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Polymorphisms Detected in Actin-related Sequences of Rats (*Rattus norvegicus*)

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Southern blot hybridization of *Eco*RI digests of DNAs from 13 rat strains using human cardiac actin gene as a probe revealed polymorphisms in actin-related sequences of rats. *Eco*RI fragments of 11 kb, 7 kb, 6 kb, 5 kb, 4.5 kb and 4 kb detected in several strains were absent in the remaining strains. The presence of these fragments was suggested to be due to presence of extra sequences homologous to the actin genes, such as processed pseudogenes, in the particular strains. The 13 strains were assigned to each of 7 specific patterns of the polymorphic *Eco*RI fragments. It was concluded that the polymorphisms of actin-related sequences should be useful for genetic monitoring of laboratory rats.

Mammalian actin genes constitute a complex gene family which encodes a group of proteins highly conserved in evolution. There are six distinct actin isotypes found in mammals [18], namely, skeletal muscle α -actin, cardiac muscle α -actin, two smooth muscle actins, and cytoskeletal β - and γ -actins. These isotypes differ from each other by only few amino acids. In addition to the genes encoding the six actin isotypes, a large number of actin-related sequences, most of which are believed to be pseudogenes, are present in certain mammalian genomes [4, 8, 14]. These actin-related sequences are known to be dispersed in human and mouse chromosomes [2, 12, 16].

Several polymorphisms among inbred mouse strains have been detected in such actin-related sequences [11, 15]. Polymorphisms in the actin-related sequences are also expected to be detected in other animals, and such polymorphisms should be useful for genetic

characterization of strains of laboratory animals. In the present study, polymorphism of the actin related-sequences in laboratory rats (*Rattus norvegicus*) was thus examined to determine whether it can be used for the genetic monitoring of the laboratory rat strains.

DNA samples from 13 rat strains, WKS/lar, LET/lar, NIGIII/lar, WM/lar, LEO/lar, IS/lar, BEG/lar, COP/lar, F1R/lar, TOM/lar, TU/lar, TX/lar, and lar : WKA which had been maintained by Imamichi Institute for Animal Reproduction, were used in this study. Among these strains, lar : WKA is an outbred stock, whereas the other 12 strains are inbred strains. Animals were kept in a room at $24 \pm 2^\circ\text{C}$ with a relative humidity of 50 to 60%, and given commercial diet (MM-2, Funabashi Farm K. K.) and tap water *ad libitum*.

High-molecular weight genomic DNAs were prepared from rat liver as described [7]. The DNA samples were digested with restriction endonuclease *Eco*RI, *Bam*HI, *Hind*III, or

Fig.1 Autoradiograms of *Eco*RI fragments in genomic DNAs from 13 inbred strains hybridized with human cardiac actin cDNA. Liver DNAs from inbred rats were digested with *Eco*RI and analyzed as described in the text. 1. WKS/Iar; 2. LET/Iar; 3. NIGIII/Iar; 4. F1R/Iar; 5. WM/Iar; 6. LEO/Iar; 7. IS/Iar; 8. COP/Iar; 9. TOM/Iar; 10. TX/Iar; 11. TU/Iar; 12. BEG/Iar; 13. Iar: WKA. Arrows on the left indicate the polymorphic *Eco*RI fragments. Lengths of marker fragments are shown on the right.

*Pst*I (Takara), and the digests were separated by 0.7% agarose gel electrophoresis in Tris-Boric acid-EDTA buffer (pH 8.3). After denaturation and neutralization, DNAs were transferred to a Nylon-membrane filter (Pall) and fixed by the method of Southern [17].

Plasmid pHM α A-1 containing human cardiac actin gene cDNA [6] was digested with *Pvu*II and *Pst*I and 0.6 kb fragment of inserted sequence was purified by electrophoresis. The fragment was labeled by random priming [5] to a specific activity of 2×10^6 cpm/ng DNA using [α - 32 P] dCTP (NCI). The filters were hybridized with a hybridization solution (50% formamide, $5 \times$ SSC, 1 mM EDTA, 0.5% SDS, $5 \times$ Denhardt's solution [3], and 100 μ g/ml of sonicated herring sperm DNA) containing cardiac actin cDNA probe at 40°C for 24 hours.

The hybridized filters were washed twice in $1 \times$ SSC, 0.1% SDS, and once in $0.1 \times$ SSC, 0.1% SDS at 50°C for 30 minutes, and exposed to Kodak XAR-5 film.

When the DNAs from the rat strains were digested with *Eco*RI and hybridized with the human cardiac actin cDNA as a probe, a large number (11 to 16) of hybridizing fragments were observed (Fig. 1). These fragments ranged from 1 kb to 20 kb in size, and showed varying intensities of hybridization. The results corresponded to the results of Nudel *et al.* [3], in which skeletal muscle α -actin gene had been used as a probe.

Furthermore, when chicken cytoskeletal β -actin cDNA was used as a probe, various *Eco*RI fragments identical to the above-described *Eco*RI fragments were obtained (data not

Table 1. Distributions of the polymorphic *EcoRI* fragments hybridized to the human cardiac actin probe in 13 rat strains.

strain	<i>EcoRI</i> fragments					
	11 kb	7 kb	6 kb	5 kb	4.5 kb	4 kb
WKS/Iar	+	—	+	+	+	+
TOM/Iar	+	—	+	—	—	+
BEG/Iar	—	+	+	—	—	—
F1R/Iar	—	+	+	—	—	—
NIGIII/Iar	—	+	+	—	—	—
WM/Iar	—	—	+	+	—	+
IS/Iar	—	—	+	—	—	+
COP/Iar	—	—	+	—	—	—
LEO/Iar	—	—	+	—	—	—
LET/Iar	—	—	+	—	—	—
TU/Iar	—	—	+	—	—	—
TX/Iar	—	—	+	—	—	—
Iar:WKA	—	+	—	—	+	—

+ : presence, — : absence

shown). Among these fragments, the 8 kb and 5.7 kb fragments represent cytoskeletal β -actin and skeletal muscle α -actin genes, respectively [1]. The various *EcoRI* fragments hybridized to the cardiac actin cDNA probe are thus considered to represent various actin genes or its pseudogenes rather than parts of cardiac actin genes. The low levels of intensities may reflect low level of homology between the probe and the fragments.

Among these *EcoRI* fragments, interstrain variation was observed in six fragments. 11 kb fragment detected in WKS/Iar and TOM/Iar was absent in the remaining 11 strains. In the same manner, 7 kb, 6 kb, 5 kb, 4.5 kb and 4 kb fragments detected in several strains were absent in the remaining strains. Consequently, numbers of the hybridizing fragments were different among the 13 strains. For example, WKS/Iar gave 16 *EcoRI* fragments, whereas LEO/Iar gave only 11 *EcoRI* fragments. Further variable *EcoRI* fragments are likely to be observed, but they were hard to clearly distinguish under such electrophoretic conditions. The remaining *EcoRI* fragments were common to all strains. In repeated tests, individual DNA samples always showed the same electrophoretic pattern, and no variation

was found in the *EcoRI* fragments from the DNA samples of rats of the same strain. The variations in the *EcoRI* fragments are thus considered to be the polymorphisms in the rat strains.

Distributions of these polymorphic six *EcoRI* fragments in the 13 strains are shown in Table 1. No correspondence among distribution patterns of six polymorphic fragments was observed, indicating that the presence or absence of these fragments represent independent polymorphism of rats.

Other restriction endonucleases, *Bam*HI, *Hind*III, and *Pst*I also gave variable fragments, and most of these fragments showed the same distribution patterns as one of the distribution patterns of the *EcoRI* fragments (data not shown). However, these variable restriction fragments were less distinguishable than the *EcoRI* fragments.

In the case of the polymorphisms of the actin-related sequence, the polymorphic fragments which gave intense bands were detected in particular strains while no corresponding fragment was detected in the remaining strains, suggesting that the presence of the polymorphic fragments may reflect the presence of extra sequences homologous to the actin genes in the particular strains. The generation of novel fragments homologous to a particular gene is known to be caused by several mechanisms including integration of cDNA copy of the particular gene which generate a processed pseudogene [10]. Consistent with this, polymorphism caused by the difference in number of processed pseudogene was reported in human dihydrofolate reductase genes [1]. The polymorphic fragments detected in particular rat strains are likely to be caused by the difference in number of the actin-processed pseudogenes, since most of the actin-related sequences of human and mouse are known to represent processed pseudogene of the cytoskeletal β - and γ -actin genes [9] and variation in number of the pseudogene is reported in inbred mouse strains [11]. However, the possibilities that the polymorphisms may represent variations in length of restriction fragments and the absence of the several bands in particular strains may be due to overlapping of common intense bands can not be excluded.

The polymorphisms of the actin-related sequences may be useful for the genetic

monitoring of the laboratory animals, since a number of variable restriction fragments are simultaneously detected. As shown in Table 1, the 13 rat strains were assigned to each of 7 specific patterns of the polymorphic *EcoRI* fragments, although identical patterns were observed among BEG/Iar, F 1 R/Iar, and NIGIII/Iar and among COP/Iar, LEO/Iar, LET/Iar, TU/Iar and TX/Iar. It was thus concluded that the patterns of the *EcoRI* fragment detected by the human cardiac actin probe have a certain degree of strain specificity to characterize particular rat strains and should be useful in the genetic monitoring of laboratory rats.

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ラットのアクチン遺伝子関連配列に検出された多型性

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ヒト心筋アクチン遺伝子の cDNA をプローブとしたサザンブロット法により、ラットにおけるアクチン遺伝子関連配列の多型性を調べた。その結果、制限酵素として *EcoRI* を用いて検出されたフラグメントのうち、11 kb, 7 kb, 6 kb, 5 kb, 4.5 kb および 4 kb のフラグメントにおいて系統間の多型性が認められた。いずれ

の多型性も、特定の系統のみに新たなフラグメントが検出されるものであり、偽遺伝子などのアクチン遺伝子関連配列の存在に起因することが推測された。これらの多型的なフラグメント、調べた13系統において7つの特異的なパターンに分類され、近交型ラットの遺伝的モニタリングに用いることが可能であると思われる。