Chromosomal localization of the rat albumin gene in normal and analbuminemic rats determined by *in situ* hybridization

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(Received July 3, 1984)

ABSTRACT

Recombinant plasmids containing rat albumin cDNA were radiolabeled and hybridized *in situ* to metaphase chromosome preparations of Wistar strain rats. Results showed that in a significantly high percentage of the hybridized cells (27%) silver grains were located on the distal portion of chromosome 2: silver grains on this portion amounted to 17% of all the chromosomal label. Thus the albumin gene locus is mapped on chromosome 2. Therefore, the sixth linkage group, including the gene loci of albumin, the plasma protein pattern (G1-1) and a coat color (h), is probably located on chromosome 2. The chromosomal location of the albumin gene was examined in an albumin-deficient strain of Sprague-Dawley rats. Of the silver grains, 18% were located on the distal region of chromosome 2. These findings support the previous conclusion that the albumin gene is not deleted from analbuminemic rats, and also indicate that the albumin gene locus is not displaced in these rats.

1. INTRODUCTION

Albumin is a major serum protein, that is known to participate in several important functions of serum (Rothschild *et al.* 1972). An albumin-deficient strain of rats has been established from a stock of Sprague-Dawley rats (Nagase analbuminemia rats, NAR). The rats are characterized by genetic lack of serum albumin (Nagase *et al.* 1979), and the analbuminemia is inherited as an autosomal recessive trait (Nagase *et al.* 1980). From a linkage study of the analbuminemia with some coat color loci, the albumin gene locus was assigned to the sixth linkage group of the rat (Shumiya and Nagase 1982). However, there are few reports on the correspondence of linkage groups with the chromosomal locations of gene loci in rats.

Recently, several single-copy genes have been mapped on specific human chromosomes by *in situ* hybridization of purified cDNA clones to metaphase chromosomes. Hybridization reactions were carried out in the presence of 10% dextran sulfate which, together with use of radiolabeled cloned probes, increases the amount of signal and allows the detection of single-copy sequences

(Harper and Saunders 1981). In the present work, we used this hybridization technique to determine the chromosomal location of the rat albumin gene. Results indicated that the albumin gene is located on chromosome 2.

2. MATERIALS AND METHODS

Preparation of chromosomes. Mitotic chromosome preparations were obtained from rat fibroblastic cells by the method of Yosida (1980). Small pieces of tail tissue were cultured in Eagle's minimal essential medium supplemented with 0.2 mM L-serine, 1 mM sodium pyruvate and 20% heat-inactivated fetal calf serum (Flow Lab., Australia). After about one week, growing cells were treated with colcemid (0.1 μ g/ml) for 30 min. The cells were removed by trypsinization, and the cell suspension was treated with 0.075 M potassium chloride at 37°C for 15 min. Then the cells were fixed in a solution of ethanol and acetic acid (3:1). The fixed cells were spread on glass slides in a humid atmosphere at 60°C. Chromosome preparations were used within 10 days for *in situ* hybridization.

Radiolabeling of probe. Recombinant plasmid prAlbI containing a 1200-nucleotide insert of rat albumin cDNA was cloned in the plasmid pBR 322 (Kioussis *et al.* 1979), which was a gift from Dr. H. Esumi (Saitama Medical School, Saitama, JAPAN). DNA prAlbI was radiolabeled by nick-translation using [3 H]dCTP (58.3 Ci/mmol), [3 H]dATP (57.5 Ci/mmol), and [3 H]dTTP (30.0 Ci/mmol) (Radiochemical Centre, Amersham) as described by Rigby *et al.* (1977). The DNA, labeled at specific activity of $1-2 \times 10^7$ cpm/ μ g, was heated in 10 mM Tris-HCl buffer (pH 7.5) containing 1 mM EDTA, 0.3 M NaCl and 1000-fold excess of sonicated salmon sperm DNA at 100°C for 3 min, and cooled rapidly.

In situ hybridization. Hybridization was carried out as described by Harper *et al.* (1981) and Trent *et al.* (1982). Chromosome preparations were treated with pancreatic ribonuclease A (Sigma Chemical Co., USA) at 100 μ g/ml in $2 \times$ SSC at 37°C for 1 h, and rinsed well with $2 \times$ SSC. The slides were dehydrated by successive immersion in 70%, 80%, and 90% ethanol for 2 min each, and then air-dried. The chromosomal DNA was denatured by treatment with 70% (v/v) formamide in $2 \times$ SSC at 70°C for 3 min, followed by dehydration in a graded series of ethanol. The hybridization mixture consisted of 3 H-labeled plasmid prAlbI (100–150 ng/ml) in $2 \times$ SSC containing 50% (v/v) formamide, 10% dextran sulfate (pH 7.0), 40 mM sodium phosphate buffer (pH 6.8) and 1000-fold excess of sonicated salmon sperm DNA. This hybridization mixture was placed on slides (60 μ l/slide), and covered with a cover glass. Incubation was performed in a moist chamber containing 50% formamide in $2 \times$ SSC for 14–16 h at 37°C. Then the slides were rinsed three times with $2 \times$ SSC con-

taining 50% formamide and once with $2\times$ SSC at 39°C, and dehydrated in an ethanol series.

Autoradiography. Slides were exposed to Sakura NRM2 nuclear track emulsion (Konishiroku Photo Ind., Tokyo) diluted with distilled water (1:1) at -80°C for 1-3 weeks. Slides were developed with Sakura Konidol X, fixed and rinsed with water.

Staining and Statistic analysis. G-Banding was accomplished by the method of Chandler and Yunis (1978). Hybridized chromosomes were treated with 0.025% trypsin (Difco) in 0.06 M phosphate buffer (pH 6.8) at 20°C for 1-2 min, and then stained with 5% Giemsa stain. The stained chromosomes were destained by treatments with 95% ethanol for 2 min, chloroform for 15 sec, 95% ethanol containing 1% HCl for 30 sec, and then 100% methanol for 2 min. The chromosomes were stained and destained once more, and finally stained with 5% Giemsa stain, and air-dried. Silver grains were identified directly and photographed under a microscope. Data on the grain distribution were converted to schematic representations of chromosomes by the method of Harper *et al.* (1983). The chromosomal nomenclature used was that recommended by the Committee for a Standardized Karyotype of *Rattus norvegicus* (1973).

3. RESULTS AND DISCUSSION

In situ hybridization of the ^3H -labeled recombinant plasmid (prAlbI) containing albumin-genomic sequences was carried out on chromosome preparations of fibroblastic cells from Wistar rats. Of the cells examined, 30% (63/210) had an average of 1.7 silver grains per cell (Fig. 1), but a significantly high proportion (17%) of the silver grains (18/104) was located on the distal region of chromosome 2 (Fig. 2). This region exhibited 70% of the grains located on chromosome 2, as illustrated by clustering of grains on chromosome 2 (Fig. 3). Other portions of chromosomes constituted less than 2% of all the labeled sites in the hybridized cells (Fig. 2). These findings indicated that the rat albumin gene was located in the distal portion of chromosome 2.

Next, *in situ* hybridization of cloned cDNA prAlbI was performed using normal and analbuminemic Sprague-Dawley rats. Of the normal rat cells examined, 28% exhibited an average of 1.4 silver grains per cell. A significantly high proportion of all the grains (17% or 9/52) showed a label on the distal portion of chromosome 2 (Fig. 4a). Of the analbuminemic rat cells, 33% of the cells analysed (27/82) showed an average of 1.6 silver grains per cell. Of 27 hybridized cells, 33% exhibited a label on the distal portion of chromosome 2. This site represented 18% (8/44) of all the labeled sites (Fig.

Fig. 1. Rat metaphase chromosomes hybridized with ^3H -labeled prAlbI, showing the rat albumin gene typically labeled on chromosome 2 (arrow). A, metaphase cell. B, labelings on the distal portion of chromosome 2 from 6 different cells hybridized *in situ* with ^3H -labeled prAlbI.

4b). Thus the albumin gene in analbuminemic rats is also located in the distal portion of chromosome 2.

In situ hybridization of chromosome preparations has been used to map human genes (Harper *et al.* 1981, and Trend *et al.* 1982). With single-copy human genes, about 10 to 30 percent of all the labeled sites were located in specific regions of the chromosomes. Thus in this study on the rat albumin

Fig. 2. Schematic presentation of chromosomal location of the albumin gene in Wistar rats. Total length of chromosomes illustrated in the standard haploid karyotype (1973) including the X and Y chromosomes was divided into 88 equal segments and the number of labeled sites from 63 cells is shown by bars in each segment.

Fig. 3. Composite of labelings with ^3H -labeled prAlbI on chromosome 2 from 63 hybridized cells in Fig. 2.

gene, the percentage of specific labeled sites was within the range observed with human genes. The technique of *in site* hybridization should thus be useful for chromosomal location of various other single-copy genes of rats besides the albumin gene.

Using the somatic cell hybrid technique, Oikawa *et al.* (1983) recently found that the gene of a rat major histocompatibility complex (RT1) was located on chromosome 14. Since the albumin gene locus is known to be linked to the sixth linkage group of the rat (Shumiya and Nagase 1982), like the plasma protein pattern (GI-1) and a coat color (Hooded, h) (Womack and sharp 1976), our results suggest that the sixth linkage group may be located on chromo-

Fig. 4. Distributions of labeled sites in hybridized cells of normal and analbuminemic Sprague-Dawley rats. *In situ* hybridization was performed with cloned DNA prAlbI (150 ng/ml) for chromosome preparations. Hybridized chromosomes were exposed to nuclear track emulsion for 18 days. a. Composite from 36 hybridized cells of normal rats; b. Composite from 27 hybridized cells of analbuminemic rats. Refer Fig. 2 to the data on labeled sites.

some 2 in the rat.

The chromosomal location of the albumin gene was found to be the same in analbuminemic rats as in normal rats. Since prAlbI clone used in this report has a 1200-nucleotide insert, which is derived from the 5' half of the albumin mRNA, the clone would not be able to detect the 3' half of the albumin gene. However, Esumi *et al.* (1983) showed that the analbuminemic rats carried an almost complete sequence of the structurally normal albumin gene, excepting 7 base pairs of deletion. These findings indicate that genomic DNA for albumin was not translocated in the analbuminemic rats. Thus the trace amount of albumin present in these rats probably comes from a single locus of the albumin gene on chromosome 2.

Recently, Szpirer *et al.* (1984) reported that the rat albumin gene was mapped on chromosome 14 by mouse-rat cell hybridization technique. This finding is not agreed with our result that the albumin gene was located on chromosome 2. The reason for this discrepancy is not clear, but it may be related to the differences in the method of gene-mapping, cDNA probes used and chromosome analysis, or to the influence of pseudogene. We should investigate the problem.

This study was carried out by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan. Contribution No. 1556 from the National Institute of Genetics, Japan.

REFERENCES

- CHANDLER, M. E. and YUNIS, J. J. (1978) A high resolution *in situ* hybridization technique for the direct visualization of labeled G-banded early metaphase and prophase chromosomes. *Cytogenet. Cell Genet.* **22**, 352-356.
- ESUMI, H., TAKAHASHI, Y., SATO, S., NAGASE, S. and SUGIMURA, T. (1983) A seven-base-pair deletion in an intron of the albumin gene of analbuminemic rats. *Proc. Natl. Acad. Sci. USA* **80**, 95-99.
- HARPER, M. E., FRANCHINI, G., LOVE, J., SIMON, M. I., GALLO, R. C. and WONG-STAAAL, F. (1983) Chromosomal sublocation of human *c-myb* and *c-fes* cellular onc genes. *Nature* **304**, 169-171.
- HARPER, M. E. and SAUNDERS, G. F. (1981) Localization of single copy DNA sequences on G-banded human chromosomes by *in situ* hybridization. *Chromosoma (Berl.)* **83**, 431-439.
- HARPER, M. E., ULLRICH, A. and SAUNDERS, G. F. (1981) Localization of the human insulin gene to the distal end of the short arm of chromosome 11. *Proc. Natl. Acad. Sci. USA* **78**, 4458-4460.
- KIOUSSIS, D., HAMILTON, R., HANSON, R. W., TILGHMAN, S. M. and TYLOR, J. M. (1979) Construction and cloning of rat albumin structural gene sequences. *Proc. Natl. Acad. Sci. USA* **76**, 4370-4374.
- MURRAY, J. C., DEMOPULOS, C. M., LAWN, R. M. and MOTULSKY, A. G. (1983) Molecular genetics of human serum albumin: Restriction enzyme fragment length polymorphisms and analbuminemia. *Proc. Natl. Acad. Sci. USA* **80**, 5951-5955.
- NAGASE, S., SHIMAMUNE, K. and SHUMIYA, S. (1979) Albumin-deficient rat mutant. *Science* **205**, 590-591.
- NAGASE, S., SHIMAMUNE, K. and SHUMIYA, S. (1980) An animal model for analbuminemia. *Exp. Anim.* **29**, 33-38.
- OIKAWA, T., YOSHIDA, M., SATOH, H., YAMASHITA, K., SASAKI, M. and KOBAYASHI, M. (1983) Provisional chromosomal assignment of the rat major histocompatibility complex. *Jpn. J. Genet.* **58**, 327-336.
- RIGBY, P. W. J., DIECKMANN, M., RHODES, C. and BERG, P. (1977) Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. *J. Mol. Biol.* **113**, 237-251.
- ROTHSCHILD, M. A., ORATZ, M., SHREIBER, S. S. (1972) Albumin synthesis. *New Engl. J. Med.* **286**, 748-757.
- SHUMIYA, S. and NAGASE, S. (1982) Linkage of the analbuminemia locus (*alb*) and the Hooded locus in the rat, *Rattus norvegicus*. *Exp. Anim.* **31**, 199-202.
- Standard karyotype of the Norway, rat, *Rattus norvegicus*: Committee for a standardized karyotype of *Rattus norvegicus*. *Cytogenet. Cell Genet.* **12**, 199-205 (1973).
- SZPIRER, J., LEVAN, G., THORN, M. and SZPIRER, C. (1984) Gene mapping in the rat by mouse-rat somatic cell hybridization: synteny of the albumin and α -fetoprotein genes and assignment to chromosome 14. *Cytogenet. Cell Genet.* **38**, 142-149.
- TRENT, J. M., OLSON, S. and LAWN, R. M. (1982) Chromosomal localization of human leukocyte, fibroblast, and immune interferon genes by means of *in situ* hybridization. *Proc. Natl. Acad. Sci. USA* **79**, 7809-7813.
- WOWACK, J. E. and SHARP, M. (1976) Comparative autosomal linkage in mammals: Genetics of esterases in *Mus musculus* and *Rattus norvegicus*. *Genetics* **82**, 665-765.
- YOSIDA, T. H. (1980) Cytogenetics of the Black Rat-Karyotype Evolution and Species Differentiation. Univ. of Tokyo Press, Tokyo and Univ. of Park Press, Baltimore.