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**Glutathione S-transferase Ya subunit is coded by a multigene family located on a single mouse chromosome**

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**ABSTRACT**

A cloned DNA probe of Ya, the major glutathione S-transferase subunit in rat liver, was used to study the organization of Ya genes in the mouse genome. Southern blot analysis of mouse genomic DNA indicates that the Ya subunit is encoded by a multigene family. The chromosomal distribution of Ya genes was determined by analysis of DNA from a panel of mouse-Chinese hamster somatic cell hybrids. All detectable Ya genes were found to be located on chromosome 9. At least some of the Ya-specific DNA sequences are clustered since, by screening a mouse genomic library, two recombinant phages, each containing two different Ya DNA sequences in the same insert, have been isolated. The finding that Ya is encoded by a cluster of different genes raises the question of the specificity of the different Ya DNA sequences.

**INTRODUCTION**

Glutathione S-transferases are a group of widely distributed drug metabolizing enzymes that catalyze conjugation of reduced glutathione (GSH) with a variety of electrophilic compounds most of which derive from the oxidative metabolism of xenobiotics (1-4). These enzymes contribute to the elimination of reactive and toxic metabolites, mutagens and carcinogens and constitute a tissue protection system (5, 6). In addition, the glutathione S-transferases act as non-specific binding proteins facilitating the transport of a variety of ligands of both endogenous (heme metabolites) and exogenous (drugs, xenobiotics) origin. At least six glutathione S-transferases have been characterized in rat liver and all are dimers of four subunits: Ya, Yb<sup>1</sup>, Yb<sup>2</sup> and Yc (7-10). The Ya subunit (Mr = 22,000), the most abundant in liver, occurs as YaYa in ligandin and as YaYc in GSH-transferase B (8, 10). Ya is multifunctional: it has a high affinity binding site for hydrophobic molecules such as bilirubin and exogenous drugs and carcinogens (5, 11), and, in addition to glutathione transferase, exhibits also steroid isomerase (12) and GSH-peroxidase (13) activities. Ya expression is regulated by xenobiotics. It is induced together with Yb<sup>1</sup> by phenobarbital (7) and selectively by 3-methylcholanthrene (14). It was

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shown that the induction of Ya synthesis by phenobarbital and polycyclic aromatic hydrocarbons is accompanied by an increase in the levels of Ya mRNA (15-17).

For an understanding of the possible mechanisms involved in the regulation of Ya gene expression we have presently studied the organization of Ya genes in the mouse genome. Using a rat Ya cDNA probe (17) we report here that in mouse the Ya-related sequences form a family of different genes located on a single chromosome, some clustered and possibly selectively expressed under the control of different inducers.

### MATERIALS AND METHODS

#### Hybridization probe

Rat liver Ya cDNA plasmid A24 (17) was digested with Bgl II and Pst I and the 291 bp Ya DNA fragment was separated by 5% acrylamide gel electrophoresis followed by electroelution and ethanol precipitation. The DNA fragment was labeled in vitro by nick translation (18) using  $^{32}\text{P}$ - $\alpha$ [dCTP] and  $^{32}\text{P}$ - $\alpha$ [dATP] (3000 Ci/mmol) from Amersham. E. coli DNA polymerase was from Boehringer-Mannheim and DNase I from Sigma.

#### Preparation and hybridization of DNA

High molecular weight DNA was isolated from cultures of the L8 rat myoblast cell line and from mouse liver as described by Bellard et al. (19) and from parental and somatic hybrid cell lines as described by D'Eustachio et al (20).

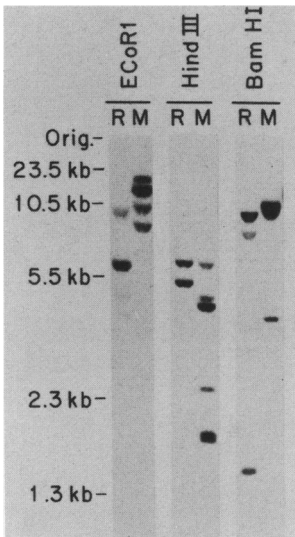
The DNAs were cleaved with various restriction endonucleases as mentioned in the legends of figures. The DNA fragments were separated by electrophoresis in a 1% agarose gel, transferred to nitrocellulose filters as described by Southern (21) and hybridized to nick-translated (18) DNA of Ya clone A24 (17) as described by Carmon et al. (22). The filters were washed with 750 mM NaCl - 75 mM Trisodium citrate at 60°C for 1 hour and subjected to autoradiography. For a more stringent wash, the filters were incubated at 70°C for 1 hour with 75 mM NaCl - 7.5 mM Trisodium citrate.

#### Screening of mouse genomic DNA library

A mouse genomic library prepared from a partial EcoRI digest of mouse sperm DNA inserted into the bacteriophage  $\lambda$ Charon 4A (a gift from D. Givol) was screened for Ya DNA sequences as described (23), using the  $^{32}\text{P}$ -labelled insert of Ya plasmid A24 (17) as a probe.

#### Mouse-Chinese hamster somatic cell hybrids

The somatic cell hybrids used to assign the Ya multigene family to mouse



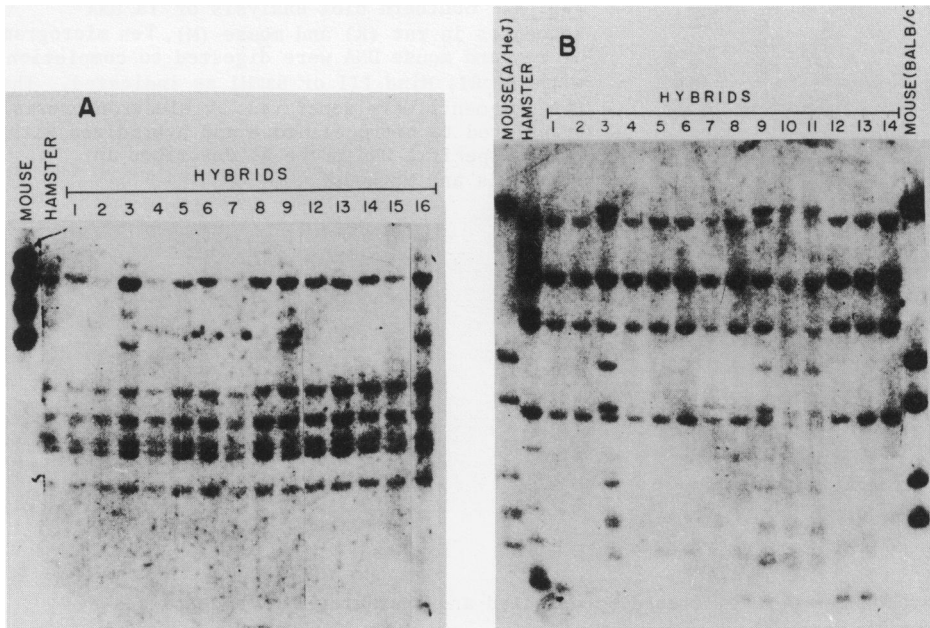
**Fig. 1:** Southern blot analysis of Ya DNA sequences in rat (R) and mouse (M). Ten micrograms of rat and mouse DNA were digested to completion with EcoRI, Hind III or BamHI as indicated. The DNA fragments were separated by electrophoresis, transferred to nitrocellulose and hybridized with the Ya-specific DNA probe as described in Materials and Methods.

chromosomes were generated, propagated and characterized as described by D'Eustachio et al. (20, 25). Somatic cell hybrids were formed between the Chinese hamster fibroblast cell line E36 and either peritoneal macrophages from A/HeJ mice (MACH hybrid series), fibroblasts from BALB/c fetal mice (BEM hybrid series), cells from a tissue culture adapted subline of methylcholanthrene-induced Meth A BALB/c mouse fibrosarcoma (MAE hybrid series), cells from a CMS4 tumor of BALB/c mouse (TuCE hybrid series), or cells from the C3H murine cell line CTIIC (hybrid ECm4e). Karyotype and isozyme analysis to determine the mouse complement of a hybrid cell line were carried out on samples from the same cell population used for DNA preparation (25).

## RESULTS

### Southern blot analysis of rat and mouse Ya DNA sequences

A rat Ya-specific (291 bp) DNA sequence (Bgl II-Pst I fragment of plasmid A24 (17)) representing the middle of the Ya coding sequence, was used to probe the rat and mouse genomes for Ya DNA sequences. Southern blots of rat and mouse DNA cleaved with various restriction enzymes (EcoRI, Hind III, BamH I) were hybridized with the radiolabeled probe. Fig. 1 shows that the Ya probe detects several genomic DNA fragments of different size in both rat and mouse. As the probe is fairly small we can assume that the number of different DNA fragments reacting with that probe reflects



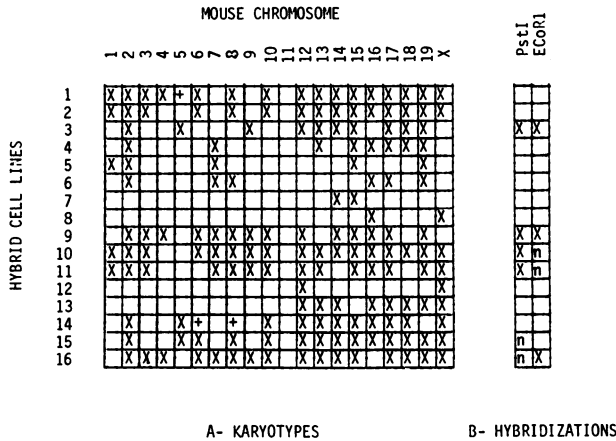
**Fig. 2:** Presence of mouse Ya DNA sequences in mouse-Chinese hamster hybrid cell lines as determined by Southern blot analysis

Ten micrograms of DNA isolated from parental and hybrid cell lines (described in Fig. 3) were restricted with EcoRI (A) or Pst I (B). The DNA fragments were separated by electrophoresis, transferred to nitrocellulose filters and hybridized with the Ya-specific DNA probe as described in Materials and Methods.

roughly the number of Ya genes (or pseudogenes). Therefore, in the mouse, Ya is encoded by a multigene family. The genes in this family seem to have very closely related DNA sequences, at least in the region that the probe detects, because the number of reacting DNA fragments does not change upon highly stringent washing conditions (see Materials and Methods). It can also be noted that the number of Ya hybridizing DNA fragments in the mouse is greater than that found in the rat.

Chromosomal assignment of Ya genes in mouse

The organization of the Ya gene family in the mouse genome was analyzed by assigning the different Ya DNA sequences to mouse chromosomes. This was achieved by using a panel of mouse-Chinese hamster somatic hybrid cell lines, each line containing different incomplete overlapping subset of mouse chromosomes on a hamster background. DNA from parental cells and from hybrid cells was cleaved with restriction endonuclease Pst I or EcoRI, the fragments were



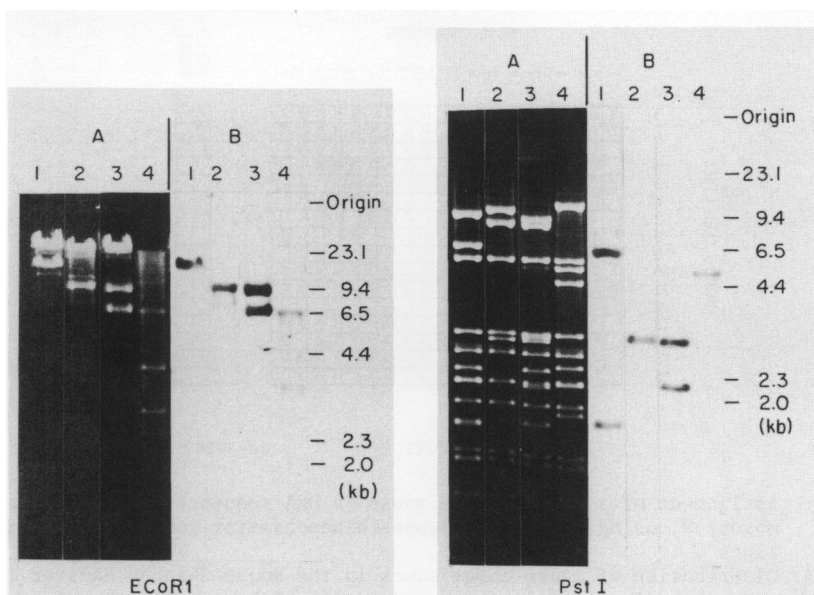
**Fig. 3:** Assignment of all detectable mouse Ya DNA sequences to mouse chromosome 9, using a panel of mouse-Chinese hamster somatic hybrid cell lines

(A) Distribution of mouse chromosomes in the mouse-Chinese hamster cell lines  $\boxtimes$  : The hybrid cell line (numbered vertically) contains the mouse chromosome (marked horizontally) in 25% or more of the cells analyzed.  $\square$  : The hybrid contains the mouse chromosome in less than 25% of the cells analyzed or has lost it altogether.  $\boxplus$  : Chromosome detected by isozyme analysis only. (B) Hybridization of the Ya-specific probe with the DNA from the various cell lines cut with PstI or EcoRI. The vertical columns indicate the hybrid cell lines tested for the presence of mouse Ya DNA sequences. The presence of mouse ligandin DNA sequences in a given hybrid cell line is indicated by an X, and its absence by an empty square. 'n' indicates that the given hybrid was not tested for the presence of mouse Ya DNA sequences. Chromosomal assignment is done by comparing the pattern of hybridization in (B) with the chromosome distribution in (A).

The hybrid cell lines used in this study were the following: 1: BEM 1-6; 2: BEM 1-4; 3: MACH 7A13-3B3; 4: MACH 4A64; 5: MACH 4A64-A1; 6: MACH 4B31A23; 7: ECm4e; 8: MAE 32; 9: MACH 2A2-B1; 10: MACH 2A2-A1; 11: MACH 2A2-C2; 12: MAE 4; 13: TuCE 12G/8; 14: TuCE 12G/5; 15: TuCE 12G/3; 16: mFE 2/1/1.

separated by agarose gel electrophoresis, blotted onto nitrocellulose filters and hybridized with the specific Ya DNA probe (Fig. 2A and B). The probe reacts with six different mouse Pst I DNA fragments (cell lines 3, 9, 10 and 11) and with four different mouse EcoRI DNA fragments (cell lines 3, 9 and 16). Examination of the hybrid cell lines indicates that all mouse Ya DNA fragments segregated together in the positive hybrids indicating that all detectable Ya-specific DNA fragments are located on a single mouse chromosome.

Out of the 16 cell lines tested, five were positive for the presence of mouse Ya DNA, 11 were negative. The Ya-specific genes (and pseudogenes) were



**Fig. 4:** EcoRI and Pst I analysis of  $\lambda$ Ch4-mouse DNA recombinants

Two  $\mu$ g DNA of the isolated recombinants  $\lambda$ mL<sub>1</sub>,  $\lambda$ mL<sub>3</sub>,  $\lambda$ mL<sub>7</sub> and  $\lambda$ mL<sub>8</sub> labeled 1 to 4 respectively, were digested with EcoRI or Pst I, the DNA fragments separated by electrophoresis on 1% agarose gels and stained with ethidium bromide (A). The gel was blotted onto nitrocellulose filters, hybridized with the <sup>32</sup>P-labeled Ya-specific probe (Materials and Methods) and autoradiographed (B).

assigned to mouse chromosomes by comparing the chromosomal composition of the different hybrid cell lines with the presence or absence of hybridizable Ya mouse DNA fragments (Fig. 3). Such an analysis indicates that all Ya DNA sequences are located on mouse chromosome 9.

Arrangement of Ya genes on the mouse chromosome

The Ya DNA sequences might be clustered in a single locus on chromosome 9 or might be dispersed along the chromosome. It is also possible that both types of organization coexist in the mouse genome. In order to investigate these possibilities we have screened a mouse genomic library, constructed by the insertion of EcoRI incomplete digests of mouse sperm DNA into bacteriophage  $\lambda$ Charon 4, with the rat Ya DNA probe. Ten phages were isolated and four were found to be different from one another. Fig. 4 shows the EcoRI and Pst I cleavage of the DNA from the four recombinant phages ( $\lambda$ mL<sub>1</sub>,  $\lambda$ mL<sub>3</sub>,  $\lambda$ mL<sub>7</sub> and  $\lambda$ mL<sub>8</sub>) and the hybridization with Ya DNA probe.

The isolated EcoRI mouse DNA inserts containing Ya sequences (7, 10 and 14 kb) correspond to three of the hybridizing DNA bands observed in the

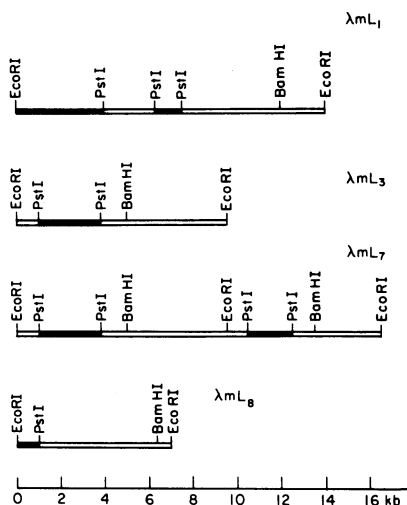


Fig. 5: Physical maps of mouse Ya clones.  $\lambda mL_1$ ,  $\lambda mL_3$ ,  $\lambda mL_7$  and  $\lambda mL_8$  DNAs (1 to 4 respectively) were cleaved with combinations of EcoRI, BamHI and Pst I. The DNA fragments were separated on 1% agarose gel, blotted onto nitrocellulose filters and hybridized with Ya DNA probe. Solid bars indicate fragments containing Ya sequences.

mouse genomic blot hybridization described in Fig. 1. Restriction map analysis of the inserts in the four phage recombinants (Fig. 5) indicates that two of them,  $\lambda mL_1$  and  $\lambda mL_7$ , carry each two different contiguous Ya DNA sequences (Fig. 5). This indicates that at least some of the Ya genes (and/or pseudogenes) are clustered.

#### DISCUSSION

The present experiments indicate that the genes coding for glutathione S-transferase subunit Ya, expressed in rat liver as the major transferase ligandin (YaYa), occur in the mouse genome as a multigene family. Southern blot hybridization of an EcoRI digest of mouse genomic DNA with Ya DNA probe has produced four hybridization bands (Fig. 1). Genomic cloning experiments have shown that three of the four bands represent five distinct genes and that some bands contain more than one Ya-specific sequence (Fig. 5). A fourth hybridization band (about 20 kb in Fig. 1) was not yet detected among the clones isolated from the mouse library.

Multigene families are known to be organized in different ways. The member genes might be clustered in a single chromosomal locus (i.e. myosin heavy chain (26, 27) ) or might be dispersed in the genome (i.e. actin 26, 28). This pattern of organization may reflect the evolutionary history of the gene family as well as the mode of expression of the gene members. In the mouse, the glutathione S-transferase Ya subunit gene family is presently found to be located exclusively on chromosome 9. At

least some of these genes are clustered since two mouse genomic fragments isolated from the mouse library contain two different contiguous Ya-specific sequences. The finding that glutathione S-transferase Ya subunit is encoded in mouse by a number of different genes (some of them probably pseudogenes) raises the question of the specificity of these genes and their selective expression. It may be speculated that different drugs and xenobiotics, which induce Ya in rat liver, act selectively activating individual Ya mouse genes. The glutathione S-transferases in mouse liver however have not yet been characterized as well as in the rat liver and their pattern of induction by drugs, xenobiotics and antioxidants is not known. The presence of a mouse ligandin (YaYa), probably induced by methylcholanthrene, was demonstrated in liver and found to be immunologically similar to that of rat liver ligandin (29). Our results support the conservation of Ya sequences between the genomes of rat and mouse. In addition to the hybridization of the rat Ya DNA probe (291 bp Bgl II-Pst I fragment of A24 clone (17) with the mouse sequences under stringent washing conditions (Fig. 1 and Fig. 4) we have observed high homology also in the 3' end of the non coding region of the gene using a 3' end-specific rat Ya probe (160 bp Hind III fragment of C70 clone) (data not shown). Some homology of sequences between rat glutathione S-transferase Ya and Yc subunits was postulated from the study of the aminoacid composition, tryptic digests and immunoreactivity (8). Although we cannot completely exclude the possibility that our Ya specific DNA probe would detect also Yc sequences, we consider it improbable since we did not observe the Ya DNA clones to select by hybridization from rat liver mRNA the Yc mRNA (17).

Further studies of the genomic sequences of Ya genes presently isolated and their specific expression will provide insight into the relationship among the members of this gene family and mechanisms involved in their activation.

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