is possible that portions of the LINE DNA family in these tissues have been modified, probably through methylation (or demethylation), so as to become resistant (or susceptible) to certain restriction enzymes and thus generate DNA bands that migrate at different positions upon gel electrophoresis.

We therefore attempted to quantitate the BL-1-specific sequence in brain, liver, and heart DNA by the PCR (19). We synthesized 20-bp oligonucleotides representing portions of BL-1 and LINE 3 sequence for use as PCR primers (Fig. 3*B*). Whereas the sequences of P2 and P4 are common to those of BL-1 and the published LINE 3 sequence (16), P3 (and P5, which has the complementary sequence of P3 with the opposite direction) is unique to BL-1 with 2 base differences from the corresponding LINE (LINE 3) sequence. P1 represents the sequence of LINE 3 corresponding to the P3 position in BL-1.

First, DNA sequences between P2 and P4 and between P3 and P4 in these tissue DNAs were amplified in test tubes by using these oligonucleotides as primers for PCR. After PCR, the DNA was electrophoresed and the degree of DNA amplification was analyzed either by hybridization with ^{32}P-

A

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  1  GAATTCAAGC AGTATTACAG AGCAATAGTG ATAAAAACTG CATGGTATTG
 51  GTACAGAGAC AGACAGATAG ACCAATGGAA TAGAATTGAA GACCCAGAAA
101  TGAACCCACA CACCTATGGT CAGTTGATAT TTAACAAAGG AGCTAAAACC
151  ATCCAATGGA AAAAAGATAG CATTTTCAGC AAATGGTGCT GGTTCAACTG
201  GAGGGCAACA TGTAGAAGAA TGCAGATCGA TCCATCCTTA TCACCCTGTA
251  CAAAGCTTAA GTCCAAGTGA ATCAAGGACC TCCACATCAA ACCAGACACA
301  CTCAAACTAA TAGAAGAAAA ACTAGGGAAG CATCTGGAAC ACATGGGCAC
351  TGGAAAAAAT TTCCTGAACA AAACAGCAGT GGCTTATGCT CTAAGATCAA
401  GAATCGACAA ATGGGATCTC ATAAAACTTA AAAGCTTCTG TAAGGCAAAG
451  GACACTGTGG GAAGGACAAA ACGGCAACCA ACAGATTGGG AAAAGATCTT
501  TACCAATCCT ACAACAGATA GAGGCCTTAT ATCCAAAATA TACAAAGAAC
551  TCAAGAAGTT AGACCGCAGG GAAACAAATA ACCCTATTAA AAAATGGGGT
601  TCAGAGCTAA ACAAAGAATT C
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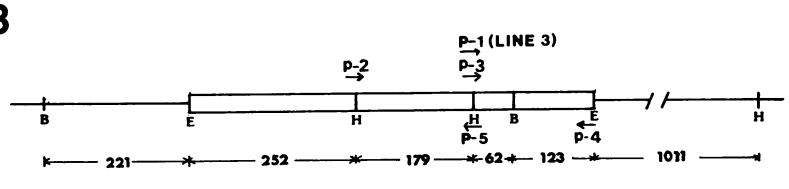
B

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P-1 : 5'-AAACTGCAAAGCTTCTGTAA-3'
P-2 : -CCTGTACAAAGCTTAAGTCC-
P-3 : -AAACTTAAAAGCTTCTGTAA-
P-4 : -GAATTCTTTGTTTAGCTCTG-
P-5 : -TTACAGAAGCTTTTAAGTTT-
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FIG. 3. Sequence of BL-1 (*A*) and major restriction sites in (or near) BL-1 and sequences of the oligonucleotides used as primers in PCR analysis (*B*). At the top in *B*, BL-1 and flanking DNA are shown diagrammatically with the *Eco*RI (E), *Hin*dIII (H), and *Bgl* II (B) sites and the distances (in base pairs) between the restriction sites. Positions and direction of the oligodeoxynucleotides are indicated by arrows. P5 has the complementary sequence of P3 (with the opposite direction). P1 has the sequence of the published LINE 3 sequence (16) at the position corresponding to P3. Sequences of the oligonucleotides are shown at the bottom. For details, see text.

labeled probes or by directly staining the gel with ethidium bromide. Although we were able to detect DNA amplification specific to brain DNA by ethidium bromide staining, which showed a faint band at ≈200 bp only with brain DNA, clearer pictures emerged after Southern hybridization. The results of PCR detected by Southern hybridization using two different template (genomic DNA) concentrations are shown in Fig. 4. Whereas the control PCR between P2 and P4 primers yielded the expected amplified 375-bp fragment in all of the three DNAs examined, PCR between the P3 and P4 primers gave a distinctive band only with brain DNA. The size (≈200 bp) of the DNA thus amplified with brain DNA was equivalent to the distance (196 bp) between P3 and P4. As a control, no brain-specific band was detected when only one of the primers (P3) was present (Fig. 4). Essentially the same pattern of amplification specific to brain DNA was observed with DNAs from several different Wistar rats, including both males and females (data not shown).

Fig. 5 shows the results of PCR with a wide range of template concentrations (1 μg to 1 pg). The 375-bp control bands obtained with primers P2 and P4 had similar intensities for brain, liver, and heart DNA up to 10 pg of template DNA (but were not detected at 1 pg of template DNA). These results provide information with respect to the amount of the brain-specific sequence between P3 and P4 relative to that of the LINE sequence between P2 and P4. It seems that the amplified (or rearranged) brain-specific sequence comprises <0.1% of the total LINE sequences. We were able to detect the same ≈200-bp band with liver and heart DNA by PCR at higher template concentrations (Figs. 4 and 5), indicating that the same sequence present in brain (lens) also exists in liver and heart DNA, although in much lower copy numbers. PCR experiments with BL-1 as a standard template DNA suggested that the copy numbers of the amplified (or rearranged) BL-1 are 50–100 for brain DNA, 5–10 for liver DNA, and 1–5 for heart DNA (data not shown).

To verify the PCR experiments described above, we performed a series of control experiments, and some of the results are shown in Fig. 6. As expected, another brain-specific DNA sequence (≈180 bp) covering a different position on the template DNA (see Fig. 3*B*) was also amplified by PCR in the presence of primers P2 and P5 (Fig. 6*A*). Pretreatment of the template DNA with *Eco*RI did not alter the brain DNA-specific amplification by PCR (Fig. 6*B*). Experiments were performed to confirm that the PCR condition used here in fact specifically recognizes the P3 sequence (versus the corresponding LINE sequence, P1). As seen in Fig. 6*C*, whereas PCR between primers P3 and P4 yielded the amplified band with BL-1 as well as with brain DNA as template DNA, PCR between P1 and P4 did not give the band with BL-1 DNA (but gave the band with all three of the tissue DNAs).

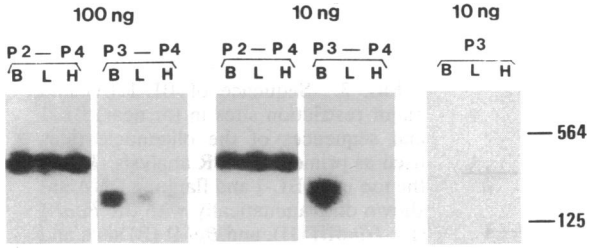

FIG. 4. PCR analysis of rat tissue DNA. DNA samples (100 ng or 10 ng each) isolated from brain (lanes B), liver (lanes L), and heart (lanes H) of an 8-week-old female Wistar rat were subjected to PCR using primers P2 and P4 or P3 and P4. In a control experiment (*Right*), only P3 was used as primer. After 25 cycles of PCR, the samples were subjected to 1.8% agarose gel electrophoresis and Southern blot hybridization using ^{32}P-labeled P4 as a probe. The amount of the template DNA as well as primers used are indicated at the top. Numbers at right indicate length of DNA in base pairs.

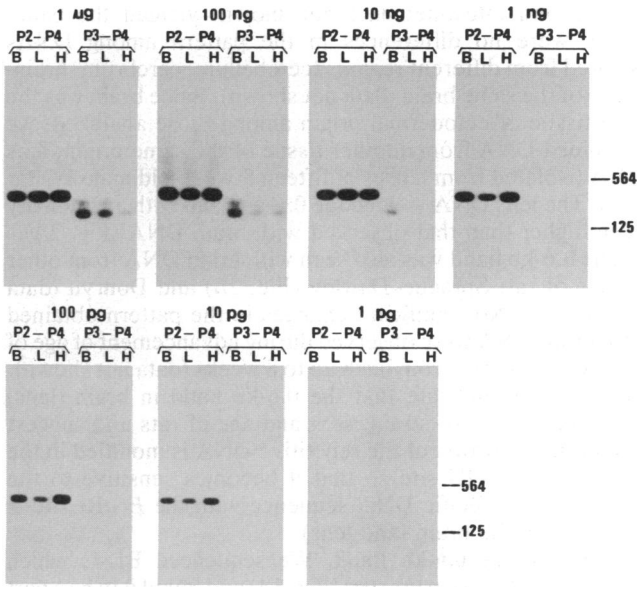

FIG. 5. PCR analysis of rat tissue DNA with various template DNA concentrations. DNA samples (1 μg to 1 pg) isolated from brain (lanes B), liver (lanes L), and heart (lanes H) of an 8-week-old female Wistar rat were subjected to PCR in the presence of primers P2 and P4 or P3 and P4 and the products were analyzed by Southern hybridization as in Fig. 4. The amount of the template DNA as well as primers used are indicated above each autoradiogram. Numbers at right indicate length in base pairs.

The PCR analyses presented above suggest that BL-1 is derived from an amplified (or rearranged) DNA sequence specific to brain rather than by DNA modification (see *Discussion* for alternative explanations of the PCR results).

DISCUSSION

A clone (BL-1) isolated from a rat brain genomic library exhibited hybridization patterns specific to DNA from brain (and lens). The patterns that emerged through the use of BL-1

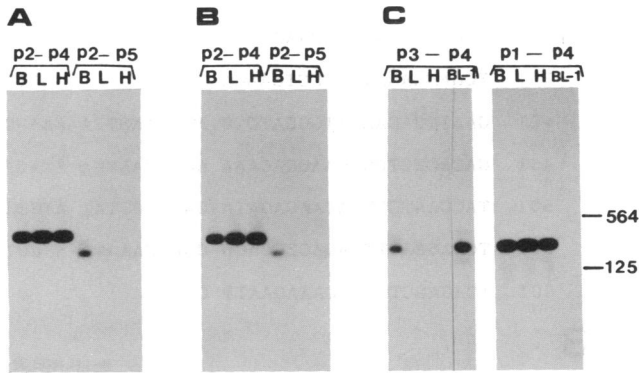

FIG. 6. PCR analysis of rat tissue DNA under different amplification conditions. DNA samples (10 ng) isolated from brain (lanes B), liver (lanes L), and heart (lanes H) of an 8-week-old female Wistar rat, and cloned BL-1 DNA (100 pg) in *C*, were subjected to PCR and the products were analyzed by Southern hybridization as in Fig. 4. (*A*) PCR was performed with primers P2 and P4 or P2 and P5. Blot was hybridized with ^{32}P-labeled P2. (*B*) PCR was performed with primers P2 and P4 or P2 and P5, with DNA samples that had been digested with *Eco*RI (200 units per 20 μg of DNA for 16 hr at 37°C in the buffer recommended by the supplier, Takara Shuzo). Southern blot hybridization was done with ^{32}P-labeled P2. (*C*) PCR was performed with primers P3 and P4 or P1 and P4. Blot was hybridized with ^{32}P-labeled P4. Primers used are indicated at top. Numbers at right indicate length in base pairs.

DNA as probes and particularly the results of PCR suggest the presence of DNA sequences—probably amplified—specific to these rat tissues.

However, these results must be interpreted carefully, since the techniques used here were rather indirect ones for characterizing genomic DNA. For example, the apparent brain-specific DNA amplification indicated by the PCR experiments may have resulted from the presence of a tissue-specific modified DNA (rather than amplified or rearranged DNA) in which the modification subtly affected the association of PCR primer with the template DNA. Besides DNA modification, other possibilities may be considered to explain these results. For example, one could argue that the DNA sequence homologous to BL-1 existed originally in all of the rat tissues (including sperm), but the sequence in most of the tissues except for brain (and lens) was not extracted by the standard DNA-extraction procedure (SDS/proteinase K/phenol) used here. Another possibility is that the DNA structure corresponding to BL-1 in most of the tissues is altered in such a way that the sequence becomes resistant to hybridization and also to PCR. The presence of tissue-specific crosslinkages between DNA strands or other unknown hybridization- or priming-resistant DNA structures would produce hybridization patterns in which it would appear that a specific band was missing. The presence of such tissue-specific or extraction or hybridization-resistant DNA structures, however, has not been reported.

The similarity of the BL-1 sequence to that of LINE is intriguing. Generally, LINE sequences exist in rodents and primates at more than 10^4 copies per genome and represent a major repetitive sequence. Most of the LINE sequences are truncated at the 5′ side, particularly in *Mus domesticus*. LINE sequences in mammalian genomes are generally considered to be inactive as a mobile element, and even if movement does occur, it seems to be a rare and random event. Thus, the repetition of the sequences observed in rodent and primate genomes is believed to reflect past events that have accumulated over many generations. The intact LINE sequences found in mammalian genomes apparently contain two open reading frames, and the putative product of the distal one has some homology with reverse transcriptases (17, 18), suggesting that it was once a retroposon. On the other hand, there are several reports providing evidence that some of the repetitive sequences are still capable of functioning as a template as well as a mobile element. Skowronski and Singer (20) found a complete LINE 1 transcript with a possible biological significance in human teratocarcinoma cells. More recently, a human LINE 1 sequence was found to be inserted within an intron of one of the c-*myc* alleles in a human breast adenocarcinoma (21). Genomic instability of the human *Alu* repetitive sequence has also been pointed out (22). These findings suggest that some of the repetitive sequences are both functional as a template and mobile.

Although the mechanism of the tissue-specific change in LINE sequence is not clear, the present observations indicate that at least some of the repetitive sequences are susceptible to amplification (rearrangement) or modification in a tissue-specific manner. The event seems to occur during embryogenesis, since we could not detect any significant changes in the hybridization pattern after birth in rats. This suggested that the mode of the tissue-specific change might be dependent upon the developmental lineages from which the tissues differentiated. In this respect, it should be noted that a similar type of DNA change was observed in brain and lens, both of which are of ectodermal origin.

The overall picture of the DNA specific to brain in rat genomes has not been fully elucidated because of the technical difficulties in characterizing a single species of repetitive DNA among an overwhelming number of similar sequences present in the genome. Nor do we have any evidence that the tissue-specific change in DNA structure has any biological significance, since it is quite possible that the change is not directly involved in developmental processes, but rather that it simply results from a change in the intracellular environment (to an amplification-prone condition) in specific tissues during development. Characterization of the transcripts that hybridize with BL-1 and located in brain, and of the overall amplified genomic structure concerning BL-1, should provide answers to these questions.

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