NOTE *Anatomy*

Mapping of rRNA Gene Loci in the Mice, *Mus musculus molossinus* (Japan) and *Mus musculus musculus* (Russia) by Double Color FISH

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ABSTRACT. Ribosomal RNA gene (rDNA) loci of Russian *Mus musculus musculus* and of Japanese *Mus musuculus molossinus* were mapped by double color FISH. The total number of rDNA loci was varied from 5 to 12, although the loci on chromosomes 12, 15, 16, 18, and 19 were common to all mice examined. Instead, polymorphisms of the rDNA loci were found on chromosomes 1, 5, 8, 9, 10, 11, 13 and 17. The novel rDNA loci of *M. m. musculus* were found in Nov/TUA strain on chromosomes 8 and 17. These observations, together with those of previous reports, suggest that the rDNA loci of *Mus musculus* species are in the evolutionary process of further translocation to other chromosomes.

KEY WORDS: chromosome, FISH, rDNA.

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According to recent sequence-based genome analysis, standard mouse laboratory strains are regarded as recombinant strains derived from four parental subspecies, *M. m. musculus*, *M. m. domesticus, M. m. castaneus* and the hybrid *M. m. molossinus* [4, 14]. The karyotype of these subspecies is identical except for the difference in sizes of the Y chromosome [10]. In the laboratory strains, the total number of rDNA loci varied from three to five, and the loci were limited to chromosomes 12, 15, 16, 18 and 19 [1, 3, 5, 9]. Although the locus polymorphism was found on chromosomes 12, 15, 16 and 19, rDNA locus on the chromosome 18 was observed consistently in all laboratory strains examined. Such variations of rDNA loci on these chromosomes were reported in the wild mice of *Mus musculus* species, as well as the polymorphism of rDNA loci on other chromosomes [11, 13].

The evolution of rDNA has been the subject of controversy for many years, as the clusters of rDNA at different chromosomal loci retain the strict sequence homology and the copy number of the gene varied about one to several hundred in mammals [12]. *Mus musculus* species is a good model system for the analysis of rDNA evolution, because of the remarkable variation of rDNA loci not only between different subspecies but also within the same subspecies. In the present study, to elucidate the evolution of rDNA loci in *Mus musculus*, we further determined rDNA loci in wild and wild mouse-derived strains of *M. m. molossinus* and wild mouse-derived strains of *M. m. musculus*.

A wild *M. m. molossinus* was captured in Machida, Tokyo, Japan. The JF1 strain of *M. m. molossinus* was derived from fancy mice and established as a strain at the National Institute of Genetics, Mishima, Japan [8]. Three wild mouse strains of *M. m. musculus* were derived from Okha, Irkutsk and Novokachalinsk in Russia and established by one of authors (T. K.) as inbred strains Okh/TUA, Irk/TUA and Nov/TUA, respectively. Lymphocytes, isolated from the spleen, were cultured, and air-dried chromosome slides were prepared following the protocol of Matsuda *et al.* [11].

A human 28S-rDNA (pHr14E3, Human Science Research Resource Bank, Japan) was labeled by nick translation with digoxigenin-11-dUTP and ethanol-precipitated with salmon sperm DNA and tRNA. This probe DNA was dissolved in 8 μl of 100% formamide and denatured at 75°C for 10 min. Biotin-labeled mouse chromosome paints (Cambio, Cambridge, UK) were denatured following the manufacturer's protocol. The labeled probes, which consist of 8 μl of rDNA and 3 μl of chromosome paints, were mixed with 11 μl of hybridization buffer (10 × SSC, 1 mg/m*l* BSA and 50% dextran sulfate). Prior to hybridization, the slides were treated with RNase (100 μg/m*l* in 2 × SSC) for 1 hr to remove the cellular rRNA. The slides were washed in 2 × SSC, dehydrated in an ethanol series, denatured at 70°C for 2 min in 70% formamide in 2 × SSC and dehydrated in the ethanol series at 4°C. Following the hybridization, the probes were visualized by avidin-FITC and anti-dig-Rhodamine as described previously [6]. The slides were counterstained with 1.5 μg/m*l* DAPI, and the fluorescence signals were captured with a CCD camera (Hamamatsu Photonics, Hamamatsu, Japan).

The overall results of the rDNA loci mapped by double-color FISH and the results of the previous reports from the literature are summarized in Fig.1 and Table 1. The chromosomes were all telocentoric except for Y chromosome, and rDNA loci were detected at the distal end of the autosomes in all the mice examined.

In the *M. m. molossinus* captured at Machida and the JF1/

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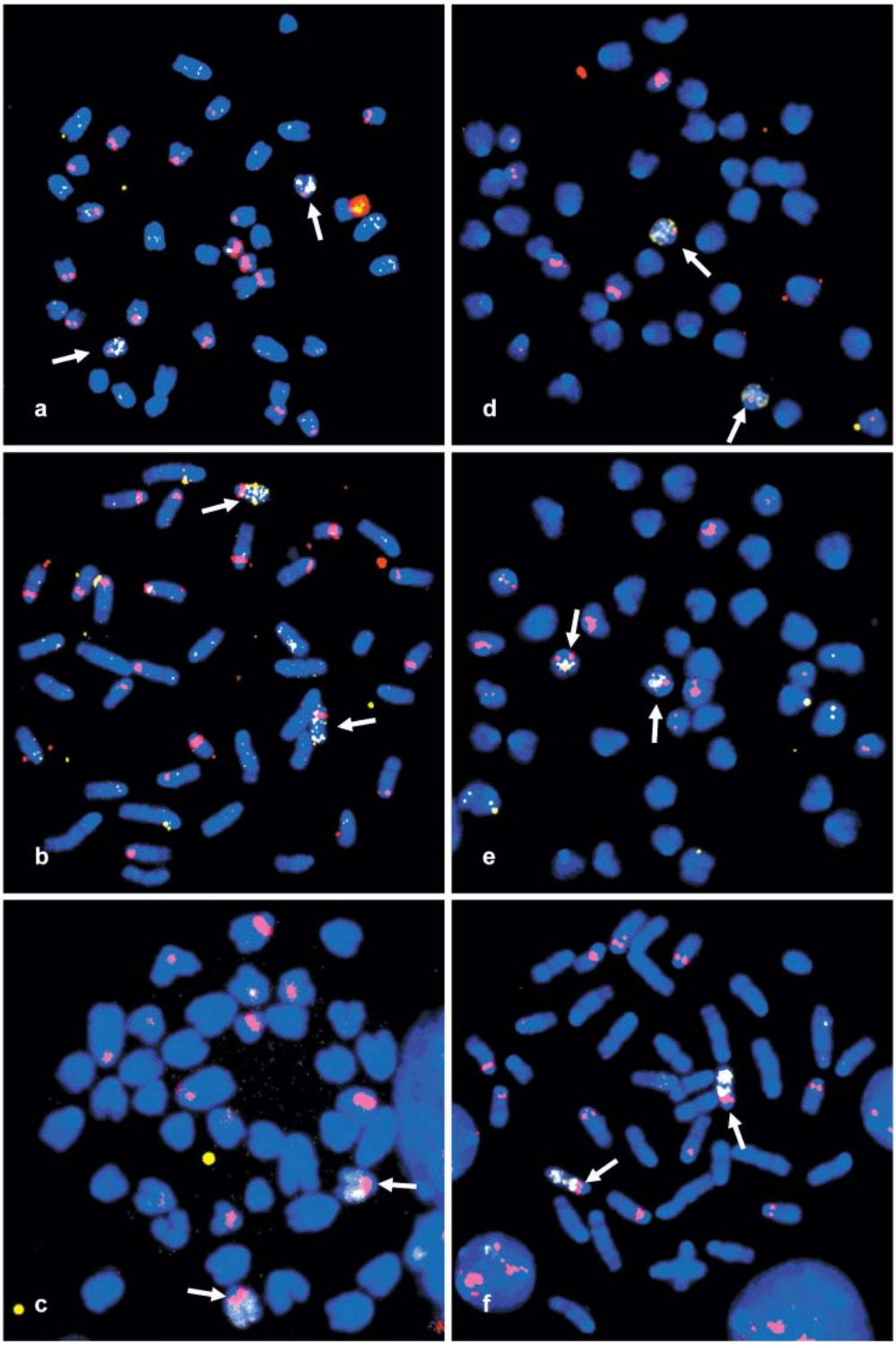


Fig. 1. Mapping of rDNA gene loci in *M. m. molossinus* (a-c) and *M. m. musculus* (d-f) by double-color FISH. Red signal: rDNA, White signal: mouse chromosome paint probes. Arrows indicate: (a) Chromosome 9 and (b) Chromosome 16 in the wild *M. m. molossinus* captured at Machida, (c) Chromosome 11 in JF1/2TUA, (d) Chromosome 16 in Okh/TUA, (e) Chromosome 11 in Irk/TUA, (f) Chromosome 17 in Nov/TUA.

Table 1. Chromosomal loci of rDNA in *Mus musculus* subspecies detected by *in situ* hybridization

Subspecies	Name	Origin	Chromosome # 1	4	5	8	9	10	11	12	13	15	16	17	18	19	References
M. m. molossinus	Wild	Machida	+	-	+	-	+	+	+	+	+	+	+	+	+	+	Present study
	JF1/2TUA	NIG	+	-	-	-	-	-	+	+	+	+	+	+	+	+	
	Wild	Hachioji	+	-	+	-	-	+	+	+	+	+	+	+	+	+	[6]
	MSM	Mishima	+	-	+	-	+	-	+	+	+	+	+	+	+	+	[11]
M. m. musculus	Okh/TUA	Okha	-	-	-	-	-	-	-	+	-	+	+	-	+	+	Present study
	Irk/TUA	Irkutsk	-	-	-	-	-	-	+	+	-	+	+	-	+	+	
	Nov/TUA	Novokachalinsk	-	-	-	+	-	-	+	+	-	+	+	+	+	+	
	Myl	Yugoslavia	-	-	-	-	-	-	+	+	-	+	+	-	+	+	[11]
Fifteen inbred strains of laboratory mice			-	-	-	-	-	-	-	±	-	±	±	-	+	±	[9]
Nine inbred strains of laboratory mice			-	-	-	-	-	-	-	±	-	±	±	-	+	±	[1] (Review)

± : polymorphic loci

2TUA, rDNA loci were mapped on nine and twelve pairs of autosomes, respectively. Among these rDNA loci, nine loci on chromosomes 1, 11, 12, 13, 15, 16, 17, 18 and 19 were invariably present in all mice, whereas the other three loci on chromosomes 5, 9 and 10 were polymorphic (Fig.1 a-c).

In the three inbred strains of Russian *M. m. musculus*, five rDNA loci in Okh/TUA, six loci in Irk/TUA and eight loci in Nov/TUA were mapped. The loci on chromosomes 12, 15, 16, 18 and 19 were common to all these strains, whereas the other loci on the chromosome 11 of Irk/TUA, and chromosomes 8, 11 and 17 of Nov/TUA were limited to these inbred strains (Fig. 1 d-f). The rDNA loci found on chromosomes 8 and 17 in Nov/TUA mouse are novel rDNA loci in *M. m. musculus*.

The loci on chromosome 17 were common in *M. m. musculus*, but polymorphic in *M. m. musculus*. The locus on chromosome 11 was found in both *M. m. musculus* and *M. m. molossinus* except for the Okh/TUA strain. In contrast, the locus on chromosome 8 was observed in the Nov/TUA strain alone.

Locus polymorphism of rDNA in the subspecies of *Mus musculus* observed in the present study, together with results from previous reports, suggests that the rDNA loci in *Mus musculus* are in the evolutionary process of further translocation to other chromosomes. In early studies using *in situ* hybridization autoradiography, it is apparent that the rDNA loci in the laboratory mouse strains are polymorphic, although the loci are limited to chromosome 12, 15, 16, 18 and 19 except for *M. m. molossinus,* suggesting the translocation of rDNA among these chromosomes of *Mus musculus* [1, 3, 5, 9].

Furthermore, in the wild *Mus musculus* species, additional rDNA loci were mapped on chromosomes 1, 4, 5, 8, 9, 10, 11, 13 and 17, as has been reported [6, 11, 13]. In *M. m. molossinus*, the loci on chromosomes 1, 11, 13 and 17 were observed in all mice examined, implying that these loci might be fixed in this subspecies. We supposed that these additional loci might have evolved by translocation of rDNA from the loci on chromosomes 12, 15, 16, 18 and 19. As the polymorphism of rDNA loci was more frequently found in *M. m. molossinus* than in other *Mus musculus* subspecies, the translocation of rDNA copies occurred repeatedly after speciation of this subspecies as a hybrid between *M. m. musculus* and *M. m. castaneus* [15].

It is striking that the locus on the chromosome 18 was consistently observed in all subspecies of *Mus musculus* without exception (Table 1). To explain this phenomenon, we propose an evolutionary scenario that, in the ancestral species of *Mus musculus*, a progenitor locus of rDNA might have been localized on chromosome 18, which thereafter translocated to chromosomes 12, 15, 16 and/or 19, and then speciation to subspecies might have occurred, accompanying with further translocation of rDNA to other chromosomes. As the location of rDNA in *Mus musculus* species is adjacent to the transition region, termed pericentromeric DNA, a hot spot for DNA recombination of repeated sequence, rDNA in *Mus musculus* might be prone to transfer to the pericentric loci of the chromosomes [2, 7].

REFERENCES

1. Akeson, E. C. and Davisson, M. T. 1996. pp. 1509–1511. *In*: Genetic Variants and Strains of the Laboratory Mouse. Lyon, M. F., Rastan, S. and Brown, S. D. M. eds., Oxford University Press, Oxford.
2. Eichler, E. E. and Sankoff, D. 2003. Structural dynamics of eukaryotic chromosome evolution. *Science* **301**: 793–797.
3. Elsevier, S. M. and Ruddle, F. H. 1975. Location of genes coding for 18S and 28S ribosomal RNA within the genome of Mus musculus. *Chromosoma* **52**: 219–228.
4. Frazer, K. A., Eskin, E., Kang, H. M., Bogue, M. A., Hinds, D. A., Beilharz, E. J., Gupta, R. V., Montogomery, J., Morensoni, M. M., Nielsen, G. B., Pethiyagoda, C. Y., Stuve, L. L., Johnson, F. M., Daly, M. J., Wada, C. M. and Cox, D. R. 2007. A sequence-based variation map of 8.27 million SNPs in inbred mouse strains. *Nature* **448**: 1050–1053.
5. Henderson, A. S., Eicher, E. M., Yu, M. T. and Atwood, K. C. 1974. The chromosomal location of ribosomal DNA in the mouse. *Chromosoma* **49**: 155–160.
6. Ito, T., Osawa, S., Shibata, H. and Kanda, N. 2007. Identifica-

tion of rRNA gene loci in the wild mouse (*Mus musculus molossinus*) captured at Hachioji, Tokyo. *J. Vet. Med. Sci.* **69**: 1277–1279.

7. Kalitsis, P., Griffiths, B. and Choo, K. H. A, 2006. Mouse telocentric sequences reveal a high rate of homogenization and possible role in Robertsonian translocation. *Proc. Natl. Acad. Sci. (U.S.A.)* **103**: 8786–8791.
8. Koide,T., Moriwaki, K., Uchida, K., Mita, A., Sagai, T., Yonekawa, H., Katoh, H., Miyashita, N., Tsuchiya, K., Nielsen, T. J. and Shiroishi, T. 1998. A new inbred strain JF1 established from Japanese fancy mouse carrying the classic piebald allele. *Mamm. Genome* **9**: 15–19.
9. Kurihara, Y., Suh, D. S., Suzuki, H. and Moriwaki, K. 1994. Chromosomal locations of Ag-NORs and clusters of ribosomal DNA in laboratory strains of mice. *Mamm. Genome.* **5**: 225–228.
10. Matsuda, Y., Moriwaki, K., Chapman, V. M., Hoi-sen, Y., Akbarzadeh, J. and Suzuki, H. 5S rRNA genes by direct R-banding fluorescence *in situ* hybridization. *Cytogenet. Cell Genet.* **66**: 246–249.
11. Matsuda, Y. and Chapman, V. M. 1995. Application of fluorescence *in situ* hybridization in genome analysis of the mouse. *Electrophoresis* **16**: 261–272.
12. Nei, M. and Rooney, A. P. 2005. Concerted and birth-and-death evolution of multigene families. *Annu. Rev. Genet.* **39**: 121–154.
13. Suzuki, H., Kurihara, Y., Kanehisa,T. and Moriwaki, K. 1990. Variation in the distribution of silver-staining nucleolar organizer regions on the chromosomes of the wild mouse, *Mus musculus*. *Mol. Biol. Evol.* **7**: 271–282.
14. Yang, H., Bell, T. A., Churchill, G. A. and Villena, F.P. 2007. On the subspecific origin of the laboratory mouse. *Nat. Genet.* **39**: 1100–1107.
15. Yonekawa, H., Moriwaki, K., Gotoh, O., Miyashita, N., Matushima, Y., Shin, L., Cho, W. S., Zhen, X. L. and Tagashira, Y. 1998. Hybrid origin of Japanese mice "*Mus musculus molossinus*": Evidence from restriction analysis of mitochondorial DNA. *Mol. Biol. Evol.* **5**: 63–78.