Taq I, Eco RI, Bgl II, and Pst I, but not in DNA of normal colon from the same patient.
23. Sequences of the intron-exon borders of the 17 exons containing the coding region of MCC, and the primers used for amplifying these sequences, are available from the authors upon request or electronically from the NCBI data repository by anonymous ftp from the pub/apc directory at ncbi.nlm.nih.gov (numerical address 130.14.20.1). The eight exons examined in FAP patients included those containing MCC nt 392 to 1195, 1576 to 1863, and 2306 to 2406. PCR and RNase protection analysis were performed as described (*21*).
24. Sequences of the intron-exon borders of the eight exons containing APC nucleotides (nt) 835 to 8960, and the primers used for amplifying these sequences, are available from the authors upon request or electronically from the NCBI data repository by anonymous ftp from the pub/apc directory at ncbi.nlm.nih.gov (numerical address 130.14.20.1). The entire 3′ end of the APC cDNA (nt 1959 to 8955) was encoded in one exon, as indicated by restriction endonuclease mapping and sequencing of cloned genomic DNA. The three exons examined in FAP patients included those containing nt 835 to 933, nt 934 to 1312, and the first 300 nt of the large 3′ exon (nt 1959 to 2259).
25. I. Nishisho *et al.*, unpublished data.
26. R. M. Myers and T. Maniatis, *Cold Spring Harbor Symp. Quant. Biol.* **51**, 275 (1986).
27. PCR products were cloned into a Bluescript vector modified as described in T. A. Holton and M. W. Graham, *Nucleic Acids Res.* **19**, 1156 (1991). A minimum of 100 clones were pooled and sequenced, as described (*16*).
28. J. Bonadio, F. Ramirez, M. Barr, *J. Biol. Chem.* **265**, 2262 (1990); C. Beldjorn *et al.*, *Nucleic Acids Res.* **16**, 4927 (1988); H.-c. Kuo, F.-u. Nasim, P. J. Grabowski, *Science* **251**, 1045 (1991).
29. The exons examined included nt 835 to 933, 934 to 1312, and 1409 to 1548.
30. A cDNA library was made from the RNA of SW480 cells by the technique described by U. Gubler and B. J. Hoffman in *Gene* **25**, 263 (1983) and cloned into a Lambda Zap vector (Stratagene). The nucleotide sequence of the entire coding region of MCC and 70% of the APC coding region were determined from cDNA clones derived from this library.
31. DNA was purified from paraffin sections as described in S. E. Goelz, S. R. Hamilton, B. Vogelstein, *Biochem. Biophys. Res. Commun.* **130**, 118 (1985). PCR was as described (*21*), with the primers 5′-GTTCCAGCAGTGTCACAG-3′ and 5′-GGGAGATTTCGCTCCTGA-3′.
32. J. M. Bishop, *Cell* **64**, 235 (1990); E. J. Stanbridge, *Annu. Rev. Genet.* **24**, 615 (1990); R. A. Weinberg, *Cancer Res.* **49**, 3713 (1989).
33. E. Elmore, T. Kakunaga, J. C. Barrett, *Cancer Res.* **43**, 1650 (1983); W. S. Kendal and P. Frost, *ibid.* **46**, 6131 (1986); R. Seshardi, *ibid.* **47**, 407 (1987); D. Kaden *et al.*, *ibid.* **49**, 3374 (1989); L. A. Loeb, *ibid.* **51**, 3075 (1991).
34. J. Harwood, A. Tachibana, M. Meuth, *Mol. Cell. Biol.* **11**, 3163 (1991).
35. Analysis of colorectal cancers with anonymously derived probes has demonstrated that point mutations resulting in the creation of new restriction sites or in the ablation of existing sites occur at $<5 \times 10^{-6}$ per base pair [S. J. Baker *et al.*, *Science* **244**, 217 (1989); B. Vogelstein *et al.*, *ibid.*, p. 207]. The prevalence of "random" point mutations can also be estimated from the frequency of tumors that contain two separate point mutations within the same allele. It can be assumed that even if one such point mutation is functional, the other is more likely to be inconsequential. Sequencing studies of the p53 gene in colorectal and other human cancers [reviewed in M. Hollstein, D. Sidransky, B. Vogelstein, C. C. Harris, *Science* **253**, 49 (1991)] have revealed only one case in which two such mutations occurred, and in this case the second "mutation" may have been a germ line variant rather than a somatic mutation [S. J. Baker *et al.*, *Cancer Res.* **50**, 7717 (1990)]. Because the aggregate number of base pairs sequenced in these tumors was $>2 \times 10^5$, the frequency of such mutations was $<5 \times 10^{-6}$ per base pair.
36. From the number of base pairs examined, corrected for the sensitivity of mutation detection (35% for RNase protection analysis and 100% for sequencing of pooled clones), the prevalence of point mutations was estimated to be 6×10^{-5} per base pair and 4×10^{-5} per base pair in the exons and flanking intronic sequences of MCC and APC, respectively.
37. W. R. Waddell and R. W. Loughry, *J. Surg. Oncol.* **24**, 83 (1983); W. R. Waddell, G. F. Gansen, E. J. Cerice, R. W. Loughry, *Am. J. Surg.* **157**, 174 (1989).
38. M. Leppert *et al.*, *N. Engl. J. Med.* **322**, 904 (1990).
39. I. Chiba *et al.*, *Oncogene* **5**, 1603 (1990).
40. Supported in part by grants from the Minister of Education, Culture and Science, the Princess Takamatsu Cancer Research Fund, the Uehara Memorial Foundation, the Kato Memorial Bioscience Foundation, the Damon Runyon–Walter Winchell Cancer Fund, the Clayton Fund, The McAshan Fund, and NIH grants CA06973, CA35494, CA44688, GM07309, and GM07184. The authors thank T. Gwiazda for expert preparation of the manuscript; J. M. Jessup, J. Willson, J. Trent, M. Brattain, C. Paraskeva, B. Boman, and M. Schwab for providing tumors and cell lines; S. Booker for help with organizing the Johns Hopkins polyposis registry; and R. White and colleagues for exchanging information prior to publication.

13 June 1991; accepted 3 July 1991

Genes for Epilepsy Mapped in the Mouse

MATTHEW L. RISE, WAYNE N. FRANKEL, JOHN M. COFFIN, THOMAS N. SEYFRIED*

The neurological mutant mouse strain El is a model for complex partial seizures in humans. The inheritance of epileptic seizures with seven conventional chromosomal markers and over 60 endogenous proviral markers was studied by means of backcrosses of El with two seizure-resistant strains, DBA/2J and ABP/LeJ. The major gene responsible for this epileptic phenotype (*El-1*) was localized to a region distal with respect to the centromere on chromosome 9. At least one other gene, *El-2*, linked to proviral markers on chromosome 2, also influences the seizure phenotype. In addition, a potential modifier of seizures was detected in the DBA/2J background. The location of *El-1* on distal chromosome 9 may allow identification of an epilepsy candidate gene in humans on the basis of conserved synteny with human chromosome 3.

EPILEPSY IS A SIGNIFICANT HEALTH problem in humans and, with the exception of stroke, it is the most common human neurological affliction. The epilepsies have been divided into two broad etiological categories: (i) idiopathic epilepsy, which includes those convulsive disorders that are genetically determined but cannot be associated with observable structural or biochemical pathology, and (ii) symptomatic epilepsy, which includes those disorders arising from definable injury, disease, or neurostructural abnormality (*1–3*). The phenomenon of genetic heterogeneity has hindered attempts to understand the modes of inheritance (single gene or polygenic) of various idiopathic epilepsies in humans (*4, 5*).

The El (epilepsy) mouse, discovered in 1954 (*6*), is considered a genetic model for human temporal lobe epilepsies or complex partial seizures with secondary generalization (*7*). El seizures can occur spontaneously at 80 to 100 days of age or can be induced at earlier ages by vestibular stimulation (*8–11*). The seizures in El mice appear to originate in the hippocampus or deep temporal lobe structures and then spread to other brain regions (*9*). El seizures are accompanied by electroencephalographic abnormalities, loss of postural equilibrium, urinary incontinence, excessive salivation, and head, limb, and chewing automatisms (*8, 10, 11*) resembling temporal lobe seizures in humans (*12*). El mice also display a seizure-associated hippocampal gliosis that is not associated with an observable neuronal loss (*13*). Phenytoin and phenobarbitol, the anticonvulsant drugs of choice for the treatment of human partial epilepsies, can also inhibit seizure activity in El mice (*14, 15*). The genetic basis of the El seizures has not been clearly established (*16*).

We outcrossed El mice to either ABP/LeJ (ABP) or DBA/2J (D2) mice (*17*) to study the segregation of El seizure genes. ABP and D2 are suitable controls because they do not seize spontaneously and have low susceptibilities to seizures induced by vestibular stimulation (Fig. 1). At 30 days of age, all parental, F1, and backcross mice were tested by vestibular stimulation twice each week for 10 weeks (for a total of 20 tests). The relative frequency of seizures was used for assessment of seizure susceptibility. The F1 mice were reciprocally backcrossed to the respective ABP or D2 parents. The progeny of both backcrosses showed a somewhat continuous distribution of seizure susceptibility (Fig. 1), indicating that multiple genetic factors influence the manifestation of

M. L. Rise and T. N. Seyfried, Department of Biology, Boston College, Chestnut Hill, MA 02167.
W. N. Frankel and J. M. Coffin, Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, MA 02111.

*To whom correspondence should be addressed.

El seizures. In addition, El seizure susceptibility appears to be inherited as a partially dominant trait because all F1 mice were susceptible to seizures, but to a lesser degree than the El parents (Fig. 1).

To identify genes responsible for this complex pattern of inheritance, we used a multilocus method of gene mapping (*18, 19*) where many independent marker loci were scored simultaneously. In addition to analyzing several conventional markers, we used a large, dispersed series of polymorphic DNA markers, the nonecotropic murine leukemia proviruses (MLV) (*20*) (Fig. 2). Most of the MLV proviruses in the common inbred strains have now been mapped (*20, 21*) and, as our study progressed, we found that about two-thirds of the endogenous proviruses in El mice were identical to those in the common strains. To map epilepsy genes, three conventional markers (*b, d,* and *Bgl*) and 41 proviruses were scored in the backcross to the D2 parent, and six conventional markers (*b, se, Bgl, wa-1, p,* and *bt*) and 38 proviruses were scored in the backcross to the ABP parent (*22*). By examining both crosses, we covered most regions in all but one of the 19 mouse autosomes.

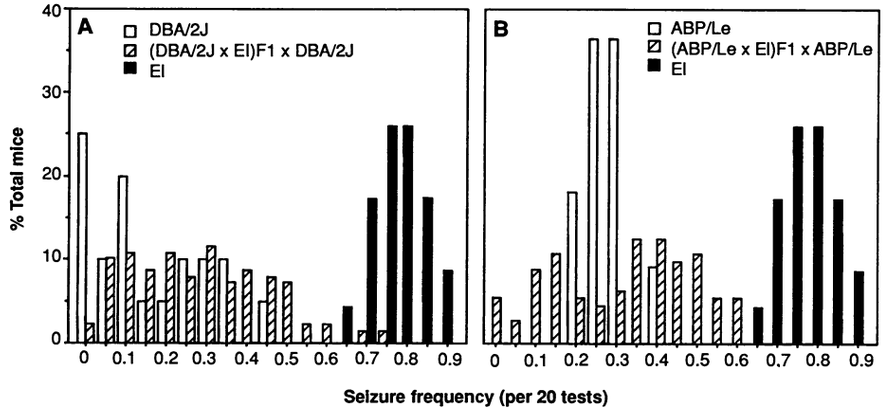

Fig. 1. Frequency distributions for seizure susceptibility in the parental strains (El, D2, and ABP) and in the D2 backcross and ABP backcross populations. Seizure susceptibility was taken as the frequency of seizure per 20 tests (vestibular stimulations), administered twice each week beginning at 30 days of age. The progeny in both backcrosses displayed continuous variation with regard to seizure susceptibility. The mean seizure frequencies ± SD and total numbers of mice tested per class (in parentheses) were as follows: D2, 0.155 ± 0.141 (20); ABP, 0.273 ± 0.056 (11); El, 0.780 ± 0.067 (23); D2 backcross, 0.282 ± 0.173 (139); and ABP backcross, 0.320 ± 0.171 (112). The values for the reciprocal F1 hybrids (data not plotted) were as follows: ElD2 F1, 0.566 ± 0.150 (19) and ElABP F1, 0.531 ± 0.075 (*16*).

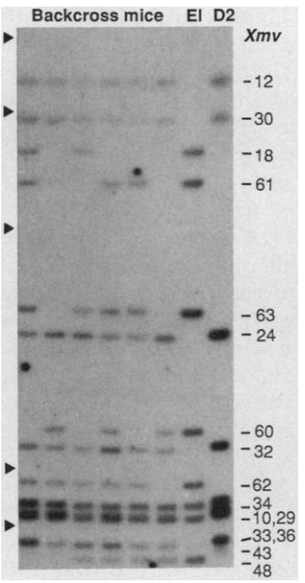

Fig. 2. Segregation of endogenous *Xmv* proviruses in backcross mice. Spleen DNAs from El, D2, and selected D2 backcross progeny were digested with Pvu II, separated by electrophoresis in agarose gels, and hybridized to radiolabeled oligonucleotide probes identifying xenotropic (*Xmv*) proviruses, as described (*20, 35–37*). Modified polytropic (*Mpmv*) and polytropic (*Pmv*) proviruses, along with ABP and ABP backcross mice, were analyzed in a similar fashion (*27*). Fragments are numbered as proviral loci at the right of each panel. Proviruses specific to El mice were scored as one-versus-zero copy (either the provirus-positive or the provirus-null allele inherited from the F1); those from D2 were scored by gene dosage with the use of fragments common to both strains (*Xmv-34* in this figure) as internal homozygous standards. Proviruses that could not be scored in the Pvu II digest, due to fragment comigration, were scored in an Eco RI digest (*27*). Whenever possible, segregation patterns were confirmed with both digests. All D2 proviruses were identified previously; proviruses of El or ABP mice were identified by the sizes and segregation of junction fragments in both backcrosses. *Xmv-48* (Pvu II, 1.86 kb; Eco RI, 6.50 kb), *Xmv-60* (Pvu II, 2.65 kb; Eco RI, 7.60 kb), *Xmv-61* (Pvu II, 5.60 kb; Eco RI, 3.96 kb), *Xmv-62* (Pvu II, 2.40 kb; Eco RI, 3.40 kb), *Xmv-63* (Pvu II, 3.56 kb; Eco RI, 7.00 kb), *Xmv-64* (Pvu II, 3.85 kb; Eco RI, 2.01 kb) (*27*), *Mpmv-43* (Pvu II, 3.00 kb; Eco RI, 3.10 kb) (*27*), *Mpmv-44* (Pvu II, 2.75 kb; Eco RI, 2.51 kb) (*27*), and *Pmv-68* (Pvu II, 9.20 kb; Eco RI, 2.11 kb) (*27*) are El proviruses that have not been previously defined in other inbred strains. Arrowheads show positions of Hind III–digested λ DNA molecular size standards: 9.4 kb, 6.6 kb, 4.3 kb, 2.3 kb, and 2.0 kb.

We initially used two independent tests to analyze the association between seizure susceptibility and the various genetic markers in the backcrosses. In the first test, mean seizure frequencies for F1-like (heterozygous) and recurrent parental-like (homozygous) mice were calculated at each marker locus and compared by *t* test (Table 1). If a given genetic marker and El seizure susceptibility segregated independently, the mean seizure frequencies of the F1-like and parental-like marker phenotypes would be approximately equal, and the *t* value would be insignificant. In the second test, median seizure frequencies of 0.275 and 0.325 were used as thresholds in the D2 and ABP backcrosses, respectively (Fig. 1 and Table 2). In each backcross, mice with seizure frequency values below the threshold were designated "seizure-resistant," whereas mice with values above the threshold were designated "seizure-susceptible." If a genetic marker and seizure susceptibility segregated independently, the numbers of backcross mice in each genotypic class would be approximately equal. Given linkage between seizure susceptibility and a marker locus, the difference between "parentals" and "recombinants" (analyzed by a χ^2 test) would be significant. To reduce the number of false positive associations, we selected $P < 0.01$ as the minimum level of significance (*23*).

Significant χ^2 values or *t* values in these tests suggested several chromosomal locations of genes influencing the El seizure phenotype (Tables 1 and 2). Strong associations were observed between seizure susceptibility and several chromosome 9 markers in both backcrosses (Tables 1 and 2). Significant χ^2 values and *t* values (at $P < 0.001$) corresponded to the chromosome 9 markers *Mpmv-27, se, d,* and *Bgl*. These data indicate that a major gene affecting El seizure susceptibility, which we designate *El-1*, lies towards the distal region of mouse chromosome 9.

An interval-mapping procedure was used to position *El-1* relative to the chromosome 9 markers. This involved a direct comparison of the mean seizure frequencies for the parental and recombinant haplotypes in the 24-centimorgan (cM) interval between the *d-se* locus and the locus for β-galactosidase (*Bgl*). In the ABP backcross, the mean seizure frequencies of both recombinant classes [0.33 for *se/se*, high β-galactosidase activity; 0.32 for *+/se,* low *Bgl*] fell between the mean seizure frequencies of the parental classes (0.23 for *se/se,* low *Bgl*; 0.38 for *+/se,* high *Bgl*). Likewise, in the D2 backcross the mean seizure frequencies of both recombinant classes (0.30 for *d/d,* high *Bgl*; 0.36 for *+/d,* low *Bgl*) fell between the mean seizure frequencies of the parental

classes (0.20 for *d/d*, low *Bgl*; 0.37 for *+/d*, high *Bgl*; Table 1). These data suggest that *El-1* maps between *d-se* and *Bgl*.

The apparent recombination frequencies between *El-1* and either the *d-se* complex (about 0.297, Table 2) or the *Bgl* locus (about 0.262) were much greater than expected if *El-1* were located within the 24-cM interval between the markers. This is presumably due to the segregation of unlinked seizure-modifying genes that distort the difference between the true and apparent recombination frequencies. Because the true and apparent recombination frequencies between a marker locus and a complex phenotypic trait are proportional (*24*), we estimate that *El-1* is located 13 cM distal to *d-se* {24[0.297/(0.297 + 0.262)]} and 11 cM proximal to *Bgl* on mouse chromosome 9.

We also analyzed these data with the MAPMAKER–Quantitative Trait Locus (QTL) computer program (*18, 25*). This program divides the genome into intervals between mapped loci and then derives maximum likelihood estimates across each interval for the presence of a QTL. The peak of the *El-1* likelihood curve is located in about the same place as the map position we deduced above, that is, midway between the *d-se* and *Bgl* loci (Fig. 3). Log likelihood (LOD) scores of 4.43 and 5.88 in the ABP and D2 backcrosses, respectively, are well above LOD 2.4, which represents an appropriate level of significance for MAPMAKER-QTL (*18*).

A highly significant association was also observed between seizures and the chromosome 2 marker *Mpmv-28* (*26*) in the ABP backcross (Tables 1 and 2). This association was significant at $P < 0.001$ using both the χ^2 test and the *t* test. Furthermore, the MAPMAKER-QTL maximum LOD of 5.20 peaks at *Mpmv-28*, which was the most distal marker used (*27*). These data are consistent with a chromosome 2–associated seizure gene in the El strain, which we designated *El-2*. The lack of a strong association with *Mpmv-28* in the D2 cross was

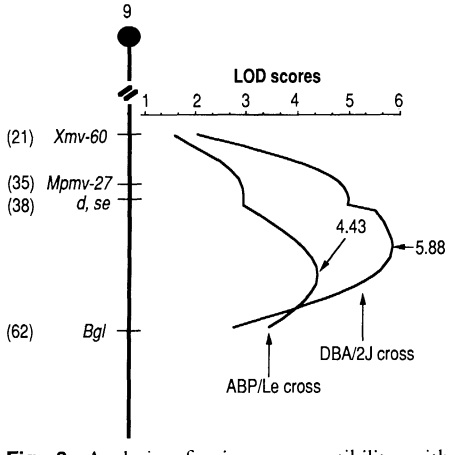

Fig. 3. Analysis of seizure susceptibility with chromosome 9 markers using the MAPMAKER-QTL program (*18, 19, 25*). Consensus map positions are shown in parentheses to the left of the markers *Xmv-60, Mpmv-27, d, se,* and *Bgl*. The centromere is represented as a ball on the end of the chromosome. The LOD scores and curves generated by the MAPMAKER-QTL computer program are shown for the ABP and D2 backcrosses. The peaks at 4.43 and 5.88 indicate that a major gene for seizures (*El-1*) is located about halfway between the *d-se* and *Bgl* loci.

Table 1. Association of seizure frequency with conventional and proviral genetic markers. Results from the D2 backcross are shown on the left side of the table; those from the ABP backcross are on the right. Listed by chromosome (Chr) are *Mpmv, Pmv,* and *Xmv* nonecotropic proviruses and their strain origins, as well as conventional markers *b* (brown), *d* (dilute), *Bgl* (β-galactosidase), *wa-1* (waved-1), *p* (pink-eyed dilution), *se* (short-ear), *bt* (belted). Also shown is *Mtv-11,* assayed as described (*34*). *E* denotes the number of backcross mice heterozygous for the El allele of a given marker, and *A* and *D* denote the number of backcross mice homozygous for ABP or D2 alleles, respectively. M^e, M^a, and M^d, respectively, are mean seizure frequency values for mice of these genotypes at a given marker locus. The absolute value of *t* calculated with student's *t* test for the difference between mean seizure frequencies is denoted $|t|$; an asterisk shows significance at the $P < 0.01$ level, and a double asterisk shows significance at the $P < 0.001$ level. Newly mapped proviruses, marked with a superscript, x, are as follows ($\underline{X} \pm$ SE is shown in centimorgans): Chr 2, *Mpmv-3–Pmv-7*, 8/54 recombinants (14.81 ± 4.83); *Mpmv-43–Pmv-7*, 5/54 recombinants (9.26 ± 3.94), *Xmv-48–Mpmv-43*, 2/77 recombinants (2.6 ± 1.81). Chr 4, *b–Mpmv-44*, 2/77 recombinants (2.61 ± 1.81). Chr 6, *Xmv-61–Xmv-24*, 1/44 recombinants (2.27 ± 2.25). Chr 8, *Xmv-12–Xmv-62*, 7/84 recombinants (8.33 ± 3.02). Chr 9, *Xmv-60–se*, 9/66 recombinants (13.64 ± 4.22); *Xmv-60–d*, 17/87 recombinants (19.54 ± 4.25). Chr 11, *Xmv-63–Pmv-2*, 4/81 recombinants (4.94 ± 2.41). Backcross data for previously mapped proviruses have been published (*21*).

Chr	Locus	Provirus origin	D	E	Mean seizure frequency M^d	M^e	$\|t\|$
1	*Mpmv-16*	D2	39	48	0.25	0.35	1.91
1	*Xmv-32*	D2	39	48	0.26	0.35	1.91
1	*Mpmv-25*	D2	35	48	0.27	0.34	1.11
2	*Xmv-48*^{x}	EI	37	50	0.25	0.37	3.04 *
2	*Mpmv-43*^{x}	EI	38	49	0.26	0.37	2.85 *
2	*Mpmv-28*		41	46	0.28	0.35	1.76
3	*Pmv-38*	D2	30	31	0.35	0.30	1.05
3	*Pmv-39*	D2	31	32	0.33	0.31	0.33
4	*b*		71	68	0.32	0.24	2.77 *
4	*Mpmv-44*^{x}	EI	42	35	0.36	0.26	2.18
4	*Pmv-23*	D2	25	19	0.38	0.30	1.35
4	*Pmv-19*	EI	35	28	0.37	0.26	2.25
4	*Mpmv-19*	D2	24	20	0.37	0.32	0.95
4	*Pmv-25*	EI	29	32	0.32	0.33	0.21
5	*Pmv-40*	D2	35	28	0.32	0.33	0.22
5	*Mpmv-23*	D2	48	39	0.31	0.32	0.01
6	*Xmv-61*^{x}	EI	33	40	0.33	0.31	0.40
6	*Xmv-24*^{x}	D2	42	45	0.32	0.31	0.32
7	*Pmv-4*	D2	32	30	0.31	0.33	0.28
7	*Xmv-30*	D2	43	44	0.32	0.31	0.18
7	*Xmv-33*	D2	19	24	0.30	0.38	1.39
7	*Pmv-31*	EI	28	35	0.32	0.32	0.16
8	*Xmv-12*^{x}	D2	44	43	0.35	0.28	1.68
8	*Xmv-62*^{x}	EI	50	37	0.34	0.29	1.34
8	*Mpmv-21*	D2	49	37	0.33	0.30	0.68
9	*Xmv-60*^{x}	EI	38	49	0.27	0.35	2.02
9	*Mpmv-27*	EI	38	49	0.24	0.38	3.76 **
9	*d*		68	71	0.21	0.35	5.26 **
9	*Bgl*		53	54	0.25	0.35	3.07 *
10	*Mpmv-12*	D2	45	42	0.32	0.32	0.03
11	*Xmv-63*^{x}	EI	43	44	0.32	0.32	0.05
11	*Pmv-2*	D2	31	30	0.36	0.30	1.24
11	*Pmv-22*	EI	20	24	0.37	0.33	0.70
11	*Mpmv-15*	EI	45	32	0.32	0.31	0.13
12	*Mpmv-11*	D2	20	22	0.32	0.36	0.57
12	*Mpmv-24*	D2	19	24	0.38	0.32	1.00
13	*Pmv-41*	D2	31	29	0.32	0.34	0.30
14	*Mtv-11*	D2	34	42	0.25	0.36	2.59
15	*Pmv-42*	D2	29	34	0.30	0.34	0.64
16	*Pmv-35*	D2	28	32	0.31	0.32	0.21
16	*Pmv-14*	EI	32	31	0.33	0.31	0.31
18	*Xmv-29*	D2	41	45	0.32	0.32	0.01
18	*Pmv-20*	D2	32	31	0.30	0.34	0.72
19	*Xmv-18*	EI	35	38	0.30	0.33	0.86
un	*Pmv-45*	EI	34	28	0.33	0.31	0.44
un	*Pmv-68*	EI	27	36	0.30	0.33	0.59

Chr	Locus	Provirus origin	A	E	Mean seizure frequency M^a	M^e	$\|t\|$
1	*Bxv-1*	ABP	29	36	0.31	0.34	0.72
2	*Mpmv-3*^{x}	EI	38	24	0.31	0.35	0.83
2	*Pmv-7*^{x}	ABP	28	26	0.30	0.36	1.32
2	*Mpmv-43*^{x}	EI	31	31	0.27	0.37	2.29
2	*Mpmv-28*		25	36	0.22	0.40	4.68 **
3	*Pmv-39*	ABP	37	25	0.31	0.33	0.43
3	*Pmv-26*	EI	34	28	0.30	0.35	0.97
3	*Mpmv-9*	EI	33	28	0.30	0.35	1.14
4	*Pmv-30*	EI	31	24	0.32	0.35	0.74
4	*b*		68	44	0.33	0.30	1.00
4	*Mpmv-44*^{x}	EI	37	21	0.36	0.29	1.31
4	*Pmv-19*	EI	31	25	0.36	0.26	2.15
4	*Pmv-25*	EI	27	27	0.33	0.29	0.66
4	*Xmv-8, 14, 44*	ABP	33	33	0.34	0.31	0.66
5	*Xmv-17*	ABP	28	31	0.36	0.31	0.95
5	*Xmv-34*	EI	32	34	0.35	0.30	1.27
5	*Mpmv-13*	ABP	31	33	0.35	0.30	1.27
5	*Pmv-11*	ABP	27	35	0.37	0.28	1.98
6	*wa-1*		54	58	0.37	0.28	2.87 *
6	*Xmv-61*^{x}	EI	36	30	0.31	0.34	0.63
7	*Pmv-18*	EI	21	38	0.37	0.30	1.39
7	*Pmv-15*	ABP	16	25	0.37	0.25	2.24
7	*Xmv-30*	ABP	24	36	0.38	0.31	1.15
7	*p*		45	67	0.31	0.32	0.29
7	*Pmv-31*	EI	20	38	0.30	0.34	0.74
8	*Xmv-26*	ABP	34	29	0.33	0.32	0.27
8	*Xmv-62*^{x}	EI	36	30	0.33	0.32	0.21
9	*Xmv-16*	ABP	29	29	0.27	0.40	2.87 *
9	*Xmv-60*^{x}	EI	32	34	0.27	0.38	2.48
9	*se*		54	58	0.26	0.38	3.79 **
9	*Bgl*		41	62	0.24	0.36	3.88 **
10	*Mpmv-26*	ABP	33	32	0.27	0.39	2.62
11	*Mpmv-18*	ABP	32	24	0.32	0.36	0.71
11	*Mpmv-4*	ABP	31	26	0.31	0.37	1.08
11	*Xmv-42*	ABP	28	30	0.32	0.35	0.64
11	*Mpmv-8*	ABP	28	29	0.33	0.34	0.30
11	*Mpmv-15*	EI	25	35	0.32	0.34	0.38
12	*Pmv-37*	ABP	33	28	0.33	0.32	0.30
13	*Xmv-13*	ABP	34	32	0.30	0.35	1.34
15	*Pmv-17*	EI	30	33	0.34	0.31	0.58
15	*bt*		50	62	0.32	0.32	0.12
15	*Pmv-42*	ABP	24	34	0.31	0.32	0.08
16	*Pmv-16*	ABP	34	26	0.32	0.34	0.52
18	*Pmv-20*	ABP	29	33	0.28	0.35	1.54
un	*Pmv-45*	EI	29	26	0.31	0.42	1.29
un	*Pmv-68*	EI	24	30	0.29	0.36	1.46
un	*Xmv-64*	ABP	20	24	0.34	0.25	1.68

Table 2. Association of seizure susceptibility with conventional and proviral genetic markers. The median frequency of seizure between the parental and F1 genotypes was used to distinguish seizure-resistant from seizure-susceptible mice in the backcross generation. Only those markers are included that have χ^2 values or *t* test (Table 1) values significant at the $P < 0.01$ level or better. The χ^2 values with one degree of freedom are for parental versus recombinant classes. Shown is the genotype at the marker locus (M) or the putative seizure locus (S), with "+" allele from either the D2 or ABP parent, depending on the cross, and "e" from the El parent. Deduced seizure genotypes are based on the threshold model described in the text. "Parentals" and "recombinants" refer to mice that fall into these categories assuming linkage between seizure susceptibility and the marker locus. Also shown are apparent recombination frequencies (rec freq) between the marker locus and seizures. At the brown (*b*) locus, DBA is *b/b* and El is +/+. (ABP is also *b/b*, but we did not observe significant association between seizure susceptibility and *b* in the cross.) At the waved-1 (*wa-1*) locus, ABP is *wa-1/wa-1* and El is +/+. At the short-ear (*se*) locus, ABP is *se/se* and El is +/+. At the dilute (*d*) coat color locus, D2 is *d/d* and El is +/+. At the *Bgl* locus, both D2 and ABP have low activity and El has high activity (*22*).

Chr	Locus	Back-cross	Parental		Recombinant		Apparent rec freq	χ^2 (1 df)
			M^+S^+	M^eS^e	M^+S^e	M^eS^+		
2	*Mpmv-28*	ABP	15	25	12	9	0.333	11.97
4	*b*	D2	26	24	45	44	0.640	10.95
4	*Mpmv-44*	D2	7	9	27	20	0.746	14.29
4	*Pmv-19*	D2	9	10	26	18	0.698	9.94
6	*wa-1*	ABP	15	26	39	32	0.634	8.04
9	*Xmv-16*	ABP	17	23	12	6	0.310	8.36
9	*Mpmv-27*	D2	25	36	13	13	0.299	14.09
9	*se*	ABP	35	46	19	12	0.277	22.33
9	*d*	D2	47	48	21	23	0.317	18.72
9	*Bgl*	ABP	30	47	11	15	0.252	25.26
9	*Bgl*	D2	36	42	17	12	0.271	22.45

confounding, but it could result from seizure modifiers that differ in the genetic backgrounds of the control strains. Suggestive associations found with the use of the *t* test (Table 1; $P < 0.01$) and MAPMAKER-QTL (LOD = 2.22; *27*) with a more proximal chromosome 2 marker, *Xmv-48,* support the possibility that *El-2* has a measurable effect in the D2 cross, but that the chromosomal position is obscured by background modifiers.

Significant negative associations were observed in the D2 backcross, but not in the ABP backcross, between seizure susceptibility and several chromosome 4 markers, including *b* (Tables 1 and 2). This effect was expressed as a lower ratio of parental to recombinant types than would be expected for independent gene assortment (Table 2). Furthermore, backcross mice homozygous for *b* had significantly higher mean seizure susceptibilities than backcross mice heterozygous at this locus (Table 1). Although associations at $P < 0.01$ or better were found with the *b* locus with both conventional tests, the best score obtained with the MAPMAKER-QTL test was 1.66, which is merely suggestive. Although it is premature to speculate on the exact mode of inheritance, this modifier could be either a seizure-resistance gene from El or an enhancer from D2. Neumann and Seyfried reported tight linkage between the *b* locus and *Asp-2,* a modifier of audiogenic seizure susceptibility (*28*). Thus, there is a possibility that *Asp-2* and this modifier of *El-1* are the same gene, which acts as a general modifier of seizure susceptibility.

In sweeping greater than 80% of the mouse genome, we identified at least two genes, *El-1* and *El-2*, that contribute significantly to the El seizure phenotype. When these two QTLs are considered together in the ABP backcross, over 50% of the phenotypic variance can be explained with MAPMAKER-QTL. However, this also suggests that undetected seizure genes still exist. Potential associations were observed in the ABP backcross with *wa-1* on chromosome 6 and *Mpmv-26* on chromosome 10, and with one marker in the D2 backcross, *Mtv-11*, on chromosome 14 (Tables 1 and 2). The significance of these results can be further tested in the future with additional DNA markers for these sparsely marked regions.

The importance of mapping epilepsy genes in mice lies in the application of this knowledge to the human condition. Approximately 37% of the mouse autosomal genome shows synteny with the human autosomal genome, and gene orders in the syntenic segments are frequently conserved (*29, 30*). Comparative genetic maps between man and mouse have been used for prediction of locations of human disease genes on the basis of their locations in the mouse genome (*30, 31*). Because the region of mouse chromosome 9 that most likely contains *El-1* is highly syntenic with human chromosome 3, it may be possible to determine whether some inherited forms of human epilepsy are determined by syntenic genes in this region. The finding that zinc deprivation results in increased seizure susceptibility in El mice (*32*) implies that proteins with zinc-binding capability could be candidates for the *El-1* defect. Although no such genes have yet been mapped to distal mouse chromosome 9 or human chromosome 3, several heavy metal–binding proteins are encoded by syntenic genes in these regions, including *Cp* (ceruloplasmin-copper), *Ltf* (lactotransferrin-iron), and *Trf* (transferrin-iron) (*33*). More refined genetic mapping should allow us to test the significance of this observation and to determine the feasibility of chromosomal walking to *El-1* with existing DNA probes as landmarks.

REFERENCES AND NOTES

1. T. N. Seyfried, in *The Mouse in Biomedical Research,* H. L. Foster, J. D. Small, J. G. Fox, Eds. (Academic Press, New York, 1982), pp. 97–204.
2. W. A. Hauser, in *Genetic Basis of the Epilepsies,* V. E. Anderson, W. A. Hauser, J. K. Penry, C. F. Sing, Eds. (Raven, New York, 1982), pp. 3–9.
3. R. J. Baumann, in *ibid.*, pp. 11–20.
4. V. E. Anderson, W. A. Hauser, S. S. Rich, in *Advances in Neurology*, A. V. Delgado-Escueta, D. M. Treiman, A. A. Ward, Jr., D. M. Woodbury, R. J. Porter, Eds. (Raven, New York, 1986), pp. 59–75.
5. A. V. Delgado-Escueta, D. M. Treiman, G. O. Walsh, *N. Engl. J. Med.* **308**, 1508 (1983).
6. K. Imaizumi *et al.*, *Exp. Anim. (Tokyo)* **8**, 6 (1959).
7. T. N. Seyfried and G. H. Glaser, *Epilepsia* **26**, 143 (1985).
8. J. Suzuki, *Experientia* **32**, 336 (1976).
9. ———, Y. Nakamoto, Y. Shinkawa, *Brain Res.* **266**, 359 (1983).
10. T. N. Seyfried, G. H. Glaser, R. K. Yu, S. T. Palayoor, in *Advances in Neurology,* A. V. Delgado-Escueta, D. M. Treiman, A. A. Ward, Jr., D. M. Woodbury, R. J. Porter, Eds. (Raven, New York, 1986), pp. 115–133.
11. M. Kurokawa, H. Naruse, M. Kato, *Prog. Brain Res.* **21a**, 112 (1966).
12. G. H. Glaser, *Pract. Med.* **10**, 1 (1977).
13. J. V. Brigande, A. Wieraszko, M. D. Albert, T. N. Seyfried, *Epilepsia* **30**, 652 (1989).
14. T. Honda, *Brain Dev.* **6**, 22 (1984).
15. E. Sugaya *et al.*, *Epilepsia* **27**, 354 (1986).
16. Y. Fueta, S. Matsuoka, T. Mita, *J. UOEH* (suppl.) **8**, 417 (1986).
17. The inbred El mice we used were obtained originally as a gift from J. Suzuki, Psychiatric Research Institute of Tokyo, and are now maintained in the Boston College animal facility. The ABP/LeJ and DBA/2J strains were purchased from JAX (Bar Harbor, Maine).
18. E. S. Lander and D. Botstein, *Genetics* **121**, 185 (1989).
19. A. H. Paterson *et al.*, *Nature* **335**, 721 (1988).
20. W. N. Frankel, J. P. Stoye, B. A. Taylor, J. M. Coffin, *Genetics* **124**, 221 (1990).
21. W. N. Frankel *et al.*, *Mouse Genome* **89**, 266 (1991).
22. El tissues exhibit high β-galactosidase activity, whereas both ABP and D2 tissues have low concentrations of the enzyme. Because this activity is inherited in a simple Mendelian fashion, the coinheritance of seizure susceptibility with *Bgl* in the D2 backcross and the ABP backcross could be analyzed. β-galactosidase concentrations in the liver tissues of parental, F1, and backcross mice were assayed according to the procedure of J. Felton, M. Meisler,

and K. Paigen [*J. Biol. Chem.* **249**, 3267 (1974)].
23. At face value, $P < 0.05$ is not stringent enough for this study because for ~100 markers one expects at least five positives to occur by chance alone. Similarly, at the $P < 0.01$ level, a few false positives could occur. Thus, we mainly describe results that (i) were significant at $P < 0.001$, or (ii) were significant at $P < 0.01$ and were confirmed by both statistical tests. Other associations are suggestive, pending further analysis with additional markers.
24. P. E. Neumann and R. L. Collins, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
25. E. S. Lander *et al.*, *Genomics* **1**, 174 (1987).
26. Although *Mpmv-28* was not scored initially in either cross because the provirus is not present in El, D2, or ABP mice, preliminary associations with proximal 2 markers in both crosses prompted us to score the *Mpmv-28* integration site as a standard restriction fragment length polymorphism by using a unique flanking cellular DNA probe (*27*).
27. M. L. Rise, W. N. Frankel, J. M. Coffin, T. N. Seyfried, unpublished data.
28. P. E. Neumann and T. N. Seyfried, *Behav. Genet.* **20**, 307 (1990).
29. J. H. Nadeau and B. A. Taylor, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 814 (1984).
30. J. H. Nadeau, *Trends Genet.* **5**, 82 (1989).
31. A. G. Searle *et al.*, *Genomics* **1**, 3 (1987).
32. M. Fukahori and M. Itoh, *Brain Res.* **529**, 16 (1990).
33. V. S. Baranov *et al.*, *Chromosoma* **96**, 60 (1987).
34. W. N. Frankel, C. Rudy, B. T. Huber, J. M. Coffin, *Nature* **349**, 526 (1991).
35. J. P. Stoye and J. M. Coffin, *J. Virol.* **62**, 168 (1988).
36. W. N. Frankel, J. P. Stoye, B. A. Taylor, J. M. Coffin, *ibid.* **63**, 1762 (1989).
37. ———, *ibid.*, p. 3810.
38. We thank J. Peterson and M. Cote for technical expertise, P. Neumann for valuable assistance and comments on mapping the epilepsy genes, E. Lander and S. Lincoln for advice and data analysis using MAPMAKER/QTL, and an anonymous reviewer for several helpful suggestions. This research was submitted to Boston College in partial fulfillment of the M.S. degree (M.L.R.) and was supported by PHS grants to T.N.S. (NS 23355) and NIH grants to J.M.C. (R35CA44385). W.N.F. is a special fellow of the Leukemia Society of America.

11 December 1990; accepted 26 April 1991

Alteration of the Phase and Period of a Circadian Oscillator by a Reversible Transcription Inhibitor

UMA RAJU, CONSTANTINOS KOUMENIS, MARTA NUNEZ-REGUEIRO, ARNOLD ESKIN*

A function for transcription in the mechanism of a circadian oscillator was investigated with the reversible transcription inhibitor 5,6-dichloro-1-β-D-ribobenzimidazole (DRB). Two-hour treatments with DRB shifted the phase of the circadian rhythm of the isolated eye of *Aplysia*, and continuous treatments of DRB lengthened the free running period of this rhythm. Camptothecin, an inhibitor of transcription that is structurally unrelated to DRB, had similar effects on the circadian rhythm. These results suggest that transcription may be part of the circadian oscillating mechanism.

A REMARKABLE PROPERTY OF CELLS is their ability to generate endogenous rhythms with periods close to 24 hours. For example, a small piece of a pineal organ exhibits a circadian rhythm of melatonin secretion (*1*). Elucidation of the oscillator mechanism responsible for such rhythms requires identification of its components and determination of the regulatory processes by which these components interact.

Progress is being made in the identification of putative components of circadian oscillators. Researchers have identified a number of proteins by tracing environmental entrainment information to the oscillator (*2*), by tracing output pathways back to the oscillator (*3*), and by looking for proteins that are rhythmically synthesized (*4*). Moreover, genes that alter circadian rhythms have been identified (*5*). Some candidates for the cellular regulatory processes that link the putative oscillator components are protein phosphorylation (*6, 7*), Ca^{2+} regulation (*7, 8*), and protein synthesis (*9*). Protein synthesis is the most widely implicated regulatory process. For example, several inhibitors of protein synthesis shift the phase and change the period of the circadian rhythm associated with the isolated eye of *Aplysia* (*10, 11*). Furthermore, inhibitors of protein synthesis block some effects of entraining agents on the *Aplysia* eye rhythm (*2*). These entraining agents also change the synthesis of a number of eye proteins (*2*). Experimental results that implicate translation are also consistent with the participation of transcription in the circadian oscillator mechanism.

The results of a number of recent studies indicate that changes in transcription regulate circadian rhythms (*12*). In addition, transcription inhibitors abolish circadian rhythms, but these findings were difficult to interpret because irreversible transcription inhibitors were used in these experiments (*13*). We have investigated the effects of a reversible inhibitor of transcription, 5,6-dichloro-1-β-D-ribobenzimidazole (DRB), on the circadian rhythm of spontaneous nerve impulses from the isolated eye of *Aplysia*. DRB inhibits the synthesis of heterogeneous nuclear RNA at the level of transcription initiation by interfering with the RNA polymerase II function (*14*).

Isolated *Aplysia* eyes were treated with DRB for 2 hours during circadian time (CT) 06 to 08 hours, and a dose-dependent shift in the phase of the circadian rhythm was observed (*15*) (Fig. 1A). Treatment with DRB at 10^{-7} M did not produce phase shifts, whereas 10^{-6} M and 10^{-5} M DRB produced delay phase shifts of 1.8 ± 0.2 hours ($n = 4$) and 4.4 ± 0.4 hours ($n = 4$), respectively. To examine the sensitivity of the rhythm to DRB throughout a circadian cycle, we generated a phase response curve (PRC) by treating isolated eyes with DRB (10^{-4} M) for 2 hours at different phases of the rhythm. The effects of DRB on the rhythm were phase-dependent (Fig. 1B).

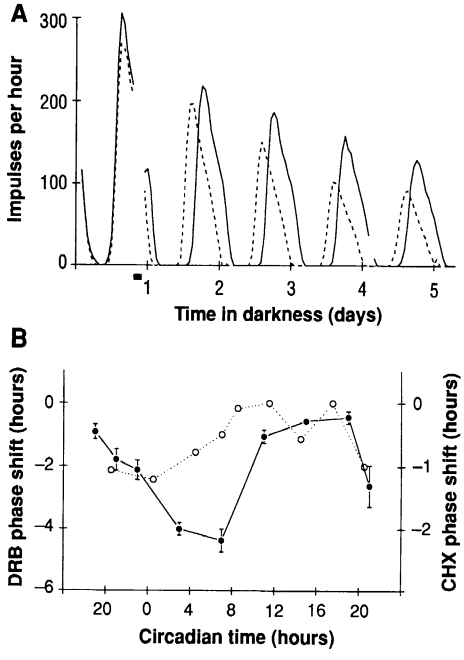

Fig. 1. Effect of DRB on the phase of the ocular circadian rhythm. (**A**) A delay shift in the phase of the rhythm was produced by a 2-hour treatment with DRB (10^{-4} M) during CT 06 to 08 hours, shown by the dark bar under the x axis (dashed line, control; solid line, DRB). The frequency of spontaneous optic nerve impulses from two isolated eyes of the same animal is plotted as a function of the time the eyes were in constant darkness. (**B**) Phase shifts of the rhythm are plotted as a function of the time of exposure of isolated eyes to DRB (closed circles). The error bars represent SEMs. The number of eyes exposed at different phases to DRB were the following: six (CT 18 to 20), five (CT 20 to 22), four (CT 22 to 24), four (CT 02 to 04), four (CT 06 to 08), four (CT 10 to 12), four (CT 14 to 16), five (CT 18 to 20, second cycle), and four (CT 20 to 22, second cycle). Data for the phase shifts of the rhythm produced by 1-hour CHX treatments (open circles) were derived from (*11*).

Department of Biochemical and Biophysical Sciences, University of Houston, Houston, TX 77204-5934.

*To whom correspondence should be addressed.