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Genetics

Distribution of ribosomal gene length variants among mouse chromosomes

(restriction fragment polymorphism/concerted evolution/repeated sequences/nontranscribed spacer/linkage maps)

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ABSTRACT **The ribosomal genes (rDNA) in mouse inbred strains have a multichromosomal distribution. Using a structural feature of rDNA [variable length rDNA segment (VrDNA)] that shows length polymorphism within and among inbred strains, we studied the chromosomal distribution of the variant ribosomal gene types through genetic analysis. Our results show that five of the length variant classes can be divided into three discrete linkage groups. The variants present on a particular chromosome pair appear to be unique to that pair and absent from nonhomologous chromosomes. The chromosomal location of particular variants appears to be the same in two unrelated inbred strains suggesting that the observed linkage patterns predate the origin of inbred mice. The nonrandom chromosomal distribution of these rDNA classes suggests that only a limited degree of genetic exchange occurs among nucleolus organizer regions on nonhomologous chromosomes. We have localized one particular VrDNA linkage group to chromosome 12. These and other restriction fragment polymorphisms can be used in the construction of detailed mouse linkage maps.**

In mouse ribosomal genes (rDNA), a region [variable length rDNA segment (VrDNA)] 5′ to the origin of transcription varies between 600 and 3,000 base pair(s) (bp) in length among ribosomal genes because of differences in the number of copies of a smaller (≈135 bp) sequence (1) (Fig. 1). An inbred strain usually has 4–6 major size classes of VrDNA regions. These are distributed among a number of chromosome pairs (3–6). Using mouse recombinant inbred strains, we carried out a genetic analysis of the chromosomal linkage relationships among these polymorphic regions. The results suggest that genetic exchanges among rDNA sequences on nonhomologous chromosomes are rare. The linkage relationships defined for the rDNA polymorphism provide an opportunity for mapping other mouse genes.

MATERIALS AND METHODS

Restriction enzymes were purchased from commercial sources and the recommended digestion conditions were employed. Five micrograms of liver DNA was digested in a reaction volume of 30 μl for 20–24 hr. DNA isolation, agarose gel electrophoresis, transfer to nitrocellulose filters, preparation of nick-translated probe, and autoradiography were as described (7). Hybridization and washing were performed in 0.45 M sodium chloride/0.045 M sodium citrate, pH 7, at 65°C. Fisher's Exact Probability test was used in the statistical analysis of the linkage data.

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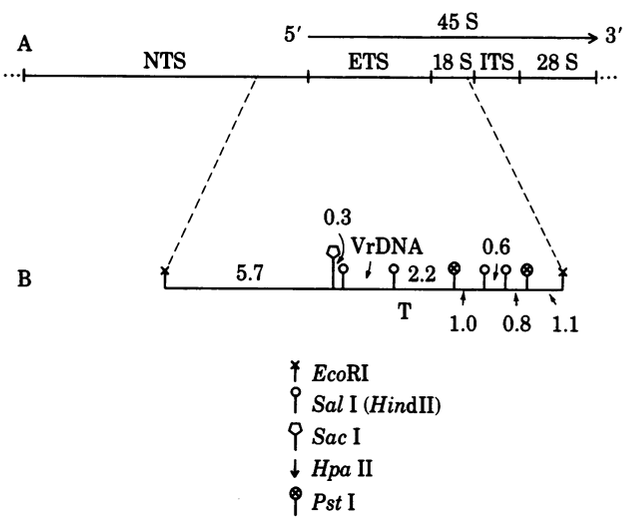

FIG. 1. Schematic representation of a mouse ribosomal gene repeating unit. (*A*) NTS, nontranscribed spacer; ETS, external transcribed spacer; ITS, internal transcribed spacer. The region transcribed into 45 S is also shown. (*B*) Restriction enzyme map of the rDNA fragment cloned in λgtWES (2). The distances between restriction enzyme sites are given in kilobase(s) (kb). T denotes the approximate location of the origin of transcription.

RESULTS

Linkage Relationships Among VrDNA Polymorphic Forms. Earlier rDNA mapping experiments showed that the VrDNA fragments are cut out of the rDNA repeats by *Hin*dII and can be detected in Southern transfer and hybridization experiments by using a cloned VrDNA fragment as a probe (1). Among the hundreds of copies of ribosomal genes in the mouse strain C57BL/6J, five major VrDNA size classes exist (Fig. 2, lane B). Fig. 2 (lane H) also shows the VrDNA pattern for C3H/HeJ mice which differs from C57BL/6J: fragments A, E, and F in C57BL/6J mice are either completely absent or significantly decreased in C3H/HeJ, whereas fragments B and G in C3H/HeJ are absent or significantly decreased in C57BL/6J. All of the fragments are present in the F_1 hybrid (lane F_1). These different rDNA genotypes offered the opportunity to study the linkage relationships among the VrDNA size classes by using standard genetic techniques. Our approach was to use recombinant inbred (RI) strains (8). One set of RI strains—the BXH strains (9)—was established by crossing C57BL/6J (B) with C3H/HeJ (H). Pairs of F_2 individuals from this cross were used to

Abbreviations: rDNA, ribosomal gene; VrDNA, variable length rDNA segment; bp, base pair(s); kb, kilobase(s); RI, recombinant inbred.

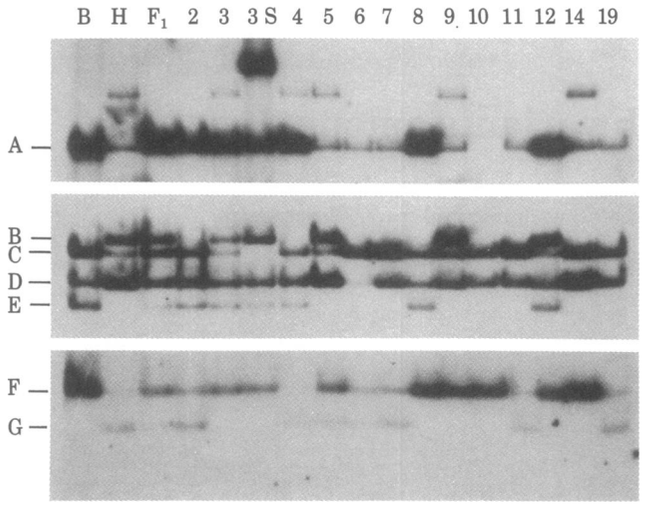

FIG. 2. VrDNA patterns in progenitor, F_1, and BXH RI strains after *Hin*dII digestion (B, C57BL/6J; H, C3H/HeJ; F_1, BXH F_1). The numbers of the remaining samples indicate the specific RI strains examined. To clearly observe all of the VrDNA bands both long and short autoradiographic exposures were needed. This figure is a composite of a 1-day (*Middle*) and a 12-day (*Top* and *Bottom*) autoradiographic exposure of the same hybridization filter. A *Sal* I digest of BXH-3 (3 S) is shown to demonstrate the absence of VrDNA band C after *Sal* I digestion, whereas this band is present after *Hin*dII digestion (see also Fig. 3). The sizes of the VrDNA fragments in kb pairs are: A, 2.7; B, 1.75; C, 1.60; D, 1.33; E, 1.2; F, 0.77; G, 0.65. Hybridization of *Hin*dII genomic digests (5 μg) was with ^{32}P-labeled VrDNA probe. This probe is $\approx$1,700 bp and contains 13 repeating units, each 135 bp in length (1). The location of the VrDNA region in the ribosomal gene is shown in Fig. 1*B*.

establish independent lines that have been propagated by brother–sister matings for more than 30 generations. The resulting RI strains have fixed genetic features from both progenitor strains; each individual RI strain randomly fixing chromosomal segments originally derived from C3H/HeJ or C57BL/6J. The linkage relationships of the VrDNA fragments can be studied by using these RI strains. For example, VrDNA bands A and E are major fragments in C57BL/6J. If A and E are tightly linked on a single chromosome, all of the BXH RI strains will either have major amounts of A and E like C57BL/6J mice or, like C3H, have minor amounts of A and no E. No RI strain would be expected to have a major amount of A without E or a minor amount of A with a major amount of E. If A is on one chromosome and E is on a nonhomologous chromosome, all four genotypes would be expected among the RI strains. If every nucleolus organizer carried representatives of both the A and E classes, then only the relative amounts of these two VrDNA classes would vary among the RI strains.

The VrDNA genotypes of 13 BXH RI strains are shown in Fig. 2 and the data are summarized in Table 1. Analysis of these data reveals that five VrDNA size classes fall into three distinct linkage groups, as discussed below. The sixth VrDNA class, D, was present in equal amounts in both C57BL/6J and C3H/HeJ and could not be studied by using these strains. The seventh class, F, showed significant quantitative variation among the RI strains and definitive conclusions about its linkage relationships cannot be made.

VrDNA Linkage Group A–E. With one exception, every RI strain had either the C57BL/6J or C3H/HeJ genotype with respect to these two VrDNA fragments, indicating that A and E are tightly linked in C57BL/6J ($P < 0.008$). The one exceptional strain (BXH-10) appeared to have no A. However, trace amounts of this fragment can be detected on the original autoradiograph. In addition to indicating that the major amounts of A and E are linked in C57BL/6J, the results also suggest that the same chromosome carries the minor amount of A in C3H/HeJ. If this were not true, one-quarter of the RI strains would have been expected to have no A band at all. Additional data (see *Discussion*) also support this conclusion. The A and E bands segregated randomly with respect to the other VrDNA classes, indicating that these other fragments were associated with rDNA on different linkage groups.

Table 1. The VrDNA genotypes of 13 BXH RI strains

BXH strain	VrDNA fragment				
	A	B	C	E	G
2	B	B	B	B	H
3	B	H	H	B	B
4	B	B	B	B	H
5	H	H	H	H	H
6	H	B	B	H	H
7	H	B	B	H	H
8	B	B	B	B	B
9	H	H	B	H	B
10	H	B	B	H	B
11	H	B	B	H	H
12	B	H	H	B	B
14	H	B	B	H	B
19	H	B	B	H	H
F_1	B	H	B	B	H
C3H/HeJ	H	H	H	H	H
C57BL/6J	B	B	B	B	B

Summary of the parental origin (B or H) of VrDNA bands A, B, E, and G seen after *Hin*dII digestion of DNA from BXH RI strains (Fig. 2). The data on the VrDNA class C were obtained by using *Sal* I (Fig. 3) and not *Hin*dII (see text). Our technique allows us to be confident that when we define a VrDNA class as being absent, it is there in less than one copy per haploid genome.

VrDNA Linkage Group B–C. Fig. 2 shows that after *Hin*dII digestion, both C57BL/6J and C3H/HeJ have VrDNA class C. If *Sal* I rather than *Hin*dII is used to cut the VrDNA fragments out of the ribosomal genes, the identical VrDNA pattern is observed for C57BL/6J but no C band appears in C3H/HeJ (data not shown). Our unpublished results indicate that in C3H/HeJ one of the two *Sal* I sites bordering the C VrDNA region has undergone a nucleotide substitution rendering it susceptible to digestion by *Hin*dII (which recognizes G-T-Y-R-A-C, in which Y is any pyrimidine and R is any purine) but not *Sal* I (G-T-C-G-A-C). We analyzed the DNAs from the RI strains with *Sal* I. The results are summarized in Table 1 and a representative sample of the data is shown in Fig. 3. This allowed us to define the B and C linkage group ($P < 0.02$). With one exception, all of the RI strains had either C or B after *Sal* I digestion. Therefore, VrDNA fragment B in C3H/HeJ is carried on the same chromosome that carries fragment C in C57BL/6J. One strain, BXH-9, had both B and C. This can be explained either by a crossover between the B and C rDNA clusters before fixation of one chromosome or by the persistent heterozygosity of both progenitor chromosomes in BXH-9.

Three of the RI strains (BXH-3, BXH-5, and BXH-12) contained the B band but lacked the C band after *Sal* I digestion (C3H/HeJ genotype). However, each of these RI strains had a C band after *Hin*dII digestion. The presence of the C3H/HeJ B band is therefore correlated with the presence of the C3H/HeJ *Hin*dII-specific C band, suggesting that B and C are linked in C3H/HeJ. If, in C3H/HeJ, the *Hin*dII-specific C fragments were unlinked to the B band, we would have expected *Hin*dII analysis to reveal some RI strains carrying B but not C.

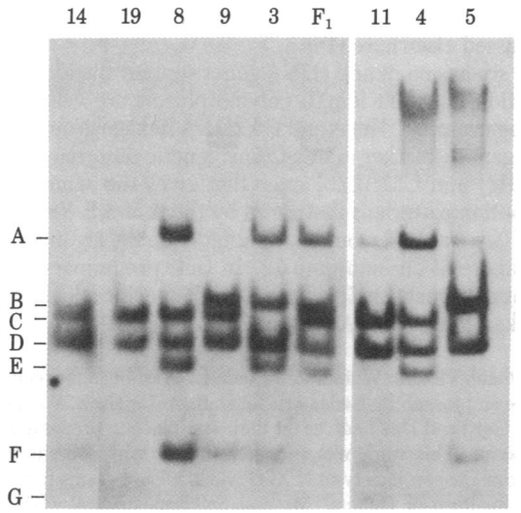

FIG. 3. VrDNA patterns of the BXH F_1 (F_1) and a representative sample of the RI strains after *Sal* I digestion. The numbers of the samples indicate the specific strains examined. The hybridizing bands above fragment A include methylated rDNA sequences (see ref. 2) and are not the result of partial digestion. Hybridization was with the VrDNA probe.

VrDNA Linkage Group G. C3H/HeJ has VrDNA class G, whereas this fragment is not detected in C57BL/6J. There appear to be only two types of BXH RI strains: those with G (C3H/HeJ genotype) and those without it (C57BL/6J). Our data show that VrDNA class G is not linked to A, E, B, or C and therefore constitutes a third linkage group.

Our results then define three independent linkage groups for the ribosomal genes carrying these VrDNA size classes in C3H/HeJ and C57BL/6J. One contains A and E, one B and C, and a third linkage group contains the G length variant. Any single chromosome has one or two major VrDNA types. The linkage analysis indicates that there is no interspersion of these five VrDNA size classes among nonhomologous chromosomes. The VrDNA types present on a particular chromosome pair appear unique to that pair and are not found on others.

The data concerning VrDNA class F are not completely unambiguous. The amount of this fragment in the RI strains is more variable than is the case with other length variants. This could be due to its presence on more than one mouse chromosome, with the individual RI strains receiving a varying number of genes carrying the F length variant. Alternatively, rDNA genes containing this fragment may have been magnified or deleted during the formation of the RI strains. Because a small amount of F is seen with the G band in all of the RI strains carrying G, as well as in the C3H/HeJ parent, we tentatively suggest that the chromosome carrying the small amount of F also carries the G fragment in C3H/HeJ. Additional studies are required to confirm this hypothesis.

The present data were compared with the accumulated data for the numerous genetic markers that have been typed in the BXH RI strains in hope of determining the exact chromosome locations of these VrDNA linkage groups. No associations were found that permitted such assignments. Regretably, there is a paucity of known centromeric-related genetic markers on three of the five chromosomes carrying nucleolar organizers. We decided to approach the chromosomal assignment of the three VrDNA linkage groups by utilizing a well-known group of Robertsonian translocations as linkage markers (10).

Assignment of the A and E VrDNA Linkage Group to Chromosome 12. A survey of several *Mus musculus* strains homozygous for different Robertsonian translocation chromosomes derived from *Mus poschiavinus* showed that one, RB5BNR/Ei, lacked VrDNA bands A and E. In this strain, the *M. musculus* acrocentric chromosomes 8 and 12 are replaced by the *Rb(8.12)5Bnr* metacentric chromosome. Because the A and E bands characteristic of most *M. musculus* inbred strains are missing in RB5BNR/Ei and because cytological studies have shown that chromosome 12—but not chromosome 8—has a nucleolus organizer region (3–6), it suggests that they are located on chromosome 12. We tested the hypothesis that chromosome 12 carries the A and E VrDNA linkage group by crossing individuals of the RB5BNR/Ei strain to C57BL/6J mice and backcrossing F_1 females to the homozygous translocation male parent. If A and E are on chromosome 12, these fragments should be absent from all translocation homozygotes and present in all heterozygotes. All 18 offspring analyzed were of parental genotypes: four were homozygous for the *Rb(8.12)5Bnr* chromosome and as expected lacked the A and E VrDNA bands (Fig. 4, lanes 1, 6, 11, and 17). The 14 remaining *Rb(8.12)Bnr/+* individuals possessed both the A and E fragments.

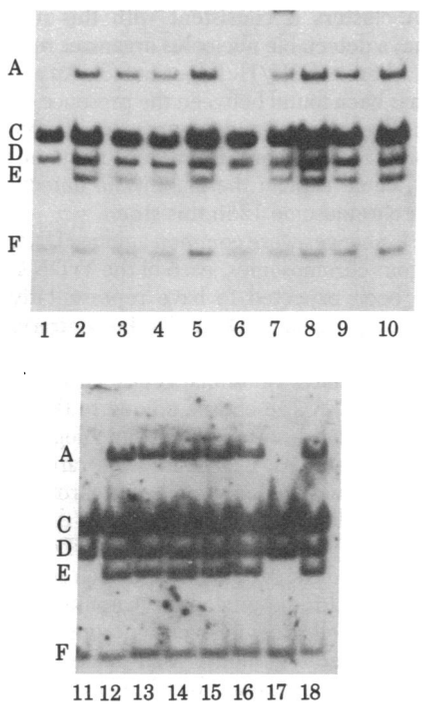

FIG. 4. VrDNA patterns of *Rb(8.12)Bnr* translocation heterozygotes and homozygotes. Five micrograms of DNA was digested with *Hin*dII. Hybridization was with the VrDNA probe. Lanes 1, 6, 11, and 17 contain DNA from *Rb(8.12)Bnr* homozygotes identified through karyotypic analysis. The remaining samples are translocation heterozygotes.

DISCUSSION

The VrDNA region of the mouse ribosomal gene varies in size among the rDNA repeating units present within a single individual. To date, each inbred strain we have examined has a unique combination of VrDNA size classes. We have analyzed the linkage relationships of five major fragment classes (A, B, C, E, and G) found in two strains and have established the existence of three linkage groups. We have localized the A and E VrDNA linkage group to chromosome 12 in C57BL/6J. In C3H/HeJ, VrDNA class A is also likely to be unique to chromosome 12 (see *Results*) but is found in significantly decreased amounts—possibly the result of a deletion. Cytological studies on both of these strains by using silver staining to detect active

major rDNA clusters is consistent with this interpretation; C57BL/6J has a detectable nucleolus organizer region on chromosome 12, whereas C3H/HeJ does not (3). An almost perfect correlation has been found between the presence of a nucleolus organizer by silver staining and the presence of rDNA by *in situ* hybridization (11). Therefore, the reduced amount of A and E in C3H/HeJ could explain the inability to detect a nucleolus organizer on chromosome 12 in this strain.

If genetic exchanges were common among rDNA clusters on nonhomologous chromosomes, each of the VrDNA size classes would have been expected to have representatives on each chromosome pair carrying rDNA. To the contrary, our results have defined three discrete linkage groups. Each nucleolus organizer appears to have ribosomal genes with one, or at most, two different VrDNA size classes unique to that chromosome pair and apparently absent from nonhomologous rDNA-containing chromosomes. An insight into the rarity of exchanges between rDNA genes on nonhomologous chromosomes can be gained by considering that in the two distantly related strains—C57BL/6J and C3H/HeJ—the VrDNA class A, although present in different amounts in each strain, appears to be on the same chromosome pair. Unpublished studies on Chinese hamster cells carrying a chromosome 12 derived from BALB/c mice (fused to a mouse X chromosome) indicate that among the six VrDNA classes found in BALB/cJ, only A and E are present on chromosome 12. Thus, both A and E may have been limited to their respective chromosomes for at least 60 yr (12) since the strains originated. Because these strains are apparently unrelated, the conservation of this linkage relationship might predate the origin of the inbred strains considerably. The simplest explanation of our results is that genetic exchanges among nucleolus organizer regions on nonhomologous chromosomes occur relatively infrequently when compared to intrachromosomal homogenization events (13).

Of course, the VrDNA regions on nonhomologous chromosomes did not evolve totally independently of one another because they are homologous in nucleotide sequence and with respect to certain restriction enzyme sites (1). As Smith has pointed out, rare interchromosomal exchanges that have occurred during mouse evolution could account for this (14). He argued that the rate of exchange need not be high in an analogy with population genetic theory, which shows that only very rare migrants are needed to maintain genetic similarity among two isolated populations of a species. In *Drosophila melanogaster*, nucleolus organizer regions are found in both the X and Y chromosomes. The evidence suggests that genetic exchanges occur rarely between rDNA sequences on these two chromosomes in this species (15–17). However, studies on human and ape rDNA suggest that from an evolutionary perspective there is no barrier to the exchange of rDNA sequences among nonhomologous chromosomes (18). Data on the distribution of human ribosomal gene variants among individual nucleolus organizers are consistent with this interpretation (19). The basis for this apparent difference in the genetic behavior of rDNA in different species is discussed elsewhere (19).

Our studies on mouse rDNA genes support the idea (20) that restriction fragment length polymorphisms are valuable tools for gene mapping. The A and E VrDNA linkage group provides a new genetic marker in mice. Any genetic difference between C3H/HeJ and C57BL/6J mice that gives the same RI strain distribution pattern as that given by the A and E VrDNA fragments (see Table 1) would undoubtedly be tightly linked to the centromere of chromosome 12. In fact, preliminary evidence (21) suggests that the Epa-1 mouse skin-specific alloantigen may be linked to this rDNA cluster.

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