SHORT COMMUNICATION

Four Paralogous Protein 4.1 Genes Map to Distinct Chromosomes in Mouse and Human

Luanne L. Peters,* Heinz-Ulrich G. Weier,† Loren D. Walensky,‡ Solomon H. Snyder,‡ Marilyn Parra,† Narla Mohandas,† and John G. Conboy†,[1]

*The Jackson Laboratory, Bar Harbor, Maine 04609; †Life Sciences Division, Lawrence Berkeley National Laboratory, University of California, Berkeley, California 94720; and ‡Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Received June 11, 1998; accepted August 11, 1998

Four highly conserved members of the skeletal protein 4.1 gene family encode a diverse array of protein isoforms via tissue-specific transcription and developmentally regulated alternative pre-mRNA splicing. In addition to the prototypical red blood cell 4.1R (human gene symbol *EPB41*,) these include two homologues that are strongly expressed in the brain (4.1N, *EPB41L1*; and 4.1B, *EPB41L3*) and another that is widely expressed in many tissues (4.1G, *EPB41L2*). As part of a study on the structure and evolution of the 4.1 genes in human and mouse, we have now completed the chromosomal mapping of their respective loci by reporting the localization of mouse 4.1N, 4.1G, and 4.1B, as well as human 4.1B. For the mouse 4.1 genes, Southern blot analysis of RFLPs in The Jackson Laboratory BSS interspecific backcross yielded the following assignments: 4.1N (*Epb4.1l1*,) chromosome 2; 4.1G (*Epb4.1l2*,) chromosome 10; and 4.1B (*Epb4.1l3*,) mouse chromosome 17. Human 4.1B was physically mapped to chromosome 18p11 using fluorescence *in situ* hybridization. All of the mouse genes mapped within or adjacent to regions of conserved synteny with corresponding human chromosomes. We conclude that a set of four paralogous 4.1 genes has been evolutionarily conserved in rodents and primates.

The protein 4.1 superfamily encompasses a group of structural proteins that play important roles in membrane biophysical processes through their interactions with actin, members of the spectrin family, and the cytoplasmic domains of integral membrane proteins (14). The prototypical member of this family is the major 80-kDa protein 4.1R isoform found in red blood cells, where it is a key component of the erythroid membrane skeleton that underlies and mechanically supports the plasma membrane. The 4.1R gene (*EPB41*) is noteworthy for elaborate alternative pre-mRNA splicing pathways by which it encodes tissue-specific protein isoforms (1, 3, 13, 16). Mutations in this gene result in membrane mechanical defects and morphological abnormalities characteristic of the red cell disorder, hereditary elliptocytosis (5). The 4.1R human gene (*EPB41*) maps to chromosome 1p33–p34.2 (4, 15), whereas the mouse 4.1R gene (*Epb4.1*) maps to a region of conserved synteny on mouse chromosome 4 (12).

Recently, three novel members of the 4.1 gene superfamily were reported. These include a neuronal homologue (4.1N or *EPB41L1*) (9, 17), a widely expressed homologue (4.1G or *EPB41L2*) (11, 18), and another brain homologue (4.1B or *EPB41L3*) (10). These new genes encode proteins that are highly homologous to the prototypical 4.1R in three key structural domains: the membrane binding domain, the spectrin–actin binding domain, and the conserved C-terminal domain. As part of our studies of 4.1 gene structure and evolution, we now report the mapping of all three mouse nonerythroid 4.1 genes, as well as the human 4.1B gene.

We mapped the mouse 4.1N gene (*Epb4.1l1*) by Southern blot analysis using 94 progeny from The Jackson Laboratory BSS interspecific backcross ((C57BL/6JEi × SPRET/Ei)F_1 × SPRET/Ei) (12). Using a 3.5-kb mouse 4.1N cDNA as hybridization probe, we identified a *Bam*HI RFLP (C57BL/6JEi, 9.9 and 2.5 kb; SPRET/Ei, 7.5 kb) with which to follow segregation of *Epb4.1l1* alleles. *Epb4.1l1* was thus mapped to mouse chromosome 2, 90 cM distal to the centromere (Fig. 1A), a region of conserved synteny with human chromosome 20q11.2–q13.1 (2), to which the human 4.1N gene (*EPB41L1*) has been mapped (7; and Weier, unpublished results).

Using a 1.5-kb fragment of mouse 4.1G cDNA (Accession No. AF044312, nt 13–1539) as the hybridization probe, we mapped the mouse 4.1G gene (*Epb4.1l2*) by the same approach employing Southern blot exper-

Typing data from this article have been deposited with the Mouse Genome Database under Accession No. J47260.

[1] To whom correspondence should be addressed at Life Sciences Division, MS 74-174, Lawrence Berkeley National Laboratory, Berkeley, CA 94720. Telephone: (510) 486-6973. Fax: (510) 486-6746. E-mail: jgconboy@lbl.gov.

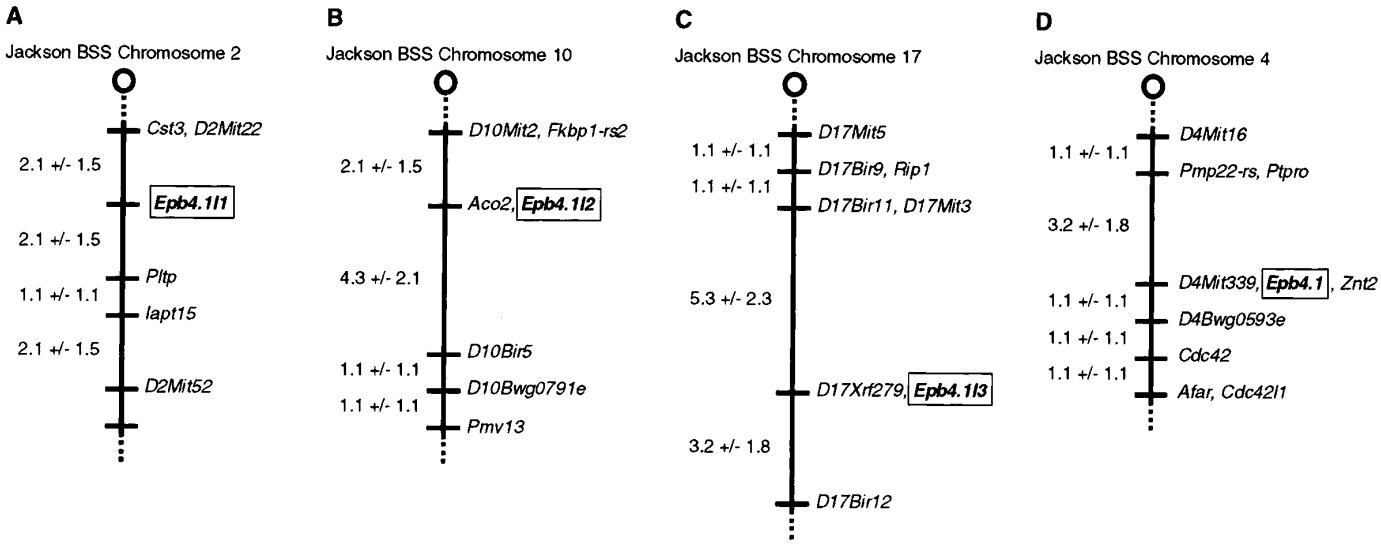

FIG. 1. Chromosomal localization of mouse 4.1 genes. (**A–C**) Localization of mouse 4.1 genes using The Jackson Laboratory BSS panel. *Epb4.1l1* mapped to chromosome 2 (**A**), *Epb4.1l2* to chromosome 10 (**B**), and *Epb4.1l3* to chromosome 17 (**C**). Genetic distances between markers (percentage recombination ± SE) are indicated to the left of each chromosome, and gene symbols are shown on the right. Missing typings in nonrecombinant animals were inferred from flanking markers. The typing data have been placed in the Mouse Genome Database (Accession No. J47260) and can be accessed through the World Wide Web (http://www.jax.org). The panel data and references for mapping the other loci are publicly available from The Jackson Laboratory Mapping Resource through http://www.jax.org/resources/documents/cmdata. (**D**) For comparison, localization of mouse 4.1R to chromosome 4 is shown.

iments with the interspecific backcross panel. Segregation of *Epb4.1l2* (mouse 4.1G) alleles was followed using an *Xba*I RFLP (C57BL/6JEi, 6.6 and 5.4 kb; SPRET/Ei, 5.0 and 4.1 kb). No recombination occurred between *Epb4.1l2* and the *Aco2* gene, localizing *Epb4.1l2* to mouse chromosome 10, approximately 20 cM distal to the centromere (Fig. 1B). This region of mouse chromosome 10 shares extensive homology with the human chromosome 6q22–q23 (2), to which the human 4.1G gene (*EPB41L2*) has been mapped (11).

The fourth member of the 4.1 gene family, protein 4.1B, has not previously been mapped in human or mouse. We mapped the mouse 4.1B gene (*Epb4.1l3*) using a *Sac*I RFLP (C57BL/6JEi, 6.8 kb; SPRET/Ei, 5.0 kb) in the interspecific backcross panel. The hybridization probe was a 1.0-kb fragment of mouse cDNA (EST clone L26705), whose sequence was confirmed in our laboratory to include a portion of the C-terminal coding domain as well as the 3′ untranslated region of mouse 4.1B. *Epb4.1l3* was nonrecombinant with the marker *D17Xrf279,* placing *Epb4.1l3* on mouse chromosome 17, approximately 42.5 cM distal to the centromere (Fig. 1C).

The human *EPB41L3* gene was localized using a multicolor fluorescence *in situ* hybridization (FISH) approach, using a 4.1B probe derived as follows. The partial sequence of human 4.1B, represented within expressed sequence tag clones AA307056, AA321945, and AA340302, was used to design PCR primers (sense, 5′-AGGAAGAACTGCTGGGCTTCCTGTG-3′; and antisense, 5′-TGTGATGCAGGTGATCGTGTA-ATGG-3′) to screen a human genomic BAC library (Research Genetics, Huntsville, AL). A 185-kb clone isolated by this approach was confirmed to include sequences encoding several internal domains of 4.1B as well as the 3′UTR. After preliminary studies localized this probe provisionally to chromosome 18, mapping was repeated using a known chromosome 18 marker, clone 945B6 from the CEPH/Généthon YAC library (20); a kind gift of Dr. J. Fung, University of California, San Francisco). The YAC probe was prepared as previously described (19). Figure 2 shows a two-color FISH (6) experiment carried out using biotin-labeled 4.1B genomic DNA (detected with avidin-FITC, green) and a digoxigenin-labeled chromosome 18 YAC marker (detected with rhodamine-conjugated anti-digoxigenin antibody, red). As shown in Fig. 2, *EPB41L3* mapped to human chromosome 18p11.32 (green signal), almost colocalizing with the control probe hybridizing to its expected location at 18p11.32. The assignment of human and mouse 4.1B genes to these loci on chromo-

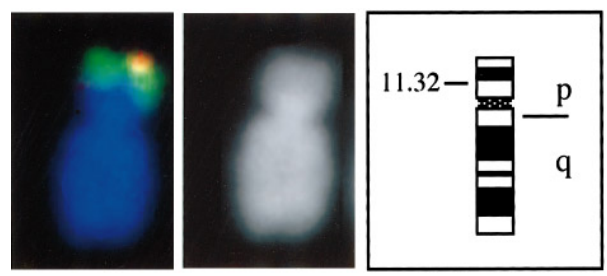

FIG. 2. Mapping of human 4.1B (*EPB41L3*) to chromosome 18 by multicolor FISH. (**Left**) Biotin-labeled 4.1B genomic DNA, detected with avidin-FITC (green), and digoxigenin-labeled YAC 945B6, detected with rhodamine conjugated anti-digoxigenin antibodies (red). (**Center**) The DAPI image suggests a map position in band p11.32 as indicated in the idiogram shown at the right. Seven metaphase spreads were examined, and all 14 chromosomes visualized were consistent in the localization of *EPB41L3* to this position.

somes 18 and 17, respectively, extends the region of conserved synteny known to exist between these two chromosomes (2).

A summary of the gene assignments in the 4.1 gene family is as follows: 4.1R, human chromosome 1/mouse chromosome 4; 4.1G, human chromosome 6/mouse chromosome 10; 4.1N, human chromosome 20/mouse chromosome 2; 4.1B, human chromosome 18/mouse chromosome 17. These gene locations are all self-consistent in that the corresponding mouse and human sites are all in or adjacent to known regions of conserved synteny.

The finding that four known protein 4.1 genes are expressed in the mammalian genome parallels similar results observed with other members of the erythroid membrane skeleton. Three distinct ankyrin genes (*ANK1*, *ANK2*, and *ANK3*), four spectrin genes (*SPTA1*, *SPTA2*, *SPTB*, and *SPTBN1*), and three band 3 genes (*AE1*, *AE2*, and *AE3*) are known, and in each case the individual family members are genetically unlinked. It is interesting to speculate that a single copy of each may have existed in the genome of a primitive ancestor and that the paralogous genes existing in modern vertebrate genomes may have arisen via tetraploidization of the genome (8). Each gene has evolved a distinct pattern of expression regulated by transcriptional and RNA splicing processes, thus encoding a family of proteins likely to play diverse roles in skeletal structures of many eukaryotic cell types.

ACKNOWLEDGMENTS

This work was supported by NIH Grants HL55321 (L.P.) and DK32094 (M.N.) and by a grant from The March of Dimes.

REFERENCES

1. Baklouti, F., Huang, S.-C., Vulliamy, T. J., Delaunay, J., and Benz, E. J., Jr. (1997). Organization of the human protein 4.1 genomic locus: New insights into the tissue-specific alternative splicing of the pre-mRNA. *Genomics* **39:** 289–302.
2. Chromosome Committee Data (1998). "Encyclopedia of the Mouse Genome, Mouse Genome Informatics Project," The Jackson Laboratory, Bar Harbor, ME.
3. Conboy, J. G., Chan, J., Chasis, J. A., Kan, Y. W., and Mohandas, N. (1991). Tissue- and development-specific alternative RNA splicing regulates expression of multiple isoforms of erythroid membrane protein 4.1. *J. Biol. Chem.* **266:** 8273–8280.
4. Conboy, J. G., Mohandas, N., Tchernia, G., and Kan, Y. W. (1986). Molecular basis of hereditary elliptocytosis due to protein 4.1 deficiency. *N. Engl. J. Med.* **315:** 680–685.
5. Delaunay, J., Alloisio, N., Morle, L., Baklouti, F., Dalla Venezia, N., *et al.* (1996). Molecular genetics of hereditary elliptocytosis and hereditary spherocytosis. *Ann. Genet.* **39:** 209–221.
6. Fung, J., Munne, S., Duell, T., and Weier, H.-U. G. (1998). Rapid cloning of translocation breakpoints: From blood to YAC in 50 days. *J. Biochem. Mol. Biol. Biophys.* **1:** 181–192.
7. Kim, A. C., Van Huffel, C., Lutchman, M., and Chishti, A. H. (1998). Radiation hybrid mapping of *EPB41L1*, a novel protein 4.1 homologue, to human chromosome 20q11.2–q12. *Genomics* **49:** 165–166.
8. Lundin, L. G. (1993). Evolution of the vertebrate genome as reflected in paralogous chromosomal regions in man and the house mouse. *Genomics* **16:** 1–19.
9. Nagase, T., Ishikawa, K., Nakajima, D., Ohira, M., Seki, N., *et al.* (1997). Prediction of the coding sequences of unidentified human genes. VII. The complete sequences of 100 new cDNA clones from brain which can code for large proteins in vitro. *DNA Res.* **4:** 141–150.
10. Parra, M., Gascard, P., Walensky, L., Snyder, S., Mohandas, N., *et al.* (1997). A family of distinct protein 4.1 genes is expressed in human tissues. *Mol. Biol. Cell* **8(S):** 177a.
11. Parra, M., Gascard, P., Walensky, L. D., Snyder, S. H., Mohandas, N., *et al.* (1998). Cloning and characterization of 4.1-G, a new member of the skeletal protein 4.1 gene family. *Genomics* **49:** 298–306.
12. Rowe, L. B., Nadeau, J. H., Turner, R., Frankel, W. N., Letts, V. A., *et al.* (1994). Maps from two interspecific backcross DNA panels available as a community genetic mapping resource. *Mamm. Genome* **5:** 253–274.
13. Schischmanoff, P. O., Yaswen, P., Parra, M. K., Lee, G., Chasis, J. A., *et al.* (1997). Cell-shape dependent regulation of protein 4.1 alternative splicing in mammary epithelial cells. *J. Biol. Chem.* **272:** 10254–10259.
14. Takeuchi, K., Kawashima, A., Nagafuchi, A., and Tsukita, S. (1994). Structural diversity of band 4.1 superfamily members. *J. Cell Sci.* **107:** 1921–1928.
15. Tang, C. J., and Tang, T. K. (1991). Rapid localization of membrane skeletal protein 4.1 (EL1) to human chromosome 1p33–p34.2 by nonradioactive in situ hybridization. *Cytogenet. Cell Genet.* **57:** 119.
16. Tang, T. K., Leto, T., Marchesi, V. T., and Benz, E. J. (1990). Heterogeneity of mRNA and protein products arising from the protein 4.1 gene in erythroid and nonerythroid tissues. *J. Cell Biol.* **110:** 617–624.
17. Walensky, L. D., Blackshaw, S. S., Conboy, J. G., Mohandas, N., and Snyder, S. H. (1997). Molecular cloning of a novel neuron-specific homologue of the erythrocyte membrane skeletal protein 4.1. *Soc. Neurosci. Abstr.* **23:** 1674.
18. Walensky, L. D., Gascard, P., Fields, M. E., Blackshaw, S., Conboy, J. G., *et al.* (1998). Immunophilin FKBP13 interacts with a novel erythrocyte membrane cytoskeletal protein 4.1. *J. Cell Biol.* **273:** 143–153.
19. Weier, H. U., George, C. X., Greulich, K. M., and Samuel, C. E. (1995). The interferon-inducible, double-stranded RNA-specific adenosine deaminase gene (DSRAD) maps to human chromosome 1q21.1–1q21.2. *Genomics* **30:** 372–375.
20. Weissenbach, J., Gyapay, G., Dib, C., Vignal, A., Morissette, J., *et al.* (1992). A second-generation linkage map of the human genome. *Nature* **359:** 794–801.