Am J Hum Genet 28:350–356, 1976

Assignment of the Structural Gene for Human β Glucuronidase to Chromosome 7 and Tetrameric Association of Subunits in the Enzyme Molecule

CHING J. CHERN[1] AND CARLO M. CROCE

INTRODUCTION

Mammalian β glucuronidase (E.C.3.2.1.31) is known to catalyze both the hydrolysis of β-D-glucosiduronic acid and transfer reactions [1]. The genetics and structure of its multiple forms in human tissues and cultured cells are not understood. Purification of human liver β glucuronidase has been reported [2]. Deficiency in β glucuronidase activity has been found in cultured skin fibroblasts derived from patients with one of the mucopolysaccharidoses [3, 4].

We have previously studied a series of hybrid clones derived from the fusion of mouse peritoneal macrophages (MPM) and SV40-transformed human cells (LN-SV) [5] which selectively retained the human chromosome 7 containing the genome of SV-40. The present paper examines a series of mouse-human hybrid clones, including the MPM × LN-SV hybrid clones, for the expression of human β glucuronidase activity.

MATERIALS AND METHODS

Twenty-seven independent somatic cell hybrids were obtained from fusions of cells of different human and mouse origins. Ten clones were derived from fusing mouse (C57BL and BALB/c) peritoneal macrophages (MPM) with SV40-transformed human fibroblasts deficient in hypoxanthine phosphoribosyltransferase (HPRT) from a patient with the Lesch-Nyhan syndrome (LN-SV) [5]. Five clones were obtained from fusing BALB/c mouse fibroblasts with HPRT deficient SV40-transformed human fibroblasts (WI8Va2) from the buccal mucosa of a normal male [6]. Seven clones were derived from fusing mouse cells (Cl-1D) deficient in thymidine kinase (TK) with LN-SV cells [7]. Four clones were obtained from fusing human fibroblasts having a balanced 10/17 translocation (46,XY,t[10:17]) with TK-deficient mouse cells (IT-22) [8]. One clone was derived from fusing human white blood cells carrying a translocation of the long arm of chromosome 15 to chromosome 17 [9] with TK-deficient mouse cells (IT-22).

The procedures for cell hybridization and selection in medium containing hypoxanthine-aminopterin-thymidine (HAT) have been described [10]. Human and mouse chromosomes

Received January 28, 1976; revised February 23, 1976.

This work was supported in part by grants from the U.S. Public Health Service (CA 10815), the National Cancer Institute (CA 16685), the Division of Research Resources (RR 05540), the Institute of General Medical Science (GM 20700), and the National Foundation (Basil O'Connor Starter grant). C. M. Croce is a recipient of a Research Career Development Award (K04CA 00143) from the National Cancer Institute.

[1] Both authors: The Wistar Institute of Anatomy and Biology, 36th Street at Spruce, Philadelphia, Pennsylvania 19104.

were identified in at least 20 metaphases from each clone by trypsin-Giemsa banding [11].

Hybrid cells were grown to confluency and harvested by trypsinization. The cells were lyophilized to dryness. Dry cell pellets (2 mg) from each clone were suspended in 0.1 ml of 0.05 M tris-citrate buffer, pH 7.5 and sonicated to release their cell contents. Cell debris was removed by centrifuging at 800 *g* for 15 min. The supernatant was concentrated to dryness by lyophilization. The sample of each clone then was taken into solution with 15 μl of 0.05 M tris-citrate buffer, pH 7.5. The solution (approximately 0.4 μl) was applied to cellogel (cellulose acetate membrane, 15 cm × 17 cm × 0.5 mm, Chemetron, Milano, Italy) with the aid of a multiple sample applicator (Shandon Southern Instrument, Inc., Sewickley, Pa.) to perform electrophoretical analysis for β glucuronidase.

Cellulose acetate electrophoresis was performed in 0.05 M tris-citrate buffer, pH 7.5, at 300 V and 4°C for 2 hr. The cellogel was then stained for β glucuronidase activity by overlaying both surfaces of the cellulose acetate membrane with filter papers saturated with a solution of 4-methylumbelliferyl-β-D-glucuronide (0.2 mg per ml of 0.5 M citrate buffer, pH 4.5). After incubating the cellogel at 37°C for at least 1.5 hr, β glucuronidase activity was visualized as fluorescence of 4-methylumbelliferone released by the enzyme. Heteropolymer bands between human and mouse enzyme bands were detected by different incubation periods.

RESULTS

Separation of human and mouse β glucuronidase by cellulose acetate electrophoresis is shown in figure 1. Three heteropolymeric bands and two parental bands of human and mouse enzymes were detected in hybrid clones (figs. 1 and 2),

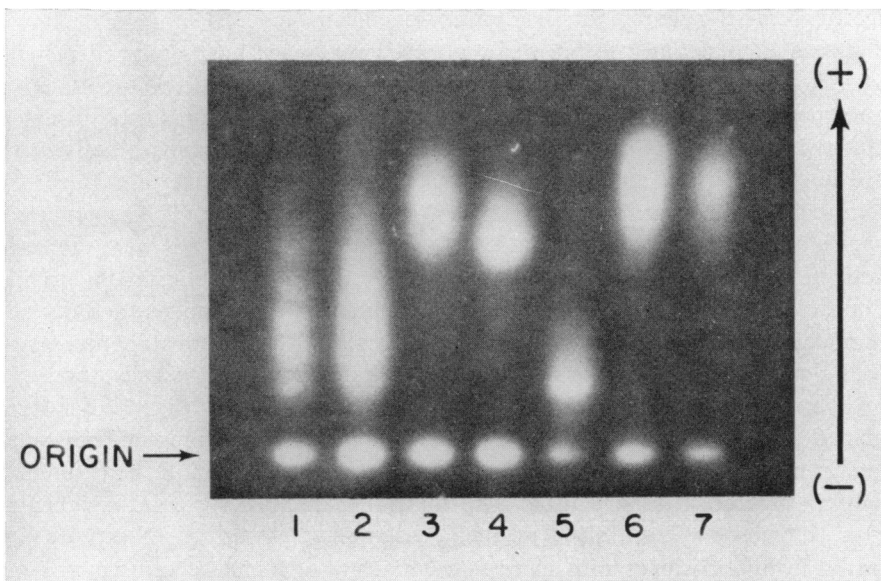

FIG. 1.—Zymograms of β glucuronidase on cellogel (cellulose acetate). Mouse parent cells (*lanes 6 and 7*); human parental cells (*lane 5*). Hybrid clones with no expression of human β glucuronidase (*lanes 3 and 4*); hybrid clones with human and mouse β glucuronidase expression (*lanes 1 and 2*). The detailed experimental procedures for electrophoresis and activity staining are described in the text. The picture was taken after incubating the gel at 37°C overnight.

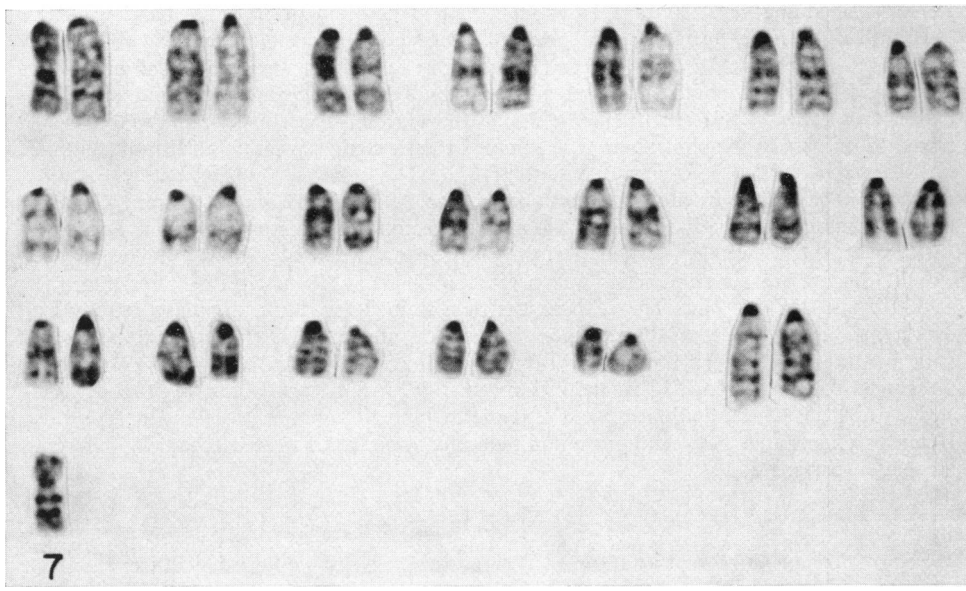

FIG. 2.—Karyotype of a hybrid cell expressing human β glucuronidase (see fig. 1). Chromosome 7 is the only human chromosome present in this hybrid clone.

suggesting that β glucuronidase is a tetrameric enzyme and the subunits of human and mouse β glucuronidase are different. Heteropolymer bands were detectable after 1.5 and 3 hr at 37°C in a hybrid clone containing five copies of human chromosome 7 per cell (fig. 3). The longer incubation periods required to detect β glucuronidase activity in clones with weak human β glucuronidase activity resulted in streaking of bands. Fluorescence origin was probably due to β glucuronidase associated with particles that persisted after centrifugation. The supernatants of sonicated samples centrifuged at 14,500 *g* (instead of 800 *g*) that were subjected to cellogel electrophoresis demonstrated markedly reduced activities at the origins.

Concordant segregation of the expression of human β glucuronidase activity and the presence of human chromosome 7 was observed in 27 independent human-mouse hybrid clones derived from fusions of different human and mouse cells (table 1). Five hybrid clones that contained only human chromosome 7 (fig. 2) displayed human β glucuronidase activity. These results imply that the presence of human chromosome 7 alone is required for the expression of human β glucuronidase activity. Therefore, the structural gene for β glucuronidase can be assigned to human chromosome 7. An effect of gene dosage on β glucuronidase activity was observed in hybrid clones with more than one copy of human chromosome 7 per cell (fig. 3) based upon densitometric intensities of activity bands in zymograms.

DISCUSSION

Genetic and physiologic information for mammalian β glucuronidase has been obtained by studying the enzymes of murine species [12]. β glucuronidase has

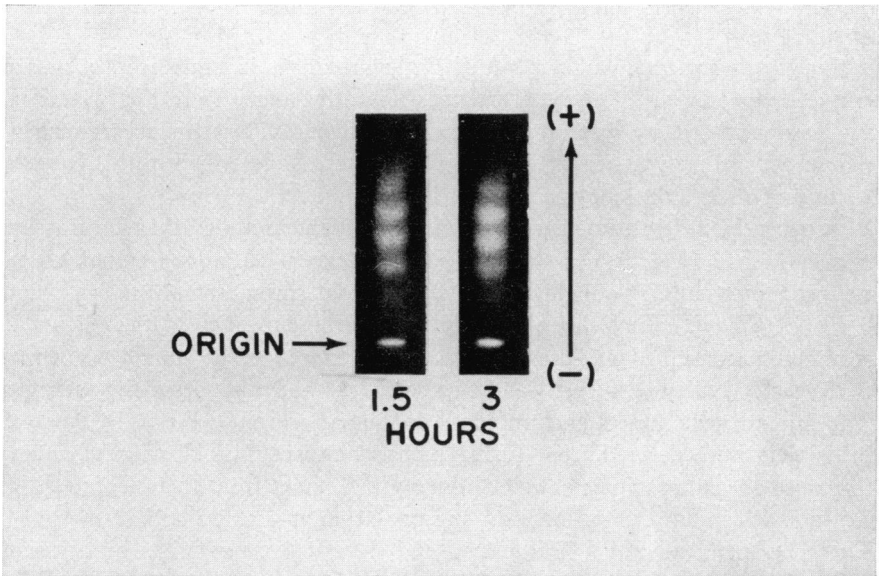

FIG. 3.—Development of β glucuronidase activity in a hybrid clone with positive expression of human and mouse β glucuronidase on cellogel at 37°C for 1.5 and 3 hr.

been purified from rat liver [13, 14], rat preputial gland [15], beef liver [16, 17], mouse liver [18], and mouse kidney [19]. The rodent lysosomal enzyme has a molecular weight of approximately 280,000 with four identical or closely similar subunits. Isoenzymes of mouse β glucuronidase and the interconversion among them upon electrophoresis have been reported [18, 20]. The microsomal form of β glucuronidase can be converted to the lysosomal form by acid, heat, or urea treatments [20]. A single structural locus on mouse chromosome 5 has been described; it codes for mouse β glucuronidase in both lysosomes and microsomes [21, 22].

Only limited information about properties of human β glucuronidase is available. The subunits of human and mouse β glucuronidase randomly associate and generate

TABLE 1

CONCORDANT SEGREGATION OF HUMAN β GLUCURONIDASE EXPRESSION WITH THE PRESENCE OF CHROMOSOME 7

	HUMAN CHROMOSOME 7	
	+	−
Human β glucuronidase:		
+	15	0
−	0	12

NOTE.—Twenty-seven independently derived human-mouse hybrid clones were studied.

three heteropolymer bands in hybrid clones which suggests that the enzyme has four subunits.

Although the intracellular location of β glucuronidase in human fibroblasts was not investigated in our study, the enzymes migrating away from the origins most likely predominated by lysosomal forms of β glucuronidase, in accordance with the conditions of enzyme extraction and electrophoresis described in this paper and the study of subcellular enzyme distribution [23].

Very recently, a "genetic element" for human β glucuronidase has been assigned to chromosome 9 [24]. In our study, five hybrid clones containing human chromosome 9 in more than 55% of their cells (but without chromosome 7) were included and found to have no activity of human β glucuronidase. Our data indicate that human chromosome 7 alone is responsible for the expression of human β glucuronidase. Recently Lalley et al. [25] and Grzeschik [26] have reported the assignment of the human gene for β glucuronidase to human chromosome 7 by analyzing hybrids that were derived from fusing human cultured fibroblasts or leukocytes with mouse or Chinese hamster cells. Four hybrid clones from this laboratory were included in this study (P. A. Lalley, personal communication, 1975).

The gene for SV40 tumor(T)-antigen and the SV40 genome has been assigned to human chromosome 7 in the LN-SV human cell line [27, 28]. The presence of this chromosome in hybrids with normal mouse cells results in the expression of the tumorigenic phenotype [29]. Since the gene for β glucuronidase is located on human chromosome 7, this enzyme may serve as a very useful linkage marker for studying the genetic control of the transformed phenotype in somatic cell hybrids.

SUMMARY

The structural locus for human β glucuronidase is assigned to chromosome 7, a localization based upon concordant segregation of the expression of the human enzyme and the presence of human chromosome 7 in somatic cell hybrid clones derived independently from fusions of different human and mouse cells. Hybrid clones containing only human chromosome 7 are included in this study. Electrophoresis of β glucuronidase also has revealed that human β glucuronidase has a tetrameric structure.

ACKNOWLEDGMENTS

We thank Patricia McBreen and Irene Kieba for their excellent technical assistance.

REFERENCES

1. WAKABAYASHI M: β-glucuronidase in metabolic hydrolysis, in *Metabolic Conjugation and Metabolic Hydrolysis*, vol 2, edited by FISHMAN WH, New York, Academic Press, 1970, pp 519–602
2. MUSA BU, DOE RP, SEAL US: Purification and properties of human liver β-glucuronidase. *J Biol Chem* 240:2811–2816, 1965
3. SLY WS, QUINTON BA, MCALISTER WH, RIMOIN DL: Beta glucuronidase deficiency: report of clinical, radiologic, and biochemical features of a new mucopolysaccharidosis. *J Pediatr* 82:249–257, 1973

4. LAGUNOFF D, NICOL DM, PRITZL P: Uptake of β-glucuronidase by deficient human fibroblasts. *Lab Invest* 29:449–453, 1973
5. CROCE CM, KOPROWSKI H: Somatic cell hybrids between mouse peritoneal macrophages and SV40-transformed human cells. I. Positive control of the transformed phenotype by the human chromosome 7 carrying the SV40 genome. *J Exp Med* 140:1221–1229, 1974
6. WEISS MC, EPHRUSSI B, SCALETTA LJ: Loss of T-antigen from somatic hybrids between mouse cells and SV40-transformed human cells. *Proc Natl Acad Sci USA* 59:1132–1135, 1968
7. CROCE CM, KNOWLES BB, KOPROWSKI H: Preferential retention of the human chromosome C-7 in human-(thymidine kinase deficient) mouse hybrid cells. *Exp Cell Res* 82:457–461, 1973
8. CHERN CJ, MELLMAN WJ, CROCE CM: Assignment of the gene for cytoplasmic glutamic-oxaloacetic transaminase to the region q24-qter of human chromosome 10. *Somatic Cell Genet.* In press, 1976
9. BANNISTER DL, ENGEL E: A G-like trisomy with a major 15 proximal supernumerary component derived from a D/E balanced maternal interchange. *J Pediatr* 86:916–918, 1975
10. CHERN CJ, CROCE CM: Confirmation of the synteny of the human genes for mannosephosphate isomerase and pyruvate kinase and of their assignment to chromosome 15. *Cytogenet Cell Genet.* 15:299–305, 1975
11. SEABRIGHT M: A rapid banding technique for human chromosomes. *Lancet* 2:971–972, 1971
12. PAIGEN K, SWANK RT, TOMINO S, GANSCHOW RE: The molecular genetics of mammalian glucuronidase. *J Cell Physiol* 85:379–392, 1975
13. DELVIN E, GIANETTO R: The purification of lysosomal rat-liver β-glucuronidase. *Biochim Biophys Acta* 220:93–100, 1970
14. STAHL PD, TOUSTER O: β-glucuronidase of rat liver lysosomes. Purification, properties, subunits. *J Biol Chem* 246:5398–5406, 1971
15. TULSIANI DRP, KELLER RK, TOUSTER O: The preparation and chemical composition of the multiple forms of β-glucuronidase from the female rat preputial gland. *J Biol Chem* 250:4770–4776, 1975
16. PLAPP BV, COLE RD: Purification and characterization of bovine liver β-glucuronidase. *Arch Biochem Biophys* 116:193–206, 1966
17. PLAPP BV, COLE RD: Demonstration and partial characterization of multiple forms of bovine liver β-glucuronidase. *Biochemistry* 6:3676–3681, 1967
18. TOMINO S, PAIGEN K, TULSIANI DRP, TOUSTER O: Purification and chemical properties of mouse liver lysosomal (L form) β-glucuronidase. *J Biol Chem* 250:8503–8509, 1975
19. LIN CW, ORCUTT ML, FISHMAN WH: Purification and characterization of mouse kidney β-glucuronidase. *J Biol Chem* 250:4737–4743, 1975
20. LIN CW: Multiple forms of β-glucuronidase: molecular nature, transformation and subcellular translocation, in *International Conference on Isozymes*, vol 1, edited by MARKERT CL, New York, Academic Press, 1975, pp 637–651
21. PAIGEN K: The effect of mutation on the intracellular location of β-glucuronidase. *Exp Cell Res* 25:286–301, 1961
22. LALLEY PA, SHOWS TB: Lysosomal and microsomal glucuronidase. Genetic variant alters electrophoretic mobility of both hydrolases. *Science* 185:442–444, 1974
23. CRISTOFALO VJ, KABAKJIAN J: Lysosomal enzymes and aging in vitro. Subcellular enzyme distribution and effect of hydrocortisone on cell lifespan. *Mech Ageing Dev* 4:19–28, 1975
24. MURNANE MJ, KUCHERLAPATI RS, CHEN S, RUDDLE FH: Assignment of a genetic

element for human β-glucuronidase to chromosome 9. *Am J Hum Genet* 27:68A, 1975

25. LALLEY PA, BROWN JA, EDDY RL, HALEY LL, SHOWS TB: Assignment of the gene for β-glucuronidase (GUS) to chromosome 7 in man. *Baltimore Conference (1975): Third International Workshop on Human Gene Mapping, Birth Defects: Original Article Series,* New York, National Foundation. In press, 1976
26. GRZESCHIK KH: Assignment of human genes: β-glucuronidase to chromosome C7, AK-1 to C9, a second enzyme with enolase activity to C12, and mitochondrial IDH to D15. *Baltimore Conference (1975): Third International Workshop on Human Gene Mapping, Birth Defects: Original Article Series,* New York, National Foundation. In press, 1976
27. CROCE CM, GIRARDI AJ, KOPROWSKI H: Assignment of the T-antigen gene of simian virus 40 to human chromosome C-7. *Proc Natl Acad Sci USA* 70:3617–3620, 1973
28. KHOURY G, CROCE CM: Quantitation of the viral DNA present in somatic cell hybrids between mouse and SV40-transformed human cells. *Cell* 6:535–542, 1975
29. CROCE CM, ADEN D, KOPROWSKI H: Tumorigenicity of mouse-human diploid hybrids in nude mice. *Science* 190:1200–1202, 1975