A Defective Ecotropic Provirus Closely Linked to the Albino Locus

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A congenic mouse strain (NFS.C) carrying the albino region of chromosome 7 from strain C58/Lw on an ecotropic virus-negative NFS background inherited a noninducible but apparently full-size provirus reactive with an ecotropic virus-specific probe. This unexpressed ecotropic provirus maps close to the albino locus but is distinct from the *Fgv-1* provirus located in the same region. The presence of this unique provirus in the albino region of chromosome 7 is potentially important, since it may provide a means of obtaining molecular clones of a chromosomal region, deletions of which are involved in profound metabolic, reproductive, and embryological abnormalities.

The chromosomally integrated DNA copies of ecotropic (mouse-tropic) retroviruses are inherited in many laboratory strains of mice. These inherited ecotropic proviruses were originally identified as Mendelian elements responsible for the spontaneous or inducible expression of ecotropic retroviruses (9). With the development of DNA hybridization probes specific for ecotropic viruses (2, 3), it became possible to identify such viruses by blot hybridization of genomic DNA. This method also allows the detection of noninducible "ecotropic" proviruses (1). This communication reports the identification of a full-size but noninducible ecotropic provirus closely linked to the albino locus (*c*) in a congenic mouse strain containing no other ecotropic proviruses. This finding is potentially important because it may allow, through cloning of the virus and flanking cellular DNA, molecular entree into a region of the genome, deletions of which are associated with severe metabolic and developmental abnormalities (6).

A congenic strain of NFS/N mice carrying the albino region of chromosome 7 from strain C58/Lw was constructed by Wallace Rowe (National Institutes of Health, Bethesda, Md.). This strain, designated NFS.C, was bred by crossing NFS mice to C58/Lw mice and serially backcrossing the progeny to NFS mice. At each generation, nonalbino (black agouti) mice were selected for further backcrossing. After 10 backcross generations, nonalbino mice were intercrossed and maintained thereafter by brother-sister mating. These mice carry the nonalbino (*C*) allele and the linked beta-globin allele (*Hbb^b*) from the C58/Lw strain (data not shown). It was not expected that these mice would carry an ecotropic provirus, because NFS mice do not have an ecotropic provirus, and the first four backcross progenitors of this strain were negative for spontaneous and inducible ecotropic virus (J. Hartley, personal communication). Nevertheless, NFS.C mice carry a single ecotropic provirus detectable by blot hybridization (11) with an ecotropic virus-specific envelope probe (2) (Fig. 1).

To confirm that this ecotropic provirus is in fact linked to the albino locus, we analyzed progeny from Mendelian crosses constructed between NFS.C (nonalbino and *Hbb^b*) and NFS/N (albino and *Hbb^{dd}*) mice. DNA was prepared from the spleens of four albino and four nonalbino mice (three each from a backcross of NFS/N × [NFS.N × NFS.C] and one each from an intercross of [NFS/N × NFS.C] × [NFS/N × NFS.C]). As expected, the four nonalbino mice had the NFS.C provirus, whereas the four albino mice did not (Fig. 1). To localize the NFS.C provirus

further, we scored a large number of additional backcross mice for inheritance of coat color and the *Hbb^b* allele from the NFS.C grandparent. Four recombinants between the albino and *Hbb* loci were identified in 164 backcross mice. Two of the recombinants were albino and carried *Hbb^{dd}* alleles, whereas two were nonalbino and carried *Hbb^{dd}* alleles. DNA was prepared from the spleens of these four recombinant mice and tested by blot hybridization. The two nonalbino recombinants carried the NFS.C provirus, whereas the two albino recombinants did not (Fig. 1). Thus, no recombinants between the albino locus and the NFS.C proviral locus were detected in a total of 12 mice, 4 of which were selected because they were recombinants between the albino and *Hbb* loci. This indicates that the NFS.C provirus is linked to the albino locus ($P < 0.005$) and is located closer to the albino locus than to the beta-globin locus.

Since NFS mice are negative for ecotropic proviruses, the NFS.C provirus was most likely inherited from the C58 strain. C58 mice carry multiple ecotropic proviruses (7), one of which appears to be the same by blot hybridization as the NFS.C provirus. Thus, restriction enzymes *Xba*I, *Sst*I, *Hind*III, and *Pvu*II generate ecotropic *env*-reactive fragments of 8.5, 8.2, 6.7, and 4.1 kilobases (kb) in NFS.C mouse DNA (Table 1) and fragments of similar size (plus others) in C58/J mouse DNA (7; N. Jenkins and N. Copeland, personal communication). The slight difference in size of the larger *Xba*I and *Sst*I fragments in NFS.C versus C58 mouse DNA probably represents calibration differences in the different laboratories. Furthermore, analysis of NZB × C58 recombinant inbred mice indicates that one of the C58 mouse proviruses, identified by an ~8-kb *Sst*I fragment, is linked to the *Hbb* locus (N. Copeland, N. Jenkins, and R. Riblett, personal communication). Taken together, these data strongly suggest that the NFS.C mouse provirus is the same as a provirus located in the albino-*Hbb* region in C58 mice.

The NFS.C provirus was not inducible from tail fibroblast cultures with iododeoxyuridine under conditions which induced the BALB/c and DBA/2 mouse proviruses. In an attempt to understand why the NFS.C mouse provirus was not inducible, DNA from NFS.C mice was cut with restriction enzymes which generate internal proviral fragments in other endogenous ecotropic proviruses. No differences were noted between ecotropic *env*-reactive fragments in DNA from AKR, C3H, and NFS.C mice by using *Pst*I, which cuts once within the long terminal repeats, or with *Bam*HI or *Kpn*I (Fig. 2). Therefore, the NFS.C mouse provirus has no

FIG. 1. Blot hybridization (11) of genomic DNA restricted with *Hind*III and electrophoresed on 0.6% agarose. The probe was a ³²P-labeled, ecotropic virus-specific probe (2). Membranes were washed in 15 mM NaCl-1.5 mM sodium citrate, 0.1% sodium dodecyl sulfate at 52°C. Coat color: +, black agouti; -, albino. Hbb phenotype was determined by hemoglobin electrophoresis (12).

obvious deletions or other alterations detectable with these enzymes which might explain its defectiveness.

Full-size but noninducible ecotropic proviruses have been identified in AKR/J and related mouse strains (1) and in transgenic mice carrying germ-line copies of Moloney virus

(10). In the case of the noninducible AKR mouse provirus designated *emv-13*, the defect lies in the P15E-U3 region (4), whereas in the transgenic mice carrying Moloney proviruses Mov-2, Mov-7, and Mov-10, defects were found in the *gag-pol* region (10). Whereas flanking cellular DNA may affect the rate of spontaneous expression of inducible proviruses, noninducibility appears to result from defects within the provirus, at least in the cases analyzed to date. Molecular cloning of the NFS.C mouse provirus and its flanking cellular sequences is being undertaken to investigate further the reasons for its noninducibility.

The NFS.C mouse provirus is located in the same region of chromosome 7 as a spontaneously expressed ecotropic provirus in C3H/Fg mice, which is designated *Fgv-1*. The *Fgv-1* provirus was mapped to within 8 (±4) centimorgans of C (8). However, these proviruses are distinct based on genetic and biochemical data. First, the presumed gene order for *Fgv-1* is *Fgv-1-Hbb-C* (8), whereas the NFS.C mouse provirus maps closer to the C than to the *Hbb* locus. Second, the restriction enzymes *Eco*RI, *Hind*III, *Pvu*I, and *Sst*I produce different-size fragments in DNA from NFS.C mice than in DNA from mice carrying the *Fgv-1* provirus (not shown). These fragments are presumed to represent virus-cellular DNA junction fragments extending to both sides of the provirus, assuming that the internal sites of these enzymes are the same as in other endogenous ecotropic proviruses (3). Close linkage between different ecotropic proviruses occurs in one other instance, the RF/J strain of mice. Crosses between RF/J and other mouse strains are associated with a high frequency of germ-line reinfections (N. Copeland and N. Jenkins, personal communication). Although it seems unlikely that proximity of the RF/J mouse proviruses, per se, is responsible for the high frequency of germ-line reinfections, it will be interesting to see if a high incidence of germ-line reinfection occurs in crosses in which the NFS.C mouse provirus is inherited in coupling with the *Fgv-1* provirus.

Linkage of the NFS.C mouse provirus to the albino locus is potentially important because it may provide a way to obtain molecular clones from the albino region of chromo-

FIG. 2. Comparison of NFS.C, AKR/N, and C3HeB/FeJ proviruses by using restriction enzymes which are believed to generate internal ecotropic *env*-reactive fragments. The band from AKR/N mouse DNA is more intense because this strain carries several proviruses which are recruited by using *Pst*I. Numbers on the left indicate restriction enzyme fragment sizes in kb.

TABLE 1. Sizes of restriction enzyme fragments from NFS.C, C58/J, AKR/N, and C3Heb/FeJ mouse DNA^a

Restriction enzyme	Sizes (kb) from DNA from mouse strains:		
	NFS.C	C58 ^b	AKR and C3H ^b
<i>EcoRI</i>	13	N.D. ^c	
<i>XbaI</i>	8.5	23, 10.5, 8.8	
<i>SstI</i>	8.2	~8.0 ^d	
<i>HindIII</i>	6.7	11.0, 10.0, 8.6, 7.6, 6.7	
<i>PvuI</i>	4.1	9.3, 8.0, 6.4, 4.1, 3.9, 3.3	
<i>KpnI</i>	4.4	N.D. ^c	4.4
<i>PstI</i>	8.2	8.2	8.2
<i>BamHI</i>	3.3	23, 3.3	3.3

^a DNA was reactive with an ecotropic provirus-specific envelope probe.^b Data from reference 7.^c N.D. Not done.^d N. Jenkins and N. Copeland, personal communication.

some 7. Deletions of DNA in the albino region are associated with profound metabolic and developmental abnormalities (6). Mice carrying the NFS.C provirus are not abnormal, indicating that this provirus does not disrupt vital genes. However, molecular cloning of the NFS.C mouse provirus and flanking cellular DNA, coupled with chromosomal "walking" experiments, could provide molecular probes to help identify genes in this chromosomal region involved in embryological development, fertility, and regulation of hepatic enzyme synthesis (6). A random piece of unique-sequence DNA from this region of mouse chromosome 7 was recently cloned by a different strategy (5). Because the region of the albino deletions is large in molecular terms (4,000 to 10,000 kb) (6) and deletions of different parts of the region are associated with different phenotypes, the identification of additional clones of unique-sequence DNA from this region of mouse chromosome 7 will be of interest.

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