An altered expression of the Yc subunit gene of rat glutathione S-transferase (GST) in the liver of the LEC rat, which is a mutant strain with spontaneous hereditary hepatitis associated with severe jaundice, has been reported. To provide further information concerning the structure of the Yc subunit gene, we carried out the Southern blot hybridization analysis of DNA samples from rats of eight different inbred strains including LEC with cDNA complementary to mRNA specific for the Yc subunit of rat liver GST as a probe. The hybridization patterns of the DNA samples from rats belonging to the different inbred strains showed interstrain variation in the length of restriction fragments with four restriction endonucleases. Since the DNA samples prepared from several rats of one inbred strain gave an identical hybridization pattern, the restriction fragment patterns for the Yc gene could be used as markers for genetic monitoring of inbred rat strains. Although the altered expression of Yc-Yc activity of GST has been observed in the liver of the LEC rat, the characteristic changes in the gene structure of the Yc subunit of LEC rat were not detected in the present hybridization analysis.
RESTRICTION FRAGMENT LENGTH POLYMORPHISM
FOR THE YC SUBUNIT GENE OF RAT LIVER
GLUTATHIONE S-TRANSFERASE

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(GST) in the liver of the LEC rat, which is a mutant strain with spontaneous
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carried out the Southern blot hybridization analysis of DNA samples from rats of
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patterns of the DNA samples from rats belonging to the different inbred strains
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structure of the Yc subunit of LEC rat were not detected in the present
hybridization analysis.

Key Words: RFLP; Yc subunit gene; rat liver GST;

INTRODUCTION

Two inbred strains designated LEC and LEA rats have been established from a
closed colony of the Long-Evans rats at the Center for Experimental Plants and
Animals, Hokkaido University17. Spontaneous fulminant hepatitis with severe jaundice
occurred in about 80% of LEC rats between 4 to 5 months of age and most of the
affected rats died within two weeks of the onset of jaundice10,17. Thus, the LEC rat
provides a new model for the study of human fulminant hepatitis. Changes in the
activities of some metabolizing enzymes in the liver of the LEC rat have been

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reported\textsuperscript{12,20}. An altered expression of liver GST, which plays important roles in drug biotransformation and xenobiotic metabolism and in binding specificity to various hydrophobic compounds including bilirubin\textsuperscript{5,7,9}, has also been reported in the liver of LEC rat\textsuperscript{11}. The rat liver GSTs are homodimers or heterodimers consisting of subunit families which are designated Ya, Yb (Yb1, Yb2) and Yc\textsuperscript{6}. The subunits are products of the separate gene families\textsuperscript{22}. Although cDNA clones which are complementary to mRNAs specific for Ya, Yb1, Yb2, and Yc subunits have been constructed by Pickett and the coworkers\textsuperscript{2,3,15,21}, gene structure of each subunit has not yet been determined. Characteristic altered expression of Yc-Yc activity of GST has been observed in the liver of LEC rat before the onset of jaundice\textsuperscript{11}. In order to get further information concerning the gene structure of Yc subunit, we carried out the Southern blot hybridization analysis using cDNA complementary to mRNA specific for the Yc subunit as a probe.

**MATERIALS AND METHODS**

**Reagents**: Restriction endonucleases and agarose were purchased from Nippon Gene Chemical Co. and [\alpha\textsuperscript{\textsuperscript{32}}P]dCTP (111TBq/mmole) from ICN Radiochemicals, USA.

**Animals**: Eight inbred strains of rats (Rattus norvegicus) at 1–2 months of age were used. ACIIHkm, LEJ/Hkm, LEA/Hkm, LEC/Hkm, BUF/Hkm, W/Hkm and WKAH/Hkm were maintained at the Institute for Animal Experimentation, Hokkaido University and BN/Hok was maintained at the Center for Experimental Plants and Animals, Hokkaido University.

**Genomic DNA and cloned DNA**: Genomic DNA was prepared from the rat spleen by treatment with proteinase K and sodium dodecyl sulfate (SDS) followed by phenol extraction. A pGTB42 clone, which contains a cDNA complementary to mRNA specific for the Yc subunit of the rat liver GST was kindly provided by Dr. Pickett\textsuperscript{21}.

![Fig. 1 Restriction endonuclease map of pGTB42.](image)

The black box represents the coding sequence for the Yc subunit of rat liver GST. The dashed line represents pBR322 sequences.

P: *PstI*; P\textsubscript{v}: *PvuII*; A: *AvaII*; Ba: *BaiI*; Bg: *BglII*; H: *HindIII*. 
The pGTB42 cloned DNA was prepared by the method of Maniatis et al. A restriction map of cDNA corresponding to Yc-mRNA is shown in Fig. 1. 

**Southern blot hybridization**: Twenty micrograms of genomic DNAs were digested with restriction endonuclease described in the legends to the figure. The DNA fragments were electrophoretically separated in 0.6% agarose gel and the DNAs were transferred onto nitrocellulose paper by the method of Southern. The pGTB42 DNA was radiolabelled as a probe by 32P-nick translation. Hybridization was carried out under the conditions described previously, i.e., 6×SSC (1×SSC is 0.15M NaCl, 0.015M sodium citrate), 5×Denhardt's solution (1×Denhardt's solution is 0.05% bovine serum albumin, 0.05% Ficoll, 0.05% polyvinyl pyrrolidone), 0.5% SDS, 100 μg/ml of yeast t-RNA (Sigma chemical Co.) and 50% formamide at 45°C for 24 hr. The hybridized filters were washed once in 2×SSC, 0.1% SDS at 42°C for 30 min and 3 times in 0.3×SSC, 0.1% SDS at room temperature for 30 min each time and exposed to Fuji RX X-ray film (Fuji photochemical Co.) with an intensifying screen at −70°C for 5–7 days.

**RESULTS**

The DNA samples were prepared from 3 to 6 separate rats of each inbred strain, digested with restriction endonucleases EcoRI, BamHI, PstI or HindIII, and analyzed by Southern blot hybridization using cDNA corresponding to the Yc subunit of rat liver GST as a probe. The DNA digests from rats of each strain gave the identical hybridization patterns within the strain (data not shown).

When the DNA samples from rats belonging to eight different inbred strains including LEC and LEA were digested with PstI, six hybridized fragments were observed in PstI-digests from each of 8 strains with Yc-cDNA as a probe, as shown in Fig. 2. Five of six fragments; 12.0, 6.6, 5.0, 3.9 and 1.8 kilobase pair (kbp) fragments, were shown to be common to all 8 strains. The fragments with different lengths were observed between PstI-digests from LEC and LEA rats (2.1-kbp for the LEA rat and 2.3-kbp for the LEC rat, Fig. 2; lane 3 & 4). The 2.1-kbp fragment was observed in the DNA sample from the WKAH (lane 8) rat, and 2.3-kbp fragments were observed in the DNA samples from ACI (lane 1), LEJ (lane 2), BN (lane 5), BUF (lane 6) and W (lane 7) rats.

The different digestion patterns were also observed between the DNA samples from LEC and LEA rats with EcoRI and HindIII (Table I). Three of five fragments (9.8, 6.6 and 4.2-kbp for the LEA rat, and 9.6, 6.4 and 3.9-kbp for the LEC rat) with EcoRI and one of four fragments (2.6-kbp for the LEA rat and 2.3-kbp for the LEC rat) with HindIII were different between the digests from LEA and LEC rats. However, no specific hybridized fragments for the digests from LEC or LEA rats were shown. The fragments which were found in the digests from LEC or LEA rats were observed in the digests from some other strains of rats (Table I). In the case of
Fig. 2 Southern blot hybridization analysis for the PstI-digests from inbred rats.

HindIII-digested lambda DNA was used as a size marker.
Arrow heads represent fragments with different sizes between the digests from LEC and LEA rats.

BamHI-digestion, the hybridization pattern of the DNA sample from the LEC rat was the same as that from the LEA rat (Table I). However, the fragments which were not found in the BamHI-digests from LEA and LEC rats were observed in the digests from the other strains of rats.
Table 1. Distribution of hybridized fragments of the Yc gene in inbred rat strains

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— : fragment was not detected.
An altered expression of the Yc gene of rat liver GST has been reported in the liver of the LEC rat\(^{11}\) which is a mutant strain with spontaneous hereditary hepatitis associated with severe jaundice\(^{17}\). To provide further information concerning the gene structure of the Yc subunit, Southern blot hybridization patterns of the DNA samples from the LEC rat were compared with those of other strains of rats, including the LEA rat which is a sibling line of the LEC rat, using cDNA complementary to mRNA specific for Yc subunit of rat liver GST as a probe. In spite of the relatively small size (860-bp) of the probe (Fig. 1), Southern blot hybridization analysis showed several hybridized fragments in the digested DNA samples with \(PstI\), \(EcoRI\), \(HindIII\) or \(BamHI\) from all strains of rats (Fig. 2, and Table I). The DNA samples prepared from several rats of one inbred strain gave an identical hybridization pattern (data not shown). On the contrary, different hybridization patterns between DNA samples from the LEC rat and the LEA rat were obtained with \(PstI\), \(EcoRI\) and \(HindIII\). However, no specific fragments for the digests from the LEC rat could be detected. Fragments with the same lengths were found in the digests from some other inbred strains of rats. Therefore, the characteristic changes in the gene structure of the Yc subunit of the LEC rat were not detected in the present hybridization analysis.

From the present study the strain-specific patterns of restriction fragment length polymorphisms (RFLPs) were shown in the DNA samples from eight different inbred strains of rats. cDNA corresponding to the Yc subunit of rat liver GST was used as a probe with several restriction endonucleases. Since intrastrain variations in the length of restriction fragments were not observed, RFLPs for the Yc gene could be used as markers for the genetic monitoring of inbred strains of rats.

The presence of a pseudogene for the Yp subunit of rat placental GST\(^{14}\), which is present at very low levels in normal liver but occurs in elevated amounts in preneoplastic nodules and hepatomas, has been reported\(^{13,18}\). Preliminary experiments using synthetic oligonucleotides specific for the coding region of the Yc gene as probes, showed several hybridized fragments in the digests with \(EcoRI\) and \(HindIII\) from LEC, LEA and BN rats (data not shown). This result suggest the presence of the pseudogene of the Yc subunit. Therefore, some hybridized fragments might be derived from the Yc pseudogene in the present study.

The nucleotide sequence of genomic Yc-DNA and its structure should be analyzed for the study of an altered expression of the Yc gene in the liver of the LEC rat.

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