Variation among Mouse Ribosomal RNA Genes Within and Between Chromosomes

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We compared nucleotide sequences of the transcription-initiation region of five mouse ribosomal DNA (rDNA) clones. Two clones were isolated from Japanese wild mice, *Mus musculus molossinus*, and the other three from BALB/c mice originating in *M. m. domesticus*. Two BALB/c clones that are derived from the same chromosome are very similar, suggesting the occurrence of intrachromosomal homogenization of rDNA repeats. However, the other clone of BALB/c was approximately as different from the former two clones as from the rDNA clones of *M. m. molossinus*. These results suggest that, compared with intrachromosomal homogenization, homogenization among rDNA repeats on nonhomologous chromosomes occurs relatively infrequently after the mouse subspecies separation.

Introduction

All animal genomes contain many multigene families, including the one that codes for the 18S and 28S ribosomal RNAs (rRNAs) (Brutlag 1980; Long and Dawid 1980; Singer 1982). Within a species, individual members of this multigene family are generally very similar, often irrespective of the family size, function, or chromosomal distribution. This remarkable feature, which is known as concerted evolution (Arnheim et al. 1980; Dover 1982), has been explained in terms of homogenization of the repeat units by mechanisms such as unequal crossover and gene conversion. The rate of homogenization within individual chromosomes appears to be higher than the rate of spreading of a given repeat unit across the whole genome. Therefore, the repeat units on a chromosome tend to form a subfamily (Arnheim et al. 1982; Jorgensen et al. 1986).

The ribosomal genes of the mouse are distributed among nucleolus organizers located on several chromosomes (Dev et al. 1977). Genetic analysis of mouse recombinant inbred strains with a polymorphic probe of rDNA spacer sequence indicates that rDNA clusters on nucleolus organizers may comprise such subfamilies (Arnheim et al. 1982). We examined the relative amount of sequence homogeneity of the rDNA repeats within a nucleolus organizer versus the amount of homogeneity between different nucleolus organizers by determining the nucleotide sequence of the transcription-initiation region of rDNA. In the present paper we compare two rDNA clones from Japanese wild mice and three clones from BALB/c mice, two of which exist on a
single nucleolus organizer, and present the sequence data on variation within and between rDNAs of nucleolus organizers.

Material and Methods

Two mouse genomic libraries were screened with an rDNA probe that spanned the SalI 3.2-kb fragment containing the transcription-initiation site of a mouse ribosomal RNA gene (Urano et al. 1980). One is a Sau3A-partial library of MOA strain derived from Japanese wild mice, Mus m. molossinus. The other is an EcoRI-partial library of BALB/c strain. Each clone isolated was subjected to subcloning and sequence analysis (Maniatis et al. 1982). Phages and plasmids were grown in the E. coli strains DP50supF and HB101, respectively. DNA sequencing was performed according to the procedure of Maxam and Gilbert (1980).

Results

In the mouse ribosomal RNA gene, an rDNA region (VrDNA) upstream of the transcription-initiation site is known to be hypervariable in length (Arnheim et al. 1982; Kuehn and Arnheim 1983). Among the several hundred copies of rDNA in the genome of the BALB/c mouse strain, four major and one minor VrDNA size classes exist; i.e., fragments A (2.7 kb), B (1.75 kb), D (1.35 kb), E (1.20 kb), and G (0.65 kb). Some of the VrDNA size classes could be unique to chromosomes carrying rDNA. Using recombinant inbred strains, Arnheim et al. (1982) have demonstrated that the rDNA repeats containing the G fragment constitute a linkage group and are not linked to those containing the A, B, C, or E fragment. We confirmed these results by examining DNA of the mice obtained by means of a backcross of the F1 hybrid between BALB/c and DDD/l mice to DDD/l (data will be published elsewhere).

Two BALB/c rDNA clones have been sequenced and reported for the transcription-initiation region (Urano et al. 1980; Bach et al. 1981; Grummt 1982). One clone (B1) contains the F VrDNA fragment, the other (B3) the G fragment. In the present study we newly isolated an rDNA clone (B2) containing the G fragment and sequenced it. Furthermore, two rDNA clones were isolated from the DNA of Japanese wild mice, Mus m. molossinus, and were sequenced for phylogenetical comparison. In figure 1, the VrDNA and transcription-initiation regions are indicated in the restriction-enzyme maps of both rDNAs (fig. 1).

![Restriction maps indicating locations of Vr sequence and the transcription-initiation region.](https://example.com/figure1.png)

**Fig. 1.**—Restriction maps indicating locations of Vr sequence and the transcription-initiation region. The Vr region is marked by the two contiguous SalI (or HincII) sites, and the transcription-initiation regions used for sequence comparison are indicated by bars above the maps. Boxed regions represent 18S and 28S gene regions. (▲) Denotes SalI; (▼) denotes EcoRI; and (▼▼) denotes BglII.
The nucleotide sequences of the transcription-initiation regions of five mouse rDNAs are compared in figure 2. Only nucleotides departing from the B1 sequence are depicted. There are 24 variable sites among the 843 sites examined. Of these 24
sites, 12 are caused by base substitutions, eight are small insertions or deletions (less than three base pairs), and four are segmental mutations. The region −103 to +41 relative to the transcription-initiation site (+1) shows an identical sequence among these clones, suggesting that the promoter region is subjected to a high selective pressure. It also suggests that the five rDNA clones are transcriptionally active. Nucleotide positions are divided into two types according to whether (type 1) they require only one genetic event (insertion, deletion, substitution) because there are only two alternative sequences for the position(s) or (type 2) they necessarily require at least two genetic events because there are three or more alternative sequences for the position(s). Insertion and deletion events may span several consecutive nucleotides. The occurrences of two types are summarized in parts A and B of figure 3 respectively. Type 1 is the most likely because the expected frequency of mutation will not give more than one hit at a single site. Type 2, requiring multiple genetic changes, is hypervariable and may be serving as a recombinational hot spot. Most of the type 2 changes are concentrated in the segments comprising simple tandem repeats, which may be generated by an unequal homologous exchange between sister chromatids. The presence of such hypervariable sequences in the genomes has been reported and discussed elsewhere (Gonzalez et al. 1985; Jeffreys et al. 1985).

Type 1 mutations can be divided into six groups, based on which clone(s) carries a different nucleotide or sequence (fig. 3A). For example, a group comprising the mutations observed at positions 43, 53, 174, 255, 399, and 593 reveals that both M1 and M2 clones show a set of nucleotides or sequences at the respective positions and that the other B1, B2, and B3 clones show a different set of nucleotides or sequences. When a phylogenetic tree is taken into consideration, the time of occurrence of these mutational events can be deduced; i.e., each mutational hit must have occurred and spread in the ancestral mice of either *M. m. molossinus* or *M. m. domesticus* after their subspecies separation (fig. 4). Similarly, except for the site −104, the other groups of mutations listed in figure 3A can be ascribed to the respective times indicated in figure 4. The exception may be accounted for by polymorphism in the ancestral mouse population, polymorphism that was inherited differently at the different sites on different chromosomes. It is noteworthy that variable sites are not shared by the rDNA copies in a subspecies.

Since the number of type 1 mutations detected between rDNA clones presumably reflects time after the separation of rDNAs, it represents a degree of evolutionary relatedness. Figure 4 summarizes the relationship of the five rDNA clones that was deduced by means of both a phylogenetic tree and the frequency of mutations. This summary does not include the data of type 2 mutations, because their frequency may not reflect the time of separation. The M1 and M2 clones are more closely related to each other than either is to the other clones, since they share 17 common nucleotide changes but show only two different nucleotides. The B2 and B3 clones are also closely related, but the B1 clone is rather different; the B1 differs by eight and 11 nucleotides, respectively, from the B2 and the B3 clones, whereas the B1 clone is different by 12 and 14 nucleotides, respectively, from the M1 and the M2 clones. Note that the B2 clone exhibits only eight nucleotides that are different from those of the M1, irrespective of subspecies difference. On the basis of these results the five rDNA clones may be better divided into three evolutionarily distinct classes: B1, B2 and B3, and M1 and M2. The higher homology observed between the M1 and M2 clones reflects the phylogenetically close relationship and possibly the same chromosomal location. However, the B2 and B3 similarity could not be explained in terms of only the phylogenetical
Fig. 3.—Classification of nucleotide changes observed in five rDNA copies. A, Type 1 changes comprising only two kinds of nucleotides or sequences present at the same positions; B, type 2 changes in which more than three kinds of nucleotides or sequences are detected at the same positions.

relationship but probably also must be explained in terms of their presence at the same nucleolus organizer, since the B1 clone of the same BALB/c strain showed as many nucleotide changes vis-à-vis the other BALB/c clones it did vis-à-vis the *M. lossinus* clones. These results strongly suggest that the rDNA repeats containing the B2 and B3 clones but not the B1 clone have undergone intrachromosomal homogenization.
Discussion

The gene and spacer regions of the ribosomal RNA gene in mammals have different characteristic evolutionary patterns; the spacer region reveals many more variations than does the gene region. Spacer sequences, therefore, have been used as probes in studies of (1) phylogenetic relationship of species and subspecies (Arnheim and Southern 1977; Kominami et al. 1983; Suzuki et al. 1986; Kominami and Muramatsu 1987) and (2) genetic exchanges of rDNA repeats within and between chromosomes (Krystal et al. 1981; Arnheim et al. 1982; Coen et al. 1982). In the present study, we have used the promoter and downstream regions (−168 to +676 relative to the transcription-initiation site) that were regarded as rather well-conserved regions, because the sequence for comparison can be identified accurately in each clone. Five rDNA sequences were compared, three and two of which were obtained from BALB/c strain and Japanese wild mice, respectively. The two BALB/c clones (B2 and B3) are assumed to be derived from the rDNA repeats on the same chromosome, since they contain the same size Vr segment that can be used to determine the chromosomal location (see the first paragraph in Results). However, recombinant-phage and plasmid populations containing internally repeated DNA cloned in E. coli tend to yield the alteration of inserts by recombination during cloning (Arnheim and Kuehn 1979; Brutlag
1980). The B2 or B3 clone (or both), therefore, may be derived from genes with a larger VrDNA segment. We consistently found that SalI digestion of the Bl and B3 clones gave a single Vr band of 1.20 kb (E fragment) and 0.65 kb (G fragment), respectively, but we cannot eliminate the possibility of Vr size being altered by recombination. The finding that the B2 and B3 rDNA repeats were much more similar to one another than were the other clones (Bl, M1, and M2) suggests that intrachromosomal homogenization of rDNA repeats occurs after the subspecies separation of Mus m. domesticus and M. m. molossinus.

Studies of the restriction-enzyme-fragment-length polymorphisms of rDNA spacer regions reveal that the rDNAs of BALB/c and its related subspecies that are present at several nucleolus organizers show a rather uniform restriction cleavage map (see fig. 1), which is distinct from that of M. m. molossinus (Kominami et al. 1983; Suzuki et al. 1986; Kominami and Muramatsu 1987). Essentially, it means a higher degree of intrasubspecies homogeneity of rDNA spacers than of intersubspecies homogeneity of rDNA. The homogeneity probably results from genetic exchanges among rDNA repeat clusters on nonhomologous chromosomes, exchanges that occurred independently in both M. m. domesticus and M. m. molossinus at some time after the subspecies separation. If such genetic exchanges occurred often, the BALB/c rDNAs (Bl, B2, and B3) should be more homologous to each other than any one of them is to the rDNAs (M1 and M2) of M. m. molossinus. The results obtained here, however, show that a BALB/c rDNA repeat (B1) is approximately as different from the other BALB/c rDNA repeats (B2 and B3) as it is from the rDNAs (M1 and M2) of Mus m. molossinus; i.e., rDNA sequences display a rather similar homogeneity intrasubspecies and intersubspecies. This is explained by relatively infrequent occurrence, compared with intrachromosomal homogenization events, of homogenization between different nucleolus-organizer regions. It therefore may be concluded that rDNA repeats (at least at the transcription-initiation region) have evolved independently on different chromosomes since subspecies divergence.

LITERATURE CITED


Ribosomal Gene Variation


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