Assignment of the Human Gene for Hexose-1-phosphate Uridylyltransferase to Chromosome 3

(mapping/galactose)

THOMAS A. TEDESCO, RICHARD DIAMOND, KATHRYN G. ORKWISZEWSKI, HERBERT J. BOEDECKER, AND CARLO M. CROCE*

Departments of Human Genetics and Pediatrics, University of Pennsylvania School of Medicine, and *The Wistar Institute, Philadelphia, Pa. 19174

Communicated by Britton Chance, June 20, 1974

ABSTRACT Mouse-human hybrid clones were tested for the presence of human hexose-1-phosphate uridylyltransferase (EC 2.7.7.12; UDPglucose: α -D-galactose-1-phosphate uridylyltransferase). Two criteria, starch gel electrophoresis and double-immunodiffusion against a human transferase-specific antibody, were used to identify human enzyme in the hybrid clones. Seventeen of 33 hybrid clones analyzed were found to contain human transferase by both criteria. Karyological analysis of the hybrid clones showed concordant segregation of human transferase with human chromosome 3. Human galactokinase was asyntenic with human transferase. We thus assign this gene to human chromosome 3.

The fusion of rodent and human somatic cells in culture to produce hybrid clones in which human chromosomes segregate has become a popular approach to linkage analysis in man (1). Unidirectional loss and segregation of human chromosomes in rodent-human hybrids (2-4) and preferential retention of certain human chromosomes in hybrids derived from fusion of thymidine kinase-deficient mouse cells and simian virus 40-transformed human fibroblasts (5) have been described.

This report provides evidence for linkage of the structural gene for hexose-1-phosphate uridylyl transferase (EC 2.7.7.12; UDPglucose: α -D-galactose-1-phosphate uridylyltransferase) to human chromosome 3 by use of mouse-human hybrid clones selected in HAT medium from four different fusion experiments between a thymidine kinase-deficient mouse cell line (Cl1D) and three different human fibroblast cell lines (W18Va2, LNSV, and KOP-2) (5).

METHODS AND MATERIALS

A description of the mouse (Cl1D) and human (W18Va2, LNSV, and KOP-2) parental cell lines, and the methods used for fusion, selection, and cloning have been reported (5).

Karyological Analysis. Slides containing chromosome preparations were treated with a solution of 0.05% trypsin and 0.02% EDTA (w/v, GIBCO) for 5 min. The slides were then washed with (a) medium containing 5% (v/v) fetal calf serum, and (b) Hanks' solution. Giemsa-banding of chromosomes was then obtained by (a) staining with 2% Giemsa at pH 6.8 for 5 min, (b) rinsing with pH 6.8 buffer (one tablet of G. T. Gurr, pH 6.8, in 100 ml of water), and (c) drying with humidified hot air. A minimum of 20 metaphases were analyzed for each hybrid cell clone. The use of this method to distinguish mouse and human chromosomes in interspecific hybrids has already been reported (6).

Preparation of Cell Extracts. Both parental cells and hybrid clones were grown to confluency in Blake bottles with Eagle's minimal essential medium supplemented with 10% fetal calf serum. The cells were harvested with trypsin-EDTA (7). The harvested cells collected by centrifugation were extracted by suspending 20×10^6 cells in 1.0 ml of lysing buffer which contained 13.3 mM Na, K phosphate, 0.8 mM Na₄EDTA, and 6.4 mM dithiothreitol, pH 7.4, followed by freezing and thawing of the cell suspension five times. The suspension was then centrifuged at $20,000 \times g$ for 15 min at 4°; the supernatant was removed and immediately lyophilized to dryness. About 1 mg of the dried extract (about 0.5 mg of protein) reconstituted in 0.04 ml of water was usually sufficient for both electrophoretic and immunodiffusion analysis.

Electrophoresis. Starch gel electrophoresis for both hexose-1-phosphate uridylyltransferase and galactokinase (EC 2.7.1.6) was performed horizontally with 11.34% Electrostarch (lot no. 371, Electrostarch Co.). The gel buffer contained 20 mM L-histidine, 3 mM Na₂EDTA, and 3 mM mercaptoethanol, pH 6.0. The electrode buffer was identical to that described by Ng *et al.* (8), except that the pH was 8.0. Electrophoresis was for 18 hr at 4° with 12 V/cm across the gel. Samples were applied to the gels by inserting 0.5×1.0 -cm strips of Whatman 3M filter paper saturated with reconstituted extract. Zones of uridylyltransferase activity were localized with the staining mixture described in ref. 8 except that the reaction mixture was layered over the gel in 0.5% Ionagar no. 2 (Consolidated Laboratories, Inc.).

Galactokinase activity was localized in the gel by modifying the uridylyltransferase reaction mixture; glycine buffer was replaced with 0.2 M Tris-HCl, pH 8.0, and galactose-1-P with 0.4 mM galactose, 6.6 mM ATP, and 0.1 unit/ml of yeast uridylyltransferase (Sigma Chemical Co.). NADPH formation in the zones of enzyme activity was made visible under long-wave ultraviolet light.

The starch gel electrophoresis method described by Spencer *et al.* (9) was used for lactate dehydrogenase A and B, and nucleoside phosphorylase. The method described by Okada and O'Brien (10) was used for the hexosaminidase A.

Double-Immunodiffusion. The preparation of a specific antibody to human transferase protein has already been described (11). Double-immunodiffusion was routinely carried out in immunodiffusion cells (IDF Cell I, Cordis Laboratories).

FIG. 1. Starch gel electrophoresis showing separation of human and mouse uridylyltransferase. (Panel A) Photograph exposed to ultraviolet light. (Panel B) Diagrammatic representation of the gel in panel A showing the fluorescent uridylyltransferase activity bands produced with extracts of human cell line LNSV (channel 5), mouse cell line Cl1D (channels 2 and 7), human uridylyltransferase-positive mouse-human hybrid clones (channels 1, 3, 6, and 8), and one human uridylyltransferase-negative clone (channel 4).

RESULTS AND DISCUSSION

Both human and mouse uridylyltransferase migrate anodally in the starch gel electrophoretic system described. Mouse uridylyltransferase migrates as a single band of enzyme activity, whereas uridylyltransferase from the human parental cell lines separates into three bands and the slower moving, most intense band moves to a position 1-cm cathodal to the mouse band. Fig. 1 shows the electrophoretic patterns of

uridylyltransferase activity in mouse and human parental cell extracts, and four clones positive and one negative for the presence of human uridylyltransferase.

We have already established that human uridylyltransferase is composed of subunits (11), but we have been unsuccessful in attempts to identify heteropolymeric forms of uridylyltransferase in clones that express both human and mouse uridylyltransferase activity. Artificial mixtures of human and mouse parental cell extracts gave electrophoretic

FIG. 2 (Panel A) Photograph of a double-immunodiffusion plate showing the immunoprecipitin reactions produced when extracts of human and mouse cells in adjacent wells diffuse against human uridylyltransferase antiserum. Extracts of mouse cell line Cl1D (wells 1, 3, and 5) and human cell line LNSV (wells 2, 4, and 6) were placed in the outer wells to diffuse against human uridylyltransferase antiserum in the center well. The formation of spurs, a distinguishable immunoprecipitin reaction between human extracts and the antiserum, indicates the presence of human uridylyltransferase. (Panel B) Diagrammatic representation of the immunoprecipitin reactions produced between human uridylyltransferase antiserum (center well) and extracts of human cell line LNSV (well 2), mouse cell line Cl1D (wells 1 and 4), human uridylyltransferase-positive mouse-human hybrid clones (wells 3 and 5), and a human uridylyltransferase-negative clone (well 6). The development of spurs usually required 2-4 days.

TABLE 1. Analysis of mouse-human hybrid clones for the presence of chromosome 3 and human uridylyltransferase

		Chromosome 3	
		+	-
Transferase	+	17	0
	-	0	16

patterns indistinguishable from extracts of clones found to contain both human and mouse uridylyltransferase. Since only the slower moving of the three human uridylyltransferase bands separates from the mouse uridylyltransferase band, and since their degree of separation is small, it is difficult to determine whether any mouse-human heteropolymers of uridylyltransferase may exist in hybrid clones that express both human and mouse enzyme.

The presence of human uridylyltransferase protein in hybrid clones was independently established by double-immunodiffusion against an antiserum prepared against purified human erythrocyte uridylyltransferase. The mouse cell uridylyltransferase crossreacts with partial identity to this antibody. Spurring occurs where human and mouse precipitin lines meet when extracts of human and mouse cells are in adjacent outer wells and human uridylyltransferase antiserum is in the center well (Fig. 2A). The formation of spurs when two antigens react with a single antiserum has been interpreted to indicate that both antigens share a common set of antigenic determinants (in this case both mouse and human uridylyltransferase), but that one of the antigens, the one that reacts to produce the spur (in this case human uridylyltransferase), possesses certain antigenic determinants that are unique (12). This technique has proved to be a sensitive method for distinguishing clones with both human and mouse

TABLE 3. Asyntenic relationships between human hexose-1-phosphate uridylyltransferase and human hexosaminidase A, lactate dehydrogenase A, lactate dehydrogenase B, and nucleoside phosphorylase

Hexosamini- dase A				Lactate dehydro- genase A			
<hr/>				<hr/>			
+ -				+ -			
Transferase	+	11	6	Transferase	+	6	1
	-	10	6		-	3	5
<hr/>				<hr/>			
Lactate dehydro- genase B				Nucleoside phospho- rylase			
<hr/>				<hr/>			
+ -				+ -			
Transferase	+	7	0	Transferase	+	6	1
	-	4	1		-	6	4

TABLE 4. *Asyntenic relationship between human galactokinase and uridylyltransferase*

Transferase		Galactokinase	
		+	-
	+	9	4
	-	8	7

is based on asyntenic relationships (Table 3) demonstrated between human uridylyltransferase and enzymes already localized to these chromosomes hexosaminidase A, 7; lactate dehydrogenase A, 11; lactate dehydrogenase B, 12; and nucleoside phosphorylase, 14 (13). Chromosome 17 was also found in the clones listed in Table 2; linkage of human uridylyltransferase to chromosome 17 was eliminated by testing four clones back-selected in BrdU that lost chromosome 17 and retained human uridylyltransferase.

Galactokinase enzymes extracted from human and mouse parental cells used in this study are easily distinguished by starch gel electrophoresis. Mouse galactokinase migrates 15–16 cm anodally, while human galactokinase migrates only 3–4 cm. Both human and mouse enzymes migrate as a single band of enzyme activity and no intermediate bands developed, suggesting that mouse-human heteropolymers of galactokinase do not form in human galactokinase-positive clones even though human erythrocyte galactokinase has been shown to contain two subunits (14). Table 4 shows that galactokinase and uridylyltransferase are asyntenic in the human cells.

The data presented here are inconsistent with those of Sun, Chang, and Chu, who have assigned the human gene for uridylyltransferase to chromosome 2 (13, 15). Since only one out of 17 of the human uridylyltransferase-positive clones listed in Table 2 displayed the presence of human chromosome 2, and since two independent lines of evidence (electrophoretic and immunodiffusion) were used to identify human uridylyltransferase in these hybrid clones, we feel that chromosome 2 is not likely to contain the human gene for uridylyltransferase. Although no reasonable explanation seems apparent at the present time, the basis of this discrepancy may be related to the use of different interspecific hybrids, i.e., mouse fibroblast-human fibroblast hybrids in this study, and galactose-negative Chinese hamster fibroblast-human lymphocyte hybrids by Sun *et al.*

Note Added in Proof. The assumption that a locus involved in the expression of hexosaminidase A is on chromosome 7 in man derives from studies reported by others [Gilbert *et al.* (1974) *Cytogenetics* 13, 96] describing the concordant segrega-

tion of human hexosaminidase A with human mannosephosphate isomerase (EC 5.3.1.8.). The gene for mannosephosphate isomerase has been assigned by McMorris *et al.* to chromosome 7 in man [Science (1973) 179, 1129]. Analyzing 54 independent hybrid clones, we have been unable to confirm the assignment of the human gene for mannosephosphate isomerase to human chromosome 7, since they were found to segregate independently. However, the evidence that the human hexose-1-phosphate uridylyltransferase is not linked to human chromosome 7 is based on the asyntenic relationship demonstrated between human uridylyltransferase and the expression of the simian virus 40 T antigen, the gene for which has been assigned to chromosome 7 in simian virus 40-transformed human cells [Croce *et al.* (1973) *Proc. Nat. Acad. Sci. USA* 70, 3617].

We have also found a positive correlation between the presence of human chromosome 17 and the expression of human galactokinase activity (Orkwiszewski *et al.*, *Nature*, in press).

This work was supported by U.S. Public Health Service Grants HD-00588, HD-04861, CA-04534, CA-10815, and RR-05540 from the National Institutes of Health; and a grant from the Pennsylvania Plan. Consultation and direction of Hilary Koprowski and William J. Mellman as well as the technical assistance of Barbara Needleman, Karen Miller, Emma de Jesus, and Irene Kieba are gratefully acknowledged.

1. Ruddle, F. A. (1973) *Nature* 242, 165–169.
2. Weiss, M. D. & Green, H. (1967) *Proc. Nat. Acad. Sci. USA* 59, 1104–1111.
3. Westerveld, A., Meera Khan, P., Visser, R. P. L. S. & Bootsma, D. (1971) *Nature New Biol.* 234, 20–24.
4. Croce, C. M., Litwack, G. & Koprowski, H. (1973) *Proc. Nat. Acad. Sci. USA* 70, 1268–1272.
5. Croce, C. M., Knowles, B. B. & Koprowski, H. (1973) *Exp. Cell Res.* 82, 457–461.
6. Croce, C. M., Kieba, I. & Koprowski, H. (1973) *Exp. Cell Res.* 79, 461–463.
7. Tedesco, T. A. & Mellman, W. J. (1969) *J. Clin. Invest.* 48, 2390–2397.
8. Ng, W., Bergren, W. R., Fields, M. & Donnell, G. N. (1969) *Biochem. Biophys. Res. Commun.* 37, 354–362.
9. Spencer, N., Hopkinson, D. A. & Harris, H. (1968) *Ann. Human Genet. (London)* 32, 9–14.
10. Okada, S. & O'Brien, J. S. (1969) *Science* 165, 698–700.
11. Tedesco, T. A. (1972) *J. Biol. Chem.* 247, 6631–6636.
12. Clausen, J. (1969) in *Laboratory Techniques in Biochemistry and Molecular Biology*, eds. Work, T. S. & Work, E. (North-Holland Publishing Co., Amsterdam), pp. 467–469.
13. Linkage assignments based on the First International Workshop on Human Gene Mapping, sponsored by the National Foundation-March of Dimes held at Yale University, New Haven, Conn. June 10–13, 1973.
14. Blume, K.-G. & Beutler, E. (1971) *J. Biol. Chem.* 246, 6507–6510.
15. Sun, N. C., Chang, C. C. & Chu, E. H. Y. (1974) *Proc. Nat. Acad. Sci. USA* 71, 404–407.