LINKAGE OF GENES FOR LAMININ B1 AND B2 SUBUNITS ON CHROMOSOME 1 IN MOUSE

ROSEMARY W. ELLIOTT, DENISE BARLOW, AND BRIGID L. M. HOGAN

Department of Molecular Biology (R. W. E.), Roswell Park Memorial Institute, 666 Elm Street, Buffalo, New York 14263, and Imperial Cancer Research Fund (D. B., B. L. M. H.), Mill Hill Laboratories, Burtonhole Lane, London NW7 1AD, United Kingdom

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SUMMARY

We have used cDNA clones for the B1 and B2 subunits of laminin to find restriction fragment length DNA polymorphisms for the genes encoding these polypeptides in the mouse. Three alleles were found for LamB2 and two for LamB1 among the inbred mouse strains. The segregation of these polymorphisms among recombinant inbred strains showed that these genes are tightly linked in the central region of mouse Chromosome 1 between Sas-1 and Ly-m22, 7.4 ± 3.2 cM distal to the Pep-3 locus. There is no evidence in the mouse for pseudogenes for these proteins.

Key words: DNA polymorphism; recombinant inbred mouse strains; parietal endoderm cells; linkage map.

INTRODUCTION

Laminin is a multidomain, extracellular matrix glycoprotein of Mr = 900,000. It is a major component of all basement membranes separating epithelial and mesenchymal cells, including those of the kidney, skin, eye and blood vessels, and of embryonic tissues such as the placenta, amnion and yolk sac (16,22,40,42). Laminin distribution is not confined to basement membranes, however. It is synthesized by cleavage stage mouse embryos (11) and may play an important role in promoting neurite outgrowth and neuronal-glial cell interactions in the central and peripheral nervous systems (3,4,12,23,26).

The precise subunit composition of laminin in different tissues is not yet known. Laminin synthesized by mouse embryo parietal endoderm (PE) cells is assembled from at least three different polypeptide chains, A (or heavy), and B1 and B2 (GP2 or light chains) (10). RNA isolated from PE cells directs the in vitro synthesis of two B1 chains, termed Bla and Blb (21), raising the possibility that there are variants of laminin analogous to the different isoforms of fibronectin (20,35). An important step towards resolving speculation about multiple forms of laminin and their involvement in human diseases affecting basement membranes or neural development, is to determine the number and chromosomal location of the genes encoding the different subunits. Progress in this direction has recently been made possible by the isolation of cDNA clones for the B chains (2). The cDNA clone, pPE9, hybridizes to a 7.4 kb mRNA in PE cells which directs the in vitro synthesis of the Mr = 185,000 B2 chain. In contrast, the cDNA pPE386 hybridizes to mRNA of only 6 kb and this directs the in vitro synthesis of both the Bla (Mr = 205,000) and Blb (Mr = 200,000) chains. pPE386 has an insert of 1.1 kb and covers 290 amino acids of C-terminal sequence, as well as the complete 3' non-coding region. pPE9 covers 217 C-terminal amino acids up to the stop codon. A third cDNA, pPE49, overlaps with pPE386 and extends a further 600 bp towards the 5' end (2). There is no significant sequence homology between any of the laminin B1 and B2 cDNAs so far isolated, and they do not cross hybridize.

The amino acid sequence of the C-terminal regions of both laminin B chains reveals a striking heptad repeat, typical of the coiled-coil a helixes found in myosin, tropomyosin, desmin and fibrin (2,29). Therefore, although the known amino acid sequences of the two subunits are different, the similarity in their secondary structure suggests that they may be evolutionarily related.
In this study we have used the cDNA clones pPE9 and pPE386 to find restriction fragment length polymorphisms (RFLPs) for the genes encoding the laminin B1 and B2 subunits in the mouse. Using the method of recombinant inbred (RI) strain linkage analysis introduced by Bailey (1) and further developed by Taylor (36), we have shown that the loci encoding the B1 and B2 subunits of laminin are closely linked on Chromosome 1 of the mouse, 7.4 ± 3.2 cM distal to the Pep-3 locus.

**METHODS**

**Animals:** Mice, 6 to 18 weeks, were obtained from the Jackson Laboratory or from our own colony. The twenty-six BXD RI strains were derived by Dr. Benjamin Taylor from C57BL/6J (strain B) and DBA/2J (strain D) by brother-sister mating commencing at the F1 generation of a cross between the progenitor strains (38). The strains are now at least at F30. The eighteen AKXL RI strains were similarly derived from a cross between AKR/J (strain A) and C57L/J (strain L) (39). They are now at least at F20. The AKXD RI strains were derived from a cross between AKR/J and DBA/2J (37).

**Isolation of plasmid DNA.** A culture (500 ml) of *E. coli* HB101 containing the plasmid was grown on L broth and ampicillin. The cells were sedimented, resuspended in 20 ml 25 mM Tris HCl buffer (pH 8.0) containing 10 mM EDTA and incubated at room temperature for 10 min. Cell lysis and plasmid extraction was performed by the alkali extraction procedure (9) as modified (6). Purification of plasmid DNA was performed using the cesium chloride-ethidium bromide centrifugation method (25).

**DNA blot analysis.** Mouse liver DNA was isolated by the method of Jeffries and Flavel (18). DNA blot analysis was performed according to Elliott and Berger (13).

**RESULTS**

**Mapping the locus LamB2:** The approach to mapping the mouse gene encoding laminin B2 involved searching for RFLPs in the DNA of progenitors of RI strains using the cDNA probe, pPE9. Once a polymorphism is found, its segregation among the individual RI strains is determined and the strain distribution pattern (SDP) is compared with those previously determined for other loci; identical SDPs for two loci indicate that they are very closely linked. The progenitor strains used in this study were A/J, AKR/J, C57L/J, SWR/J, DBA/2J, C57BL/6J, C3H/HeJ, BALB/cByJ, C58/J, NZB/BINJ and SM/J.

Of the twelve enzymes tested, *Bali*, *EcoRI*, *HinII* and *PstI* revealed polymorphisms, while *BamHI*, *BgI*, *HindIII*, *MspI*, *PstI*, *SstI* and *TaqI* gave a similar pattern for all the strains tested. Each pattern was relatively simple, containing up to three fragments, depending on the enzyme. The finding of a single *MspI* fragment suggests that only one gene for the laminin B2 polypeptide is present in the mouse genome. The *HinII*-generated RFLP between DBA/2J and the other strains is illustrated in Fig. 1. The SDP for this