Mammalian Genome 4, 618–620 (1993).

Mammalian Genome

Mapping of the neural retina leucine zipper gene, *Nrl*, to mouse Chromosome 14

Irina N. Bespalova[1,2] Qais Farjo,[3] Douglas P. Mortlock,[2] Anne U. Jackson,[2] Miriam H. Meisler,[2] Anand Swaroop,[2,3] Margit Burmeister[1,2,4]

[1]Mental Health Research Institute, University of Michigan, Ann Arbor, Michigan 48109, USA
[2]Department of Human Genetics, University of Michigan, Ann Arbor, Michigan 48109, USA
[3]Department of Ophthalmology, University of Michigan, Ann Arbor, Michigan 48109, USA
[4]Department of Psychiatry, University of Michigan, Ann Arbor, Michigan 48109, USA

Received: 6 April 1993 / Accepted: 28 June 1993

We have used genomic denaturing gradient gel electrophoresis (gDGGE) and PCR across a variable length triplet repeat element to map the mouse *Nrl* gene close to the T-cell receptor alpha/nucleoside phosphorylase/ribonuclease-1 cluster on mouse Chromosome (Chr) 14, consistent with its position on human 14q11 (Yang-Feng and Swaroop 1992). This result was obtained with the BXD and AKXD recombinant inbred (RI) strains (Taylor 1990) and confirmed in a *Mus. spretus* backcross.

We recently cloned a human cDNA for a retina-specific protein with a basic motif and sequences homologous to leucine zippers and called it neural retina leucine zipper (*NRL*, D14S46E) (Swaroop et al. 1992). Isolation and sequence of the mouse cDNA and gene structure of the mouse homolog, *Nrl*, are described elsewhere (Farjo et al. 1993). In order to map *Nrl* to a mouse chromosome, we used three methods to detect polymorphisms: RFLPs, variation in triplet repeat length, and gDGGE. Using ten different restriction enzymes, we could not find any RFLP between strains AKR/J, C57L/J, C57BL/6J, and DBA/2J. We therefore used gDGGE (Burmeister et al. 1991), a novel approach that may be useful in finding polymorphisms. We also identified a short tandem repeat in the *Nrl* sequence and found it to be polymorphic.

The sequence of the mouse *Nrl* cDNA, derived from the BALB/c strain, contains a microsatellite repeat $(AGG)_{19}$ in its 3′ untranslated sequence (Farjo et al. 1993). PCR primers flanking this repeat were synthesized; primer 1: CATCGTGGCATGCTAGAGGTTAG and primer 2: AGATTCGGTGAAAATGCAGCTTC (nucleotide 996–1019 and 1337–1314 in the mouse cDNA sequence). These primers amplify a fragment of 341 bp from BALB/c [with 250 μM NTPs, 10 pmol primers, and PEK buffer (Ponce and Micol 1992), 0.5 μl Taq polymerase; 30 cycles of 15 s 94°C, 15 s 59°C, 75 s 71°C]. To discriminate the different alleles, we cleaved the PCR product with *Alu*I, resulting in constant fragments of size 19, 76, and 111 bp and a variable fragment that is 134 bp in BALB/c. The fragments were separated by non-denaturing 15% polyacrylamide gel electrophoresis followed by ethidium bromide staining. Analysis of a variety of inbred mouse strains showed a length polymorphism consistent with variability in the repeat length. Whereas 129/ReJ, C57BL/6J, C57L/2J, AKR/J, A/J, and C3H/HEJ all showed the same allele as BALB/c, we found a CASA/Rk allele in which the variable *Alu*I fragment is about 170 bp, a SJL/L allele of about 152 bp, a *M. spretus* allele of 143 bp, and a MOLD/Rk allele of 128 bp. Most importantly for RI strain mapping, the DBA/2J allele is 3 bp larger than the common allele.

In parallel, we also used gDGGE to find polymorphisms between parental strains of recombinant inbred (RI) strains. gDGGE is a new approach that has been used successfully to find human DNA polymorphism (Burmeister et al. 1991; Gray 1992). It is estimated to detect about 50% of all point mutations (Burmeister et al. 1991). Briefly, 5 μg of genomic DNA from the six RI mouse strain progenitors A/J, AKR/J, C3H, C57L/J, C57BL/6J, and DBA/2J (obtained from The Jackson Laboratory, Bar Harbor, ME.) were each digested with the restriction enzymes *Alu*I, *Hin*fI, *Hae*III, and *Sau*3A. The resulting genomic DNA fragments were separated by polyacrylamide gel electrophoresis in the presence of a gradient of urea and formamide. Two different gradients, of 10–50% and 40–80% denaturant, were run as described (Burmeister et al. 1991) on com-

Correspondence to: M. Burmeister

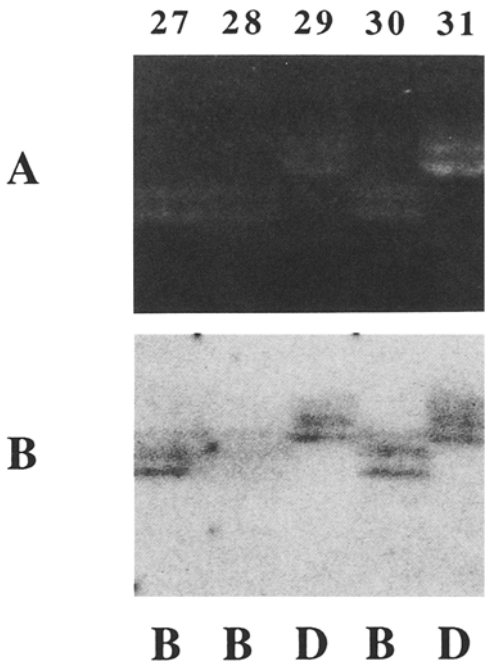

Fig. 1. Typing of *Nrl* on DNA from BXD RI lines. (A) *Nrl* was amplified by PCR, and the product was digested with *Alu*I, as described in the text. Fragments were separated on a 15% nondenaturing polyacrylamide gel and stained with ethidium bromide. (B) DNA was digested with *Hae*III, separated on a 40–80% denaturant gDGGE, electroblotted to Hybond N plus (Amersham) nylon membrane, and hybridized with a 1.1-kb *Nrl* cDNA probe. The multiple bands can be explained by partial methylation of CpG sites in the fragment because the pattern differs when different tissue sources were used (Mortlock and Burmeister, unpublished observation), and it is known that DGGE separates fragments that differ in methylation status (Collins and Myers 1987). Numbers above the lanes refer to BXD RI strains. The allele typing is indicated below the lanes.

mercially available DGGE equipment (C.B.S. Scientific Company, Del Mar, Calif.). The fragments were then electroblotted (Gray 1992) to Hybond N plus (Amersham) nylon membranes. The filters were hybridized to a radioactively labeled unique probe, a 1.1-kb *Eco*RI fragment (nucleotide 0–1095) of the mouse *Nrl* cDNA close to but not containing the AGG repeat. The analysis of the hybridizing bands showed a melting polymorphism between DBA/2J and the other strains tested when *Hae*III-digested DNA was analyzed. Because there is no *Hae*III site between the AGG repeat and the probe and also the DBA allele runs more slowly in the gel, this is likely to be the same polymorphism as the one detected by PCR.

To map *Nrl,* we typed the BXD and AKXD sets of RI strains with both detection methods (Fig. 1). The strain distribution patterns (SDPs) for both methods were identical. The SDP of BXD (Table 1) showed 0/26 recombinants with the *Tcra/Rib-1/Np-2* cluster (Dembic et al. 1985; Elliot et al. 1985; Taylor 1981) which is not separated in BXD RI strains (Nadeau and Cox 1992). The distance between *Nrl* and this cluster is thus 0 ± 1.3 cM (95% confidence interval).

The SDP of the AKXD lines confirmed the linkage of *Nrl* to mouse Chr 14, with 5/23 recombinants to the closest linked marker, *Mtv-11* (Table 1; Traina et al. 1981; Lee and Eicher 1990), indicating a distance of 8.1 ± 4.7 cM from *Mtv-11,* and 6/23 recombinants or 10.7 ± 6.2 cM from *Nfl* (Hearne et al. 1991). With the more proximal marker, *Odc-ps9,* 10/24 animals were recombinant, indicating a distance of 27.8 ± 17.9 cM, but this linkage is not significant because of the large distance. Since two strains (number 7 and 15) showed two recombination events flanking *Nrl,* we retyped the AKXD strains a second time with *Nrl,* with the same result. The current consensus map suggests the order *Odc-ps9–Mtv-11–Tcra/Nrl–Nfl* (Nadeau and Cox 1992).

Our data are more consistent with the order *Odc-ps9–Tcra/Nrl–Mtv-11–Nfl,* because the consensus order gives five rather than two AKXD strains with two recombination events. Since the BXD data place *Nrl* near *Tcra,* our results are most consistent with a location of *Mtv-11* approximately 15 cM distal to its current map position. This suggested new gene order is also consistent with the original typing of *Mtv-11* in the BXD RI strains (Traina et al. 1981; Lee and Eicher 1990). The SDP data for all markers in the BXD strains (Nadeau and Cox 1992) are consistent with placement

Table 1. SDPs of *Nrl* and closest linked markers in RI lines. References for SDPs other than *Nrl:* (1) Taylor 1981; (2) Dembic et al. 1985; (3) Elliot et al. 1985; (4) Richards-Smith and Elliot 1992; (5) Traina et al. 1981; Lee and Eicher 1990; (6) Hearne et al. 1991.

Locus		BXD																									
	Ref.	1	2	5	6	8	9	11	12	13	14	15	16	18	19	20	21	22	23	24	25	27	28	29	30	31	32
Np-2	1	B	D	B	D	D	D	D	D	B	D	D	D	D	D	B	B	B	B	B	B	B	B	D	B	D	D
Tcra,	2																										
Rib-1	3																										
Nrl		B	D	B	D	D	D	D	D	B	D	D	D	D	D	B	B	B	B	B	B	B	B	D	B	D	D
Locus		AKXD																									
	Ref.	1	2	3	6	7	8	9	10	11	12	13	14	15	16	17	18	20	21	22	23	24	25	26	27	28	
Odc-ps9	4	A	D	D	D	A	D	A	A	D	D	A	A	D	A	A	A	D	A	D	D	A	A	D	D	A	
				x		x			x	x				x				x				x			x	x	
Nrl		A	D	A	D	D	D	A	D	A	D	A	A	A	A		A	A	A	D	A	D	A	D	A	D	
		x				x								x			x			x							
Mtv-11	5	D	D	A	D	A	D	A	D	A	D	A	A	D	A	D	D	A	A	A	A	D		D	A	D	
																								x			
Nfl	6	D	D	A	D	A	D	A	D	A	D		A	D	A		D	A	A	A	A	D	A	A	A	D	

of *Mtv-11* between *D14Mit5* and *D14Byu5*, distal to the consensus position.

In addition to the two sets of RI strains, 21 backcross animals from a C57BL/6J × *M. spretus* backcross (Bain et al. 1993) were typed for Chr 14 marker *D14Mit3* and for *Nrl* with the repeat length polymorphism. We found 3/21 recombinant animals, indicating a distance of 14 ± 7 cM. This is consistent with the reported position of *D14Mit3* approximately 10 cM proximal to *Np-2* (Dietrich et al. 1992; Nadeau and Cox 1992).

Human *NRL* was previously mapped to human Chr 14q11 (Yang-Feng and Swaroop 1992). The data presented here demonstrate that *Nrl* is located on mouse Chr 14 near *Tcra* and *Np-2*. *Nrl* appears to be part of a previously described conserved linkage group that spans approximately 3 cM on mouse Chr 14 and includes seven additional loci on human Chr 14q11 (Nadeau and Cox 1992). In addition to *Nrl*, this linkage group consists of the genes for Angiogenin (*Ang;* Weremowicz et al. 1990), the cytotoxic T-lymphocyte antigen-1 (*Ctla-1;* Harper et al. 1988), the adult and fetal forms of cardiac muscle myosin heavy chain (*Myhc-a* and *Myhc-b;* Saez et al. 1987), nucleoside phosphorylase (*Np-1;* Francke et al. 1976), and the T-cell receptors α and δ (*Tcra* and *Tcrd;* Chien et al. 1987; Collins et al. 1985; Croce et al. 1985).

Acknowledgments. We would like to express our thanks to Benjamin A. Taylor for assistance in RI strain analysis and to an anonymous reviewer for pointing out the discrepancy with the *Mtv-11* position in the consensus map. This work was supported in part by grants from the Markey Charitable Trust (M. Burmeister), the Retinitis Pigmentosa and the George Gund Foundations (A. Swaroop), and U.S. Public Health Service grant GM24872 (M.H. Meisler). D.P. Mortlock is supported by the Genetics Training Program of the University of Michigan (T32GM07544), and A. Jackson by a graduate fellowship of the National Science Foundation.

References

Bain, P.A., Meisler, M.H., Taylor, B.A., Payne, A.H. (1993). The genes encoding gonadal and nongonadal forms of 3-β-hydroxysteroid dehydrogenase isomerase are closely linked on mouse chromosome 3. Genomics 16, 219–223.

Burmeister, M., diSibio, G., Cox, D.R., Myers, R.M. (1991). Identification of polymorphisms by genomic denaturing gradient gel electrophoresis: application to the proximal region of human chromosome 21. Nucleic Acids Res. 19, 1475–1481.

Chien, Y.H., Iwashima, M., Kaplan, K.B., Elliott, J.F., Davis, M.M. (1987). A new T-cell receptor gene located within the alpha locus and expressed early in T-cell differentiation. Nature 327, 677–682.

Collins, M., Myers, R.M. (1987). Alterations in DNA helix stability due to base modifications can be evaluated using denaturing gradient gel electrophoresis. J. Mol. Biol. 198, 737–744, 1987.

Collins, M.K., Goodfellow, P.N., Spurr, N.K., Solomon, E., Tanigawa, G., Tonegawa, S., Owen, M.J. (1985). The human T-cell receptor alpha-chain gene maps to chromosome 14. Nature 314, 273–274.

Croce, C.M., Isobe, M., Palumbo, A., Puck, J., Ming, J., Tweardy, D., Erikson, J., Davis, M., Rovera, G. (1985). Gene for alpha-chain of human T-cell receptor: location on chromosome 14 region involved in T-cell neoplasms. Science 227, 1044–1047.

Dembic, Z., Bannwarth, W., Taylor, B.A., Steinmetz, M. (1985). The gene encoding the T-cell receptor alpha-chain maps close to the Np-2 locus on mouse chromosome 14. Nature 314, 271–273.

Dietrich, W., Katz, H., Lincoln, S.E., Shin, H.-S., Friedman, J., Dracopoli, N.C., Lander, E.S. (1992). A genetic map of the mouse suitable for typing intraspecific crosses. Genetics 131, 423–447.

Elliott, R.W., Samuelson, L.C., Lambert, M.S., Meisler, M.H. (1986). Assignment of pancreatic ribonuclease gene to mouse chromosome 14. Cytogenet. Cell Genet. 42, 110–112.

Farjo, Q., Jackson, A.U., Xu, J., Gryzenia, M., Skolnick, C., Agarwal, N., Swaroop, A. (1993). Molecular characterization of murine neural retina leucine zipper gene, *Nrl*. Genomics, in press.

Francke, U., Busby, N., Shaw, D., Hansen, S., Brown, M.G. (1976). Intrachromosomal gene mapping in man: assignment of nucleoside phosphorylase to region 14cen leads to 14q21 by interspecific hybridization of cells with a t(X;14) (p22;q21) translocation. Somatic Cell Genet. 2, 27–40.

Gray, M.R. (1992). Detection of DNA sequence polymorphisms in human genomic DNA by using denaturing gradient gel blots. Am. J. Hum. Genet. 50, 331–346.

Harper, K., Mattei, M.G., Simon, D., Suzan, M., Guenet, J.L., Haddad, P., Sasportes, M., Golstein, P. (1988). Proximity of the CTLA-1 serine esterase and Tcr alpha loci in mouse and man. Immunogenetics 28, 439–444.

Hearne, C.M., McAleer, M.A., Love, J.M., Aitman, T.J., Cornall, R.J., Ghosh, S., Knight, A.M., Prins, J.B., Todd, J.A. (1991). Additional microsatellite markers for mouse genome mapping. Mammalian Genome 1, 273–282.

Lee, B.K., Eicher, E.M. (1990). Segregation patterns of endogenous mouse mammary tumor viruses in five recombinant inbred strain sets [published erratum appears in J. Virol. 1991 Mar;65(3):1666]. J. Virol. 64, 4568–4572.

Nadeau, J.H., Cox, R. (1992). Mouse chromosome 14. Mammalian Genome 3 (Suppl.), S206–19.

Ponce, M.R., Micol, J.L. (1992). PCR amplification of long DNA fragments. Nucleic Acids Res. 20, 623.

Richards-Smith, B.A., Elliott, R.W. (1992). Mapping of the mouse ornithine decarboxylase-related sequence family. Mammalian Genome 2, 215–232.

Saez, L.J., Gianola, K.M., McNally, E.M., Feghali, R., Eddy, R., Shows, T.B., Leinwand, L.A. (1987). Human cardiac myosin heavy chain genes and their linkage in the genome. Nucleic Acids Res. 15, 5443–5459.

Swaroop, A., Xu, J., Pawar, H., Jackson, A., Skolnick, C., Agarwal, N. (1992). A conserved retina-specific gene encodes a basic motif/leucine zipper domain. Proc. Natl. Acad. Sci. USA 89, 266–270.

Taylor, B.A. (1981). Linkage of Np-2. Mouse News Lett. 65, 28.

Taylor, B.A. (1990). Recombinant inbred strains. In *Genetic Variants and Strains of the Laboratory Mouse,* 2nd ed., M.F. Lyon and A.G. Searle, eds. (Oxford: Oxford University Press), pp. 773–796.

Traina, V.L., Taylor, B.A., Cohen, J.C. (1981). Genetic mapping of endogenous mouse mammary tumor viruses: locus characterization, segregation, and chromosomal distribution. J. Virol. 40, 735–744.

Weremowicz, S., Fox, E.A., Morton, C.C., Vallee, B.L. (1990). Localization of the human angiogenin gene to chromosome band 14q11, proximal to the T cell receptor alpha/delta locus. Am. J. Hum. Genet. 47, 973–981.

Yang-Feng, T.L., Swaroop, A. (1992). Neural retina-specific leucine zipper gene NRL (D14S46E) maps to human chromosome 14q11.1-q11.2. Genomics 14, 491–492.