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Rapid Mapping of Genomic P1 Clones: The Mouse L-Isoaspartyl/D-Aspartyl Methyltransferase Gene

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We report the mapping of the gene for the murine protein-L-isoaspartate (D-aspartate) *O*-methyltransferase (EC 2.1.1.77) from a 129 mouse strain. This gene encodes an enzyme present in all tissues that can catalyze the first step of a repair reaction in which age-damaged proteins containing abnormal L-isoaspartyl (or D-aspartyl) residues can be converted to forms containing normal L-aspartyl residues. We first mapped the restriction sites from a genomic P1 clone using a rapid method generally applicable to all bacteriophage P1 clones containing large DNA inserts. We show that a single pulsed-field electrophoresis blot can be used to map an entire 89-kb P1 clone insert for eight restriction endonucleases with an error of no more than 2% of the length of the fragment, or 1 kb at the middle of the insert. In this method, we combine complete restriction endonuclease digestion at rare sites within the P1 vector with partial restriction endonuclease digestion within the insert. After size separation by pulsed-field gel electrophoresis and blotting, the fragments are detected by Southern hybridization with probes to the vector. This method is potentially useful for restriction mapping other large DNA clones such as artificial chromosomes. We then determined the positions of the exons of the methyltransferase gene by restriction mapping of long PCR fragments. The previously unidentified exon 8, which encodes the -DEL C-terminus of the more acidic isozyme II, was sequenced and mapped 5.3 kb from the end of exon 7.

INTRODUCTION

We have been interested in determining the functional role of a protein L-isoaspartate (D-aspartate) *O*-methyltransferase (EC 2.1.1.77) that has been shown to be capable of repairing certain forms of spontaneous damage to proteins in a wide variety of organisms (Johnson *et al.*, 1991; MacLaren *et al.*, 1992; Romanik *et al.*, 1992; Mudgett and Clarke, 1993; Brennan *et al.*, 1994; Lindquist and McFadden, 1994; Galletti *et al.*, 1995; Kagan and Clarke, 1995). This repair reaction can prevent the accumulation of proteins containing isomerized and racemized aspartyl residues and may be an important component in limiting the detrimental effects of the aging process. In bacteria, it has been shown that the enzyme is crucial to stationary phase and heat-shock survival; deletion mutations decrease survival by up to 100-fold (Li and Clarke, 1992).

An attractive approach to analyzing protein function in mammals *in vivo* is to create "gene knockout" mice that lack the activity of the specific protein. This approach generally requires knowledge of the gene structure and an accurate restriction map of one or more genomic clones with insert DNA isolated from the same strain of mice used to prepare the embryonic stem cell lines.

The bacteriophage P1 system permits cloning of DNA fragments of 75–100 kb as shear-resistant circular plasmids (Pierce *et al.*, 1992; Sternberg, 1994). P1 clones are stably maintained at one copy per cell by the P1 plasmid replicon and are available for a wide variety of organisms (i.e., human, mouse, rat, and *Drosophila melanogaster*) from a commercial screening service (Genome Systems, St. Louis, MO). The clone used in our experiments was isolated from a library constructed from the DNA of a 129 mouse strain that is utilized in many embryonic stem cell experiments (Wassarman and DePamphilis, 1993; Sternberg *et al.*, 1994).

Constructing restriction maps of large recombinant DNA clones by standard methods can be a tedious task. Typically, restriction sites are mapped by digesting DNA with two or more different restriction enzymes, both singly and together. Attempts are then made to determine a uniquely ordered arrangement that accounts for the observed fragments. A variety of computational methods have been developed in an effort to increase the speed and accuracy of this standard procedure (Pearson, 1982; Fitch *et al.*, 1983). Unfortunately, their application is limited by the requirement for pre-

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cise length measurements of every fragment in the digest. A missing fragment, even a very small one, or slight inaccuracies in measurement of fragment lengths can produce an erroneous map.

An alternative to the standard multiple-enzyme digestion method for restriction mapping was initially developed in 1976 (Smith and Birnstiel, 1976). In this case, the DNA is first uniquely radiolabeled at one end of the molecule. Then partial digestion with a restriction enzyme, followed by electrophoresis and autoradiography, reveals a series of fragments that can be ordered from the labeled terminus. In 1982, the utility of using an end-labeled probe to the vector sequence was shown (Clark *et al.*, 1982). In 1984, the partial restriction map of a large insert was obtained by probing with radiolabeled oligonucleotides to either end of a λ phage vector (Rackwitz *et al.*, 1984), and mapping of larger fragments in a cosmid vector was accomplished soon after (Little and Cross, 1985; Rackwitz *et al.*, 1985). The whole *Escherichia coli* chromosome was mapped in 1987 using this partial restriction digestion method along with Southern hybridization with random-primed probes to the arms of the λ phage vector (Kohara *et al.*, 1987). Finally, a partial restriction digestion strategy combined with pulsed-field gel electrophoresis was recently used to map the 48.5-kb λ DNA molecule (Goode and Feinstein, 1990).

We have extended these methods to allow the complete restriction mapping of entire P1 clones. In this paper we utilize Southern hybridization with random-primed probes to the vector and pulsed-field electrophoresis to create a restriction map of a large (89-kb insert) P1 bacteriophage mouse genomic clone. The restriction map of a P1 clone of the mouse L-isoaspartyl methyltransferase gene is presented herein as well as an efficient method to produce restriction maps for any P1 clone. In addition, we have utilized "long PCR" (Barnes, 1994) to place exons rapidly without the need for subcloning and sequencing.

MATERIALS AND METHODS

Preparation of P1 DNA. A P1 clone (p1949) derived from embryonic stem cell line E14 (a 129/0la mouse) and maintained in *E. coli* strain NS3529 was obtained from Genome Systems (St. Louis, MO) (Pierce and Sternberg, 1992; Sternberg *et al.*, 1994). To increase the yield of DNA, the P1 clone DNA was transferred by electroporation to *E. coli* strain NS3516 (Sternberg *et al.*, 1994). These *E. coli* were grown at 37°C to 8 OD units at 600 nm in Terrific broth medium (Sambrook *et al.*, 1989) containing 25 μg kanamycin/ml for P1 plasmid selection. The P1 DNA was isolated by a modified alkaline lysis method using lysozyme and a Qiagen-tip 2500 column (Qiagen, Chatsworth, CA) as described by McCormick *et al.* (1994). These preparations were analyzed by agarose gel electrophoresis, and no evidence of RNA species was seen after ethidium bromide staining. The concentration of DNA was measured by the absorption at 260 nm assuming that one absorption unit represents a concentration of 50 ng/μl. Contaminating bacterial host DNA was detected in a few preparations as a broad smear of ethidium bromide staining in material electrophoresed in 0.5% agarose gels. However, we found no effect of such contaminating DNA in our experiments. We recovered ~30 μg of P1 DNA from a 500-ml culture of Terrific broth of the clone in NS3529 cells. When the clone was transferred to the NS3516 cells, we achieved a fivefold increase in yield to ~150 μg of P1 DNA from 500 ml of Terrific broth.

Restriction cleavage and electrophoretic separation of P1 clone DNA. Restriction enzymes were chosen based on their velocities and lack of star activity in REact 2 buffer (50 m*M* Tris–HCl, pH 8.0, 50 m*M* NaCl, 10 m*M* $MgCl_2$) (Gibco-BRL Life Technologies, Grand Island, NY) and their ability to cleave at the multiple cloning sites of common vectors, e.g., pBluescript (Stratagene, La Jolla, CA) or pGEM (Promega, Madison, WI). The restriction enzymes used in these experiments (*Cla*I, *Mlu*I, *Bam*HI, *Eco*RI, *Hin*dIII, *Not*I, *Pst*I, *Sal*I, *Xba*I, *Xho*I) were obtained from either Promega or Gibco-BRL.

Partial restriction digestion of the P1 clone DNA was performed as follows. *Cla*I and *Mlu*I restriction endonucleases (14 units each at a concentration of 10 U/μl) were added to 70 μl of a REact 2 buffer solution at 4°C containing 3.5 μg P1 clone DNA. To perform threefold serial dilutions of the partial-cutting restriction enzyme reaction, this mixture was divided into 30-, 20-, and 20-μl portions. Restriction endonuclease (0.1 units) was mixed into the 30-μl sample well. Ten microliters from the 30-μl sample was moved to the 20-μl well and mixed. Ten microliters of this mix was then transferred to the last 20-μl well and mixed. In this way, the first well contains 0.0033 U/μl, the second well 0.0011 U/μl, and the third well 0.00033 U/μl. All samples were then incubated at 37°C for 1 h and then mixed with 5 μl of 10× type I gel loading buffer (Sambrook *et al.*, 1989). A 10-μl portion of each digest was fractionated by pulsed-field gel electrophoresis on a 30-lane 1% agarose gel in 0.5× TBEX buffer (TBEX is Marathon Buffer from Gibco-BRL; 0.5× TBEX is 68 m*M* Tris base, 23 m*M* boric acid, 1.3 m*M* Na_2EDTA) run at 200 v on the CHEF-DR II pulsed-field system (Bio-Rad, Hercules, CA). TBEX buffer was used instead of TBE (Sambrook *et al.*, 1989) because the 10× solution does not precipitate in storage and because it has a greater buffering capacity to allow for longer run times. The run time and the linear ramp switching parameters are described in the figures. All of the molecular weight DNA size standards were obtained from Gibco-BRL and are as follows: high-molecular-weight ladder (S1), 1-kb ladder (S2), mixture of 100-bp ladder and 1-kb ladder (S3), and mixture of 1-kb ladder and 0.1- to 2.0-kb DNA mass ladder (S4).

Analysis of electrophoresis products. DNA fragments were transferred to uncharged nylon membranes (GeneScreen; Dupont NEN, Boston, MA) using the following rapid downward blotting protocol, a modification of the 1-h alkaline capillary transfer method of Chomczynski (Chomczynski, 1992). The gel was soaked in a denaturing solution (0.4 *M* NaOH, 3 *M* NaCl) with gentle agitation for 30 min. The gel was then soaked in the transfer solution (8 m*M* NaOH, 3 *M* NaCl) for 30 min. The nylon blotting membrane was placed in water for 5 min. The gel was placed on top of the nylon membrane, which was then placed on top of a 3-cm stack of blotting papers. Transfer of the DNA fragments from the gel to the membrane by capillary action was complete by 2 h. The transfer apparatus was not covered nor was the gel compressed with any weight. The capillary action of the transfer caused the gel to flatten. The absence of any gel compression improves the transfer of large DNA fragments and may obviate the need for DNA depurination or nicking. The nylon membrane was removed and the DNA fixed in place by UV-crosslinking the damp membrane (125 mJ of total energy at 254 nm) using the GS Gene Linker (Bio-Rad). The blot was washed for 5 min in 2× SSC and dried on a vacuum gel dryer at 60°C for 10 min. The membrane was Southern hybridized with random-primed [α-^{32}P]dCTP probes ($>10^9$ cpm/μg) in a formamide-based solution at 42°C as described (Sambrook *et al.*, 1989).

The probes to either side of the insert cloning site were generated from PCR fragments of the vector and were designed to lie adjacent to and between the *Mlu*I and *Cla*I sites, respectively. The left and right probe primer pairs (pLF-pLB2 and pRF-pRB, Table 1) were designed with a T_m of 55°C and produced fragments of 780 and 470 bp, respectively. The PCR conditions were as follows. In a final volume of 50 μl, final concentrations of 60 m*M* Tris–HCl, pH 9.0, 15

m*M* $(NH_4)_2SO_4$ buffer (Invitrogen, San Diego, CA), 2.5 m*M* of each nucleotide, 1 ng P1 clone DNA, 20 pmol of each primer, 2 units *Taq* DNA polymerase (Promega), 2 units *Taq* extender (Stratagene), and 1.5 m*M* $MgCl_2$ by HotWax Mg^{2+} beads (Invitrogen) were combined. All reactions were cycled 35 times at 4 s at 94°C, 30 s at 55°C, and 1 min at 68°C in a 96-well thermocycler plate on a PTC-100 thermocycler (MJ Research, Watertown, MA).

Exon placement and direct mapping. Exon placement of the L-isoaspartyl/D-aspartyl methyltransferase gene was determined by restriction mapping of PCR products from exon-specific primers designed to span the length between exons (Table 1). Thirty-five cycles were carried out using a 4 s, 94°C denaturation step, a 30 s, 66°C annealing step, and a 20 min, 68°C extension step. PCR primers were created from previously published sequence (Romanik *et al.*, 1992) and designed using MacVector 4.1 (Kodak, New Haven, CT) so that they had a T_m of 70 ± 1°C and a GC content from 40 to 60%. The primers were synthesized on a Pharmacia LKB gene assembler (Pharmacia Biotech, Uppsala, Sweden).

Sequencing of exon 8 from a 3.2-kb *Hin*dIII–*Bam*HI subclone in pBluescript was performed by the Sanger Dideoxy method (Sanger *et al.*, 1977) using the Sequenase 2.0 kit (USB), [α-^{32}P] labeling, and primer M8B (Table 1).

DNA fragments of P1 clone p1949 were subcloned into the Lambda DASH II vector using the Gigapack II Plus packaging extract and the XL1-Blue MRA (P2) *E. coli* strain as described (Stratagene). The λ phage DASH II vector subclones and P1 clone p1949 were further subcloned into the pBluescript II SK(+) plasmid vector (Stratagene). All dephosphorylation and ligation reactions were accomplished using shrimp alkaline phosphatase (United States Biochemical, Cleveland, OH) and T4 DNA ligase (Gibco-BRL), respectively. Plasmid subclones were transformed into DH5α (Gibco-BRL) and selected on LB-agar plates containing 100 μg ampicillin/ml. Phage and pBluescript subclone selection was done by random selection and plaque or colony Southern hybridization using random primed [α-^{32}P]-labeled probes (Sambrook *et al.*, 1989) of PCR products of specific exons of the L-isoaspartyl/D-aspartyl methyltransferase gene. DNA from the phage subclones was isolated as described (Sambrook *et al.*, 1989). The pBluescript subclone DNA was isolated by alkaline lysis using "Wizard mini-preps" (Promega).

RESULTS

Rapid Restriction Mapping of Large P1 Clones

A P1 clone (p1949) spanning the entire protein-L-isoaspartate (D-aspartate) *O*-methyltransferase gene was identified by screening of a mouse 129 genomic library (Genome Systems) using primers (DM3 and DM4; Table 1) that produce a 157-bp fragment from exon 7 (Romanik *et al.*, 1992).

As shown in Fig. 1, the rapid restriction site mapping of P1 clones is accomplished by end probing a partial digestion of the DNA. The P1 clone is completely digested with a rare-cutting enzyme (known to cut only in the vector) and partially digested with the restriction endonuclease of interest, preferably a rare- or six-cutter. This leaves a mix of different-sized DNA fragments with a fraction including the P1 cloning vector ends on either side. These fragments are separated by pulsed-field gel electrophoresis and are blotted onto a membrane. Southern hybridization analysis is performed on the blot with DNA complementary to one side of the insert or the other. The distance from the probe end of the DNA to a restriction site is shown by the length of the fragment. Thus, the distance between sites and the order of different restriction sites can be read directly from the Southern analysis.

In this work we took advantage of the mammalian genome rare-cutting restriction enzymes *Cla*I and *Mlu*I. As shown in Fig. 2, these enzymes cut the vector sequence 2.2 and 0.8 kb from the genomic DNA insertion site and provide for relatively large terminal probes. Another rare-cutting enzyme that can be used

TABLE 1

Oligonucleotide Sequences Referred to in This Paper and Their Locations in the Murine PCMT Gene or the pAd10sacBII P1 Vector

Name	Sequence 5′ → 3′	Exon[a]
For P1 library screening		
DM3	GGACCACTGCTTTTCTTTATCTG	← 7
DM4	GTTAAAGCCCGGAGGAAGATTGATATTG	→ 7
For PCMT exons		
M2F	TGAAGTGATGCTGGCTACAGACCGTTCCCAC	→ 2
M3B	GGGGCACTGATTGTTGCCTGGAAACCTG	← 3
M4B	TGAGGATTCCACTTCCAGAGCCCACGTCC	← 4
M4F	GTGGAATCCTCACCGCCTGTTTTGCACG	→ 4
M5F	CAAAAAGGATGACCCAATGCTCCTGTCCTCGG	→ 5
M6B	CCCACATGAATAGCGTCGTAAGGGGCTTCTTC	← 6
M7B	TGACCATGCAACACTTGTCACTCTTTAATGCCATG	← 7
M7F	CCAGTTAAAGCCTGGTGGAAGATTGATATTGCCAG	→ 7
M8B	GCTGTGATGCTGTTGGTTCTCAAATGCTGTAAGGAC	← 8
M8F	TGAAAGCAACGTGAGCTTGACCAGTCTATAAACCG	→ 8
For vector probes		
pLF	AAACGGAGAGCTTCAGGGAGAC	→ VL
pLB2	GGAGGCATCAAGTGACCCAAACAG	← VL
pRF	ACGAAAAATACCAAGTGCCTC	→ VR
pRB	AAATCGTTTTGTGATCTTCCAC	← VR

[a] Arrows indicate forward (→) or backward (←) orientation.

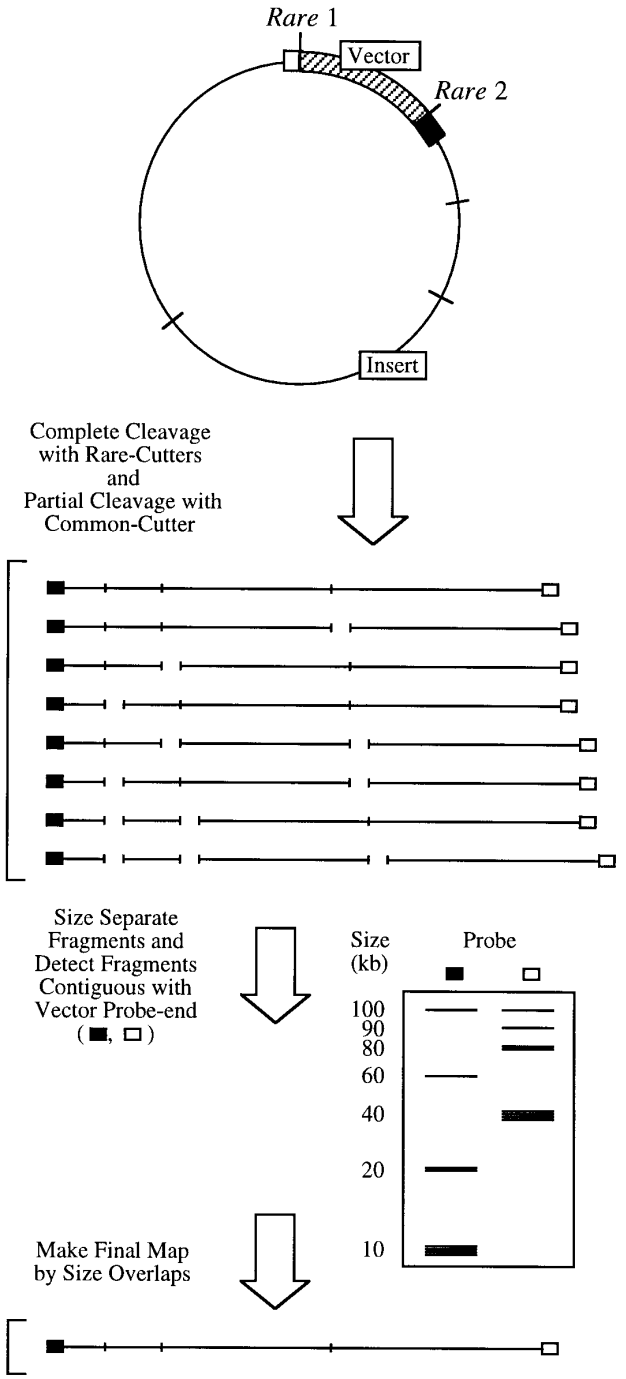

FIG. 1. General scheme for large-scale restriction mapping of P1 clones. The process consists of four steps in which: (1) the P1 clone is completely digested with enzymes that cut only within the vector (*Rare*1 and *Rare*2) and partially digested with an enzyme that cuts within the insert. The rare-cutters are chosen so that each cuts close to one side of the insertion site. (2) The digested DNA is separated by pulsed-field gel electrophoresis and blotted onto a membrane. (3) The fixed DNA is subjected to Southern hybridization using probes complementary to the cloning vector on either side of the inserted DNA (solid and open boxes). (4) Distances of insert restriction sites to the rare-cutter sites are read from the blot and the final map is made by integrating information from both sides of the insert.

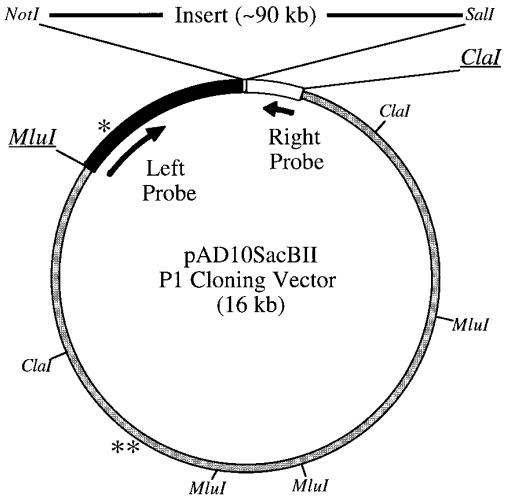

FIG. 2. Probe placement and revised map of the P1 cloning vector p*Ad*10*sac*BII (Pierce *et al.*, 1992). Primers were made to produce probes to the vector for the right and left sides of the cloning insert site for subsequent partial restriction mapping of the insert. The *Not*I, *Sal*I, *Cla*I, and *Mlu*I sites are shown. The *Mlu*I and *Cla*I sites flanking the probe regions are underlined. The cloning vector was restriction mapped and compared to the expected map from the DNA sequence (Genome Systems). We found two regions of disparity. One region was found to be missing 0.4 kb (*) and the other 0.8 kb (**).

in this method as well is *Nru*I. Two *Nru*I sites are located 391 bp to the left and 201 bp to the right of the insert site (not shown in figure). Some difficulties arose during the characterization of the p*Ad*10*sac*BII P1 cloning vector. Restriction digest and PCR analysis showed that two regions of the vector did not match the given sequence from Genome Systems. These are shown in the revised map of p*Ad*10*sac*BII in Fig. 2.

To determine the proper level of restriction digestion for end-probe mapping, four different restriction enzymes over a range of three concentrations were used to digest the p1949 P1 clone DNA. This DNA was electrophoresed, stained, and blotted onto a nylon membrane. The blot was then Southern hybridized with the random primed [α-^{32}P]-labeled right probe shown in Fig. 2. Figure 3 shows both the ethidium bromide-stained gel before blotting (Fig. 3A) and the autoradiogram of the Southern hybridized blot (Fig. 3B). The signal and size distribution of fragments over a range of concentrations shows the relationship between decreasing average fragment length and increasing enzyme concentration. Here, we show clear ladder patterns that one can use to identify the locations of the restriction sites relative to the end of the fragment. We found that the direct end labeling of the digested DNA before electrophoresis (Smith and Birnstiel, 1976; Clark *et al.*, 1982) gave a high background. This was probably due to the labeling of nick sites within the fragments and is more likely to occur with large fragments such as those found in P1 clones. Southern hybridization of random-labeled probes to the vector over-

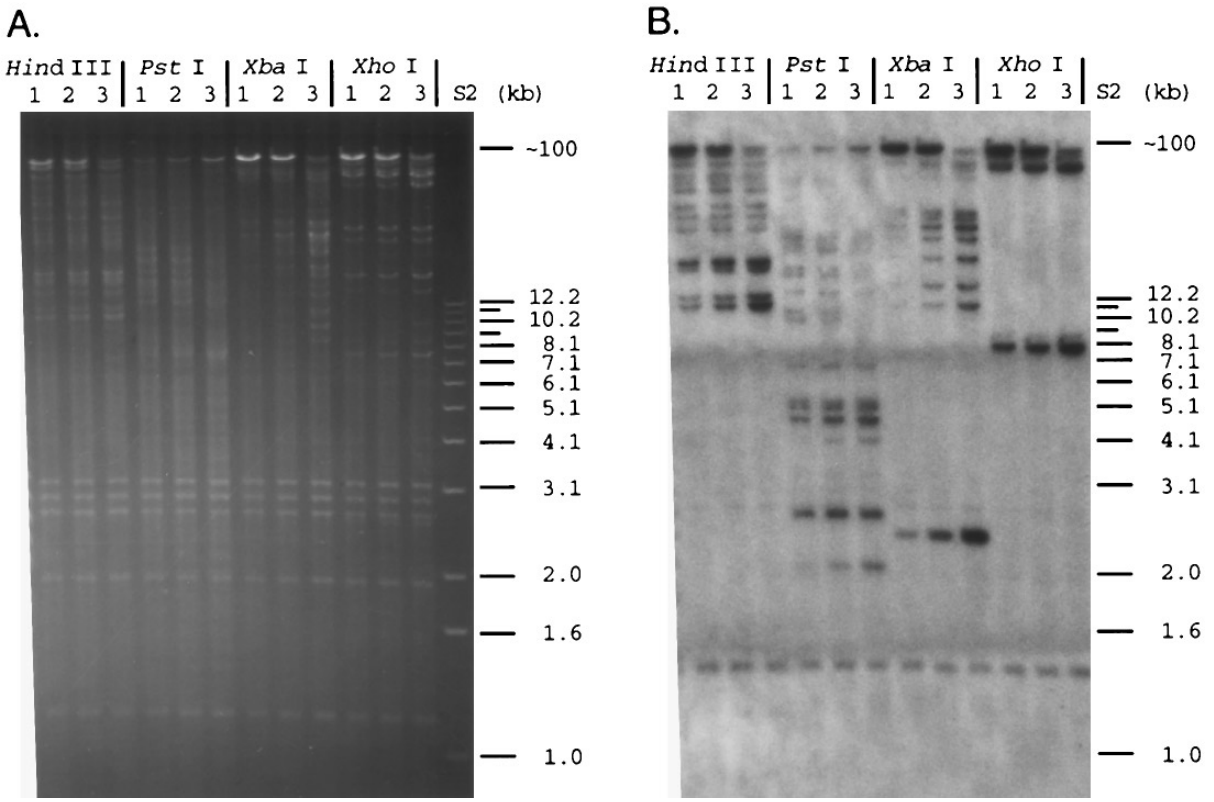

FIG. 3. Conditions for partial digestion with various restriction enzymes. Five hundred nanograms of P1 clone p1949 DNA in a final volume of 10 μl in REact 2 buffer was completely digested with 2 U each of *Cla*I and *Mlu*I and partially digested with 0.003 U (lane 1), 0.01 U (lane 2), and 0.03 U (lane 3) of the enzyme indicated for 1 h. The digests and molecular weight standard S2 were electrophoresed in an 0.8% agarose gel in 0.5× TBEX for 7 h with a linear switching ramp from 0.1 to 3.5 s. The gel was blotted and hybridized as described under Materials and Methods. Autoradiography was performed for 8 h at room temperature using Kodak X-OMAT AR film. (A) Ethidium bromide-stained gel. (B) Southern analysis of the blot of gel shown in A using the right probe shown in Fig. 2.

came the internal labeling problem and produced clean strong signals.

After optimizing enzyme amounts based on the results in Fig. 3, P1 DNA was cleaved with a set of eight partially cleaving restriction endonucleases, electrophoresed on a single gel, and transferred to a nylon membrane. The blot was then hybridized with radiolabeled probes to the vector sequence on either the left side (Fig. 4A) or the right side (Fig. 4B) of the insert site. The separation of different digestion products in adjacent lanes allows more accurate resolution of the order of restriction sites as their accurate molecular weight assignment becomes unnecessary for order determination. The data shown in Fig. 4 are representative of two independent experiments.

Creation of the entire restriction map was accomplished by aligning restriction site information from both probes. Thus, for each side, independent measures of their position relative to each end were obtained. Generally, we utilized the positional information from the closer probe site. However, for the central portion of the insert, the position of the restriction site was determined by averaging information from both ends. The method of constructing the final end-probe map by overlaying restriction site fragment length data is shown for *Bam*HI in Fig. 5A. The final map constructed from the data presented in Fig. 4 and an additional blot for *Eco*RI (not shown) is given in Fig. 5B. These figures (Figs. 4 and 5) show that a single blot can be used to map an entire P1 clone for at least eight different restriction endonucleases.

Exon Placement and Direct Mapping of the L-Isoaspartyl/D-Aspartyl Methyltransferase Gene

The spacing of the exons of the L-isoaspartate (D-aspartate) *O*-methyltransferase gene within the P1 clone p1949 was determined utilizing "long PCR" (Barnes, 1994). Generation of up to 10-kb PCR fragments were routinely successful from clone p1949 DNA. Using the exon-specific primers shown in Table 1, we obtained fragments of 1.5 kb for exons 2 to 3, 3.2 kb for exons 3 to 4, 3.6 kb for exons 4 to 5, 0.6 kb for exons 5 to 6, 2.0 kb for exons 6 to 7, and 5.3 kb for exons 7 to 8. To verify the exon separations we also performed PCR to span exons 2 to 4, 4 to 6, and 5 to 7 and found the fragment lengths consistent with that expected from the individual exon pairs. To determine the positions of these exons with respect to the previously determined restriction map of p1949, we per-

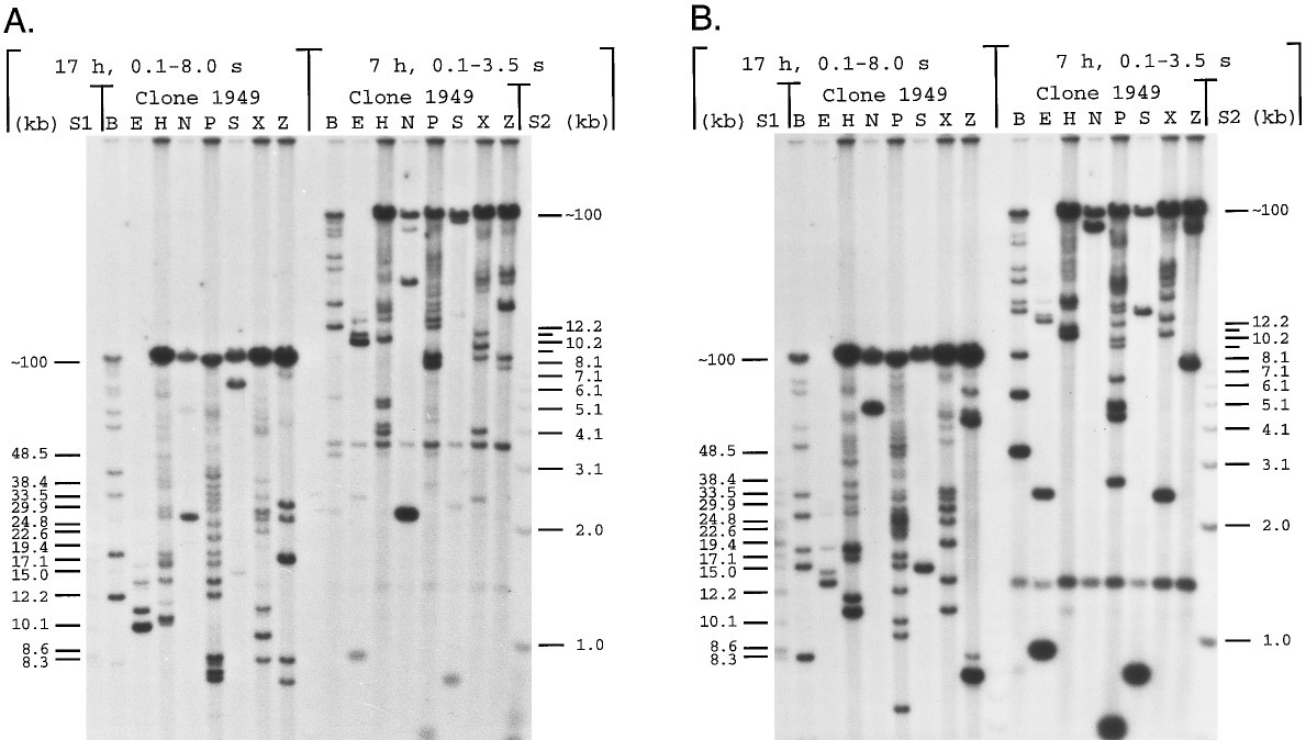

FIG. 4. End-probe Southern hybridization of partial restriction digests of P1 clone p1949. Full digestion with *Cla*I and *Mlu*I and partial restriction digestion with 0.01 U of *Bam*HI (B), *Eco*RI (E), *Hin*dIII (H), *Not*I (N), *Pst*I (P), *Sal*I (S), *Xba*I (X), or *Xho*I (Z) was as described under Materials and Methods. The digests were electrophoresed along with [α-^{32}P]-end-labeled DNA size standards (S1 and S2) and subjected to Southern hybridization as described under Materials and Methods. Total run time and switching parameters are shown at the top. (A) Southern hybridization of previously probed (right-end probe) and stripped blot using the left probe. (B) Southern hybridization of fresh blot using the right probe to the vector. Autoradiography was performed for 8 h as described in Fig. 3.

formed restriction digests on the PCR fragments obtained above. A representation of the PCR products and their restriction digest patterns used to construct a more detailed map and to place the exons is shown in Fig. 6. The only intronic gap PCR was unable to overcome was the 15-kb distance from exon 1 to exon 2. The placement of exon 1 was accomplished by restriction mapping and sequencing as follows. Exon 1 was mapped by Southern hybridization to a large *Not*I–*Bam*HI fragment that was subsequently subcloned into pBluescript. A *Not*I site in the mouse methyltransferase gene was known to lie just upstream of exon 1 by previous sequencing (Galus *et al.*, 1994). The *Not*I–*Bam*HI subclone was sequenced in from the *Not*I end, and exon 1 was found to start about 0.1 kb from the *Not*I site as expected. This *Not*I site is unique in the

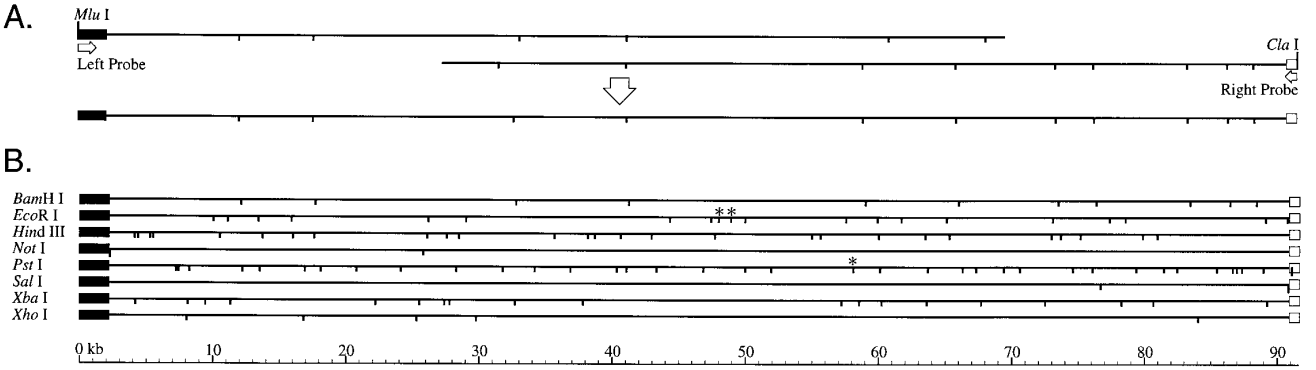

FIG. 5. Restriction maps of P1 clone p1949 using the end-probe method. The cleavage sites for each enzyme were "read" from Southern hybridization results using each end probe. The results from each end were compared and aligned to create the final map. (A) Creation of final *Bam*HI map showing restriction sites from each end probe. (B) Final end-probe map of the entire P1 clone (p1949) of the murine L-isoaspartyl/D-aspartyl methyltransferase gene. The scale is in kilobases. Solid and open boxes represent vector sequences to the left and right sides of the insert, respectively. The *Eco*RI and *Pst*I sites marked with asterisks were found after the additional analysis of the subclones (see text).

clone p1949 insert DNA, and so exon 1 was placed 15 kb upstream of exon 2.

Exon 8, previously not identified, was found to be 5.3 kb from exon 7 by "long PCR" and subclone restriction mapping. A genomic DNA subclone containing exon 8 was then sequenced. The 5′-sequence of a portion of exon 8 as well as its leading intronic DNA sequence is given in Fig. 7. The *Eco*RI site 5′ to exon 8 on the restriction map was found in the DNA sequence as shown, thus providing additional support to the placement of this exon.

Figure 8 is presented to compare the accuracy of the end-probe method with traditional subcloning and fragment-length overlap map building methods. Southern blots of complete restriction-digested clone p1949 DNA revealed the fragment sizes that particular exons lay within. We then isolated three λ phage subclones of p1949: a *Bam*HI fragment that maps between 18 and 33 kb, an *Eco*RI fragment that maps between 29 and 44 kb, and a *Bam*HI fragment that maps between 41 and 59 kb (Fig. 5). These λ phage clones were then subcloned into pBluescript II SK(+) for further analysis and fine-detail restriction mapping. For the region thus analyzed between 18 and 59 kb on Fig. 5, we found that the maximum deviation of the partial-digestion map compared to the subclone-derived map was 1 kb at sites 50 kb from the probe (Fig. 8, data not shown). Furthermore, we found that the deviation for sites closer to the insert ends was proportionately less. Thus it appears that the end-probe method gives a maximum error of 2% of the length of the fragment. Finally, these analyses indicated that only three restriction sites were missed in the end probe analysis (Fig. 5). For two cases, *Eco*RI sites were missed at 49 kb because they were close to two other *Eco*RI sites and the partially digested fragments were not adequately resolved on the pulsed-field gel. In another case, a *Pst*I fragment at 58 kb was initially missed due to a faint signal on the autoradiograph. Significantly, the end-probe analysis resulted in no false-positive site assignments. Figure 8 also compares our map with a previous map of exons 1 through 7 (Romanik *et al.*, 1992) of a genomic clone derived from B10.A mice (Benoist *et al.*, 1983; Samelson *et al.*, 1983; Chien *et al.*, 1984). The differences between these maps may reflect strain differences.

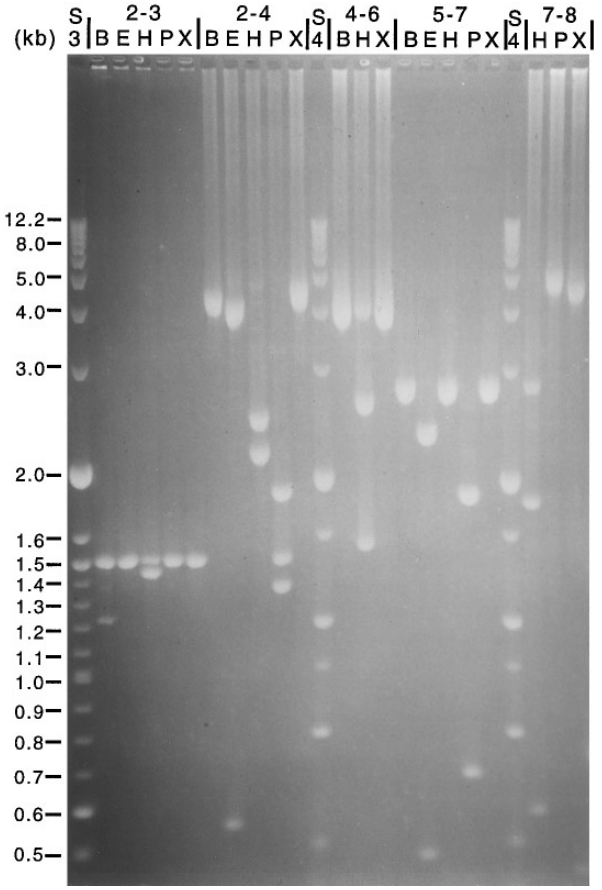

FIG. 6. Restriction digests of PCR products from P1 clone p1949. Numbers separated by dashes represent the exons spanned by the PCR products. The 2.5 μl of PCR products were digested with restriction enzymes *Bam*HI (B), *Eco*RI (E), *Hin*dIII (H), *Pst*I (P), and *Xba*I (X) and electrophoresed with DNA size standards (S3 and S4).

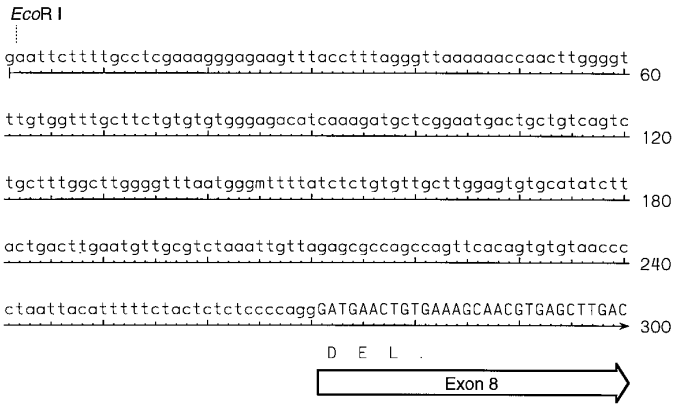

FIG. 7. Genomic sequence of exon 8 derived from a plasmid subclone of the P1 clone DNA. The exonic region corresponding to cDNA clone 1580 (Romanik *et al.*, 1992) is given in uppercase letters and the intronic sequence is given in lowercase letters. The C-terminus translation of isozyme II is shown as DEL.

DISCUSSION

Although direct DNA sequencing is readily available, restriction endonuclease analyses are still widely used in population genetics, molecular systematics studies, and investigating particular regions for functional studies (Nei, 1987). These analyses provide cheaper and faster ways to assay patterns of nucleotide differentiation across a large number of individuals. In 1990, Sternberg revealed an alternative cloning system that would provide ease of clone manipulation and clones with inserts larger than cosmid contigs (Sternberg, 1990). This P1 cloning system contains inserts from 75

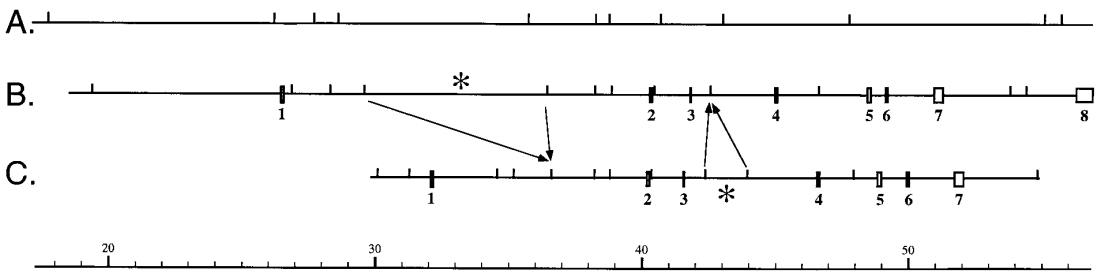

FIG. 8. Comparison of *Hin*dIII maps of the murine L-isoaspartyl/D-aspartyl methyltransferase gene. (A) Map derived using the partial digestion end-probe method of P1 clone p1949 (see Fig. 4) from a 129 mouse strain (Wassarman and DePamphilis, 1993) (Genome Systems). (B) Refinement of the map of P1 clone p1949 using traditional methods of plasmid subcloning followed by subclone restriction endonuclease digestion. Exons were placed using long PCR. (C) Previously published partial map (Romanik *et al.*, 1992) of the gene isolated from mouse strain B10.A (Chien *et al.*, 1984). The scale is in kilobases and refers to Fig. 4. (*) indicates regions of disparity.

to 100 kb long in the form of an easily manipulated supercoiled plasmid readily and stably propagated in *E. coli*. The P1 origin of replication maintains the copy number to one per cell and thus prevents any selective growth advantage to smaller clones that could arise by deletion mutations. The one-copy maintenance also reduces the possibility of recombination mutations and ensures the fidelity of a library. Genomic P1 clones are now available for many different organisms including humans, mice, rats, and fruit flies. P1 libraries are especially useful in mapping the mammalian genome, where the average gene length is about 17 kb (Lewin, 1994). As such, a 75- to 100-kb mammalian P1 clone would most likely cover all of a gene of interest.

We describe a simple and rapid procedure for obtaining a restriction map of any P1 clone. In this paper, we show the application of this method to map the entire mouse protein L-isoaspartate-(D-aspartate) *O*-methyltransferase gene (EC 2.1.1.77). This paper shows that a single pulsed-field electrophoresis blot can be used to map an entire 89-kb P1 clone genomic insert for eight restriction endonucleases. This method can also be used for any clones with the appropriate rare-cutting restriction sites within the vector. This approach has already found success with yeast artificial chromosomes (Burke *et al.*, 1987) and may also be useful for bacterial artificial chromosomes (Shizuya *et al.*, 1992) and particularly P1-artificial chromosomes (Ioannou *et al.*, 1994).

This general method can also be used to analyze a genome or clone for specific regions or sites. For example: CpG islands can be assayed using CG-sensitive enzymes (e.g., *Not*I); the general palindromic character of a region of DNA can be assayed by using a representative mix of palindromic endonucleases; and start and stop codon regions can be assayed with start and stop codon-specific enzymes (Rowland *et al.*, 1992). Any enzyme that can modify the mobility of nucleotide fragments could potentially be assayed in this manner.

Using fragments obtained from this mouse P1 clone, we are now pursuing the creation of a "knockout" mouse to determine the role of the protein L-isoaspartate-(D-aspartate) *O*-methyltransferase.

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