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Genetics of the connective tissue proteins: Assignment of the gene for human type I procollagen to chromosome 17 by analysis of cell hybrids and microcell hybrids*

(gene mapping/immunology/fibroblasts)

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ABSTRACT **Somatic cell hybrids between mouse and human cell lines have been used to identify the specific chromosome that governs the synthesis of type I procollagen. Fourteen hybrid clones and subclones were derived independently from crosses between mouse parents [LM (thymidine kinase-negative) or A9 (hypoxanthine phosphoribosyltransferase-negative)] and human cells (human diploid lung fibroblasts WI-38 or diploid skin fibroblasts GM5, GM17, and GM9). The cultures were labeled with [^{3}H]proline in modified Eagle's medium without serum. Radioactive procollagens were purified from the medium by the method of Church *et al.* [(1974) *J. Mol. Biol.* 86, 785–799]. DEAE-cellulose chromatography was used to separate collagen and type I and type III procollagen. Human type I procollagen was assayed by double immunodiffusion analysis with type I procollagen antibodies prepared by immunizing rabbits with purified human type I procollagen. These analyses combined with karyology and isozyme analyses of each hybrid line have produced evidence for the assignment of the gene for human type I procollagen to chromosome 17. A human microcell–mouse hybrid cell line containing only human chromosome 17 was positive for human type I procollagen, lending further support to the assignment of the human type I procollagen gene to chromosome 17. Finally, by using a hybrid line containing *only* the long arm of human chromosome 17 translocated onto a mouse chromosome, the type I procollagen gene can be assigned more specifically to the long arm of chromosome 17.**

Collagen is one of the most abundant proteins in higher animals, occurring in the extracellular matrix as insoluble fibers. This connective tissue protein accounts for a large part of the organic mass of skin, tendon, blood vessels, bone, testes, cornea, and vitreous humor (for reviews, see refs. 1–3). The molecular composition of collagen is now well established and at present at least four genetically distinct species of collagen have been identified in human and other mammalian species (3).

It is now well known that collagen is initially synthesized as a high molecular weight precursor, procollagen (4). This molecule undergoes several post-translational modification sequences such as hydroxylation of select prolyl and lysyl residues, glycosylation of select hydroxylysyl residues, and cleavage of the terminal extensions from the secreted procollagen molecule. Finally, lysine-derived aldehydes are formed, resulting in crosslinking of collagen fibrils (5). Each of these particular steps is enzyme mediated and thus offers many opportunities for errors in either the synthesis of the type of collagen or one or more of the post-translational modification systems; these errors occur in several inheritable collagen disorders (6–10).

The use of tissue culture in the study of procollagen/collagen synthesis in different cell lines has been shown by Church *et al.* (11–14) to be one of the best ways to understand the various post-translational modification sequences involved in collagen biosynthesis. Church and Tanzer recently (14) reported the successful purification of type I procollagen from human skin fibroblasts.

Because of the extreme importance of different collagens during the development and maintenance of structural form in humans, it is of interest to determine the specific chromosome(s) that govern the synthesis of various collagens and their modification enzymes. The technique of somatic cell hybridization has been used in mapping a large number of human genes (15, 16). This technique involves the fusion of human and rodent cells to form hybrid cell lines that preferentially eliminate human chromosomes. An assay able to distinguish the homologous gene products of the parental lines allows gene assignments to be made by correlating the expression of a human phenotype with the presence of a particular human chromosome. Recently it has become possible to introduce single or small numbers of rodent chromosomes into mammalian cells by fusing microcells (subcellular particles with a decreased amount of nuclear material) with whole cells (17). A method for the production of human microcells has also been described (18). The generation of human microcell hybrid cell lines containing single human chromosomes would obviously simplify gene mapping procedures. In this study we utilized somatic cell hybrids and a human microcell hybrid cell line to determine the human chromosome carrying the gene for type I procollagen. A brief account of this work has appeared (19).

MATERIALS AND METHODS

Cell Lines and Culture Conditions. The cells were maintained in Dulbecco's modified Eagle's medium (hereafter referred to as "medium") supplemented with 10% fetal bovine serum. When necessary, the cell lines were also maintained in the same medium supplemented with penicillin (100 units/ml) and streptomycin (100 μg/ml). Logarithmically growing cells were harvested either with Viokase (Gibco) or by scraping the dishes. Parental cell lines used for the cell hybridization, the hybrid lines that were analyzed in this study, and their origin (20, 21) are given in Table 1. Hybridization was facilitated with Sendai virus inactivated by β-propiolactone (22, 23). Hybrid cells were selected by using the hypoxanthine/aminopterin/thymidine system of Szybalski *et al.* (24) as adapted by Little-

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Abbreviations: APRT, adenine phosphoribosyltransferase; TK, thymidine kinase; HPRT, hypoxanthine phosphoribosyltransferase.

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Table 1. Hybridization cell lines for human procollagen (Type I) synthesis

Hybrid cell line	Parent crosses	Source
JFA 14a-13 JFA 14b JFA 16a-8	GM5,* human female skin fibroblast (30 yr old) carrying a 14/22 translocation × mouse L cell line A9 (APRT⁻, HPRT⁻)	(20)
AIM 3a-07 AIM 8a AIM 11a AIM 23a-3	GM17,* human male fibroblast (2½ yr old) carrying a 15/18 translocation × A9	Kucherlapati & Ruddle, unpublished data
WA Ia WA IIa WL II 24a-2a WA V	WI-38, human fetal female lung fibroblast X A9	(20)
ANOM	GM9,* human fetal (1st trimester) male skin fibroblast × A9	Sundar Raj & Church, unpublished data
NHB	GM75,* human male skin fibroblast (10 mo old)	(14)
IL-II-5	IMR-32, human neuroblastoma cells × L cell line LM(TK⁻)	(21)
TMSIII-2a	HeLa microcell × B82(mouse TK⁻)	

* Human genetic mutant cell repository culture designation, Institute for Medical Research, Camden, NJ.

field (25). Cells were collected by centrifugation, washed with phosphate-buffered saline, and stored as pellets at −90° to be used later for isozyme analyses. Extracts from the frozen cells were analyzed by starch gel electrophoresis (26) to differentiate human and mouse isozymes (27). Chromosomal preparations of the mouse–human hybrid clones were analyzed by quinacrine and alkaline Giemsa differential staining procedures (28) to identify the human chromosomes present in each hybrid clone.

Preparation of Human Microcell Hybrid Cell Lines. Human HeLa S3 microcells were prepared by a modification of the procedure of Schor *et al.* (18). HeLa S3 cells were arrested in S phase by the addition of 2.5 mM thymidine to the culture medium of logarithmically growing cells for 23 hr. The medium was then removed from the plates, the cell layer was rinsed once, and fresh medium plus 10% fetal bovine serum was added. Subdivision by extrusion (29) was induced by mitotically arresting the cultures in a pressure chamber with 5 atmospheres (1.065×10^5 Pa) of nitrous oxide for 34 hr. After this prolonged arrest, 33% of the cells had divided by extrusion, resulting in clusters of microcells. Whole cells and microcell clusters were centrifuged at $1000 \times g$ for 15 min and resuspended in 2 ml of 0.5% bovine serum albumin (fraction V, Sigma) in phosphate-buffered saline. Microcell clusters were dispersed by gentle pipetting. The resulting crude preparation, consisting of 68% microcells, was purified by unit gravity sedimentation on a linear 1–3% bovine serum albumin gradient with a total volume of 50 ml (30). After sedimentation for 3 hr at room temperature, the gradient was fractionated, and nucleated particles were analyzed by phase microscopy. The top 15 ml of the gradient was found to be composed predominantly of microcells, with less than 1% whole cell contamination. This fraction of microcells was centrifuged at $1000 \times g$ for 12 min, resuspended in 0.5 ml of medium, and used immediately for fusion.

The purified human microcells (2.5×10^6 particles) were added to a nearly confluent flask of mouse B82(TK⁻) cells (approximately 2.5×10^6 cells) along with 500 hemagglutination units of β-propiolactone-inactivated Sendai virus in 0.5 ml of medium. The flask was incubated at 4° for 1 hr and then at 37° for 1 hr. The flask was rinsed once with fresh medium and incubated for 24 hr at 37° with fresh medium plus 10% fetal bovine serum. Cells were detached by Viokase treatment and distributed to five 25-cm² flasks in hypoxanthine/aminopterin/thymidine selective medium. Two weeks later, a single hybrid clone appeared and was picked by the ring technique and grown for chromosome and isozyme analysis. A more detailed protocol for the production of human microcell hybrids will be published elsewhere.

Synthesis and Purification of Procollagen Fractions. Procollagen was isolated and purified by the method of Church *et al.* (13, 14) from both parental and hybrid clones. Briefly, the cells were grown to confluency in medium plus 10% fetal bovine serum. Radioactive labeling was carried out by using [³H]proline (1 μCi/ml) in medium without serum or antibiotics but with ascorbic acid (100 μg/ml) and β-aminopropionitrile fumarate (50 μg/ml). Harvested labeled medium was brought to 30% saturation with ammonium sulfate to precipitate collagen-related proteins. This precipitate was solubilized in pH 7.6 phosphate buffer (ionic strength, 0.4) and the solution then made 18% in ethanol. The precipitate from this step, containing collagen-related proteins, was then chromatographed on DEAE-cellulose and the eluted procollagen and collagen peaks were further analyzed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (13) and immunoreactivity to antisera against human type I procollagen.

Preparation of Antiserum for Type I Procollagen. Type I procollagen, $[\text{Pro}\alpha 1(\text{I})]_2\text{pro}\alpha_2$, from normal human skin fibroblast cells in culture was purified by DEAE-cellulose chromatography as reported (14) and was used as antigen to raise specific type I procollagen antibodies in adult rabbits (31). Individual rabbits received a subcutaneous injection of 0.25 mg of the antigen emulsified with an equal volume of Freund's adjuvant. Two weeks after the initial inoculation, 0.25 mg of the antigen in Freund's incomplete adjuvant was injected subcutaneously. Animals were bled 7 days after the booster injection and the serum was tested for activity by Ouchterlony double immunodiffusion assay. Control sera were prepared from rabbits prior to immunization. The tested immune sera were divided into 1-ml aliquots and stored at −90°.

RESULTS

A total of 14 hybrid clones and subclones were analyzed both for the presence of human chromosomes and for the production of human type I procollagen. Isozyme analysis and karyology were used to identify the human chromosomes present in each line, both before and immediately after collection of culture medium for the procollagen assay. Each line was tested at least twice for human procollagen production and chromosome constitution.

Procollagen was assayed immunologically by two procedures. In one method, about 100 ml of serum-free medium was collected after 48 hr of incubation with the cells and was concentrated 50-fold with Aquacide II (Calbiochem). These medium concentrates were then tested for the presence or absence of

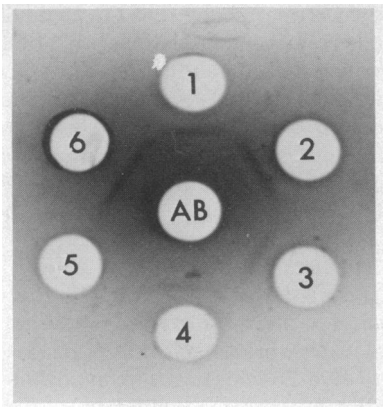

FIG. 1. Double immunodiffusion assay of various hybrid clones and procollagen standards. Agar slides were made with 1% agarose in 0.05 M Tris·HCl, pH 7.5/0.1 M NaCl/0.01% sodium azide. Center well contained human type I procollagen antiserum (AB). Outer wells are DEAE-cellulose chromatography type I procollagen peaks from: 1, AIM 8a; 2, JFA 16a-8; 3, WL II 24a-2a; 4, IL II 5; 5, purified human type III procollagen standard; and 6, purified human type I procollagen.

human type I procollagen by double immunodiffusion with agar slides and antibody to purified type I procollagen. The standard human line NHB gave a strong reaction when carried through this procedure (Fig. 1, well 6). Neither mouse parent demonstrated immunoreactivity when tested by this same procedure, although mouse types I and III procollagens were being produced (not shown). Moreover, human type I procollagen antibody did not crossreact with human type III procollagen (Fig. 1, well 5).

In the other procedure used to test for human type I procollagen, the [^{3}H]proline-labeled procollagen fraction from each of the hybrid cell lines was purified, type I and type III procollagens were separated by DEAE-cellulose chromatography, and the purified material was assayed by double immunodiffusion in agar slides against human type I and type III antibodies. Fig. 2 presents the chromatographic separation of purified collagenous protein from three hybrid clones: AIM 8a, JFA 16a-8, and WL II 24a-2a. Peak 1 (marked by arrow, fractions 60–80) was found to be type I procollagen at a purity of about 95% as judged by collagenase sensitivity. The pooled fractions were dialyzed against phosphate buffer and tested by double immunodiffusion with NHB type I procollagen antiserum. Purified type I peaks from the representative hybrids showed immunoprecipitation bands (Fig. 1, wells 1, 2, and 3).

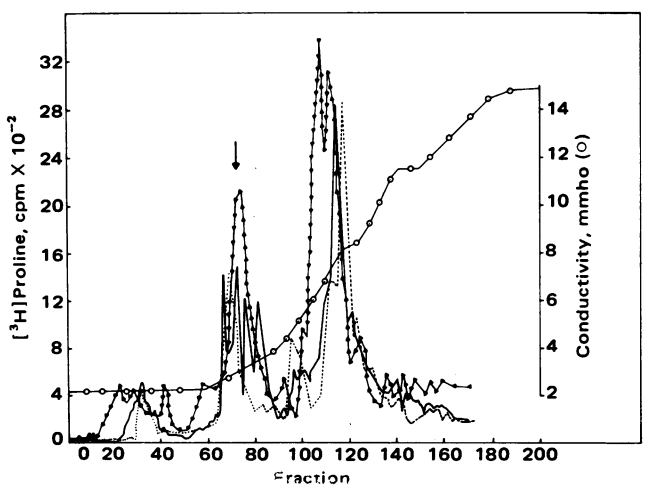

FIG. 2. DEAE-cellulose chromatography of procollagen types from mouse–human hybrids: AIM 8a (—), JFA 16a-8 (→), and WL II 24a2a (- - - -). The 18% ethanol precipitate was dissolved in 0.05 M Tris·HCl, pH 7.4/2 M urea/0.3 M NaCl (limiting buffer) and applied to a column (2.5 × 20 cm) of DEAE-cellulose. The column was developed over a linear gradient of 1500 ml with 0.3 M NaCl in the limiting buffer. The arrow indicates the procollagen peak (type I, fractions 60–80) that was pooled for immunodialysis.

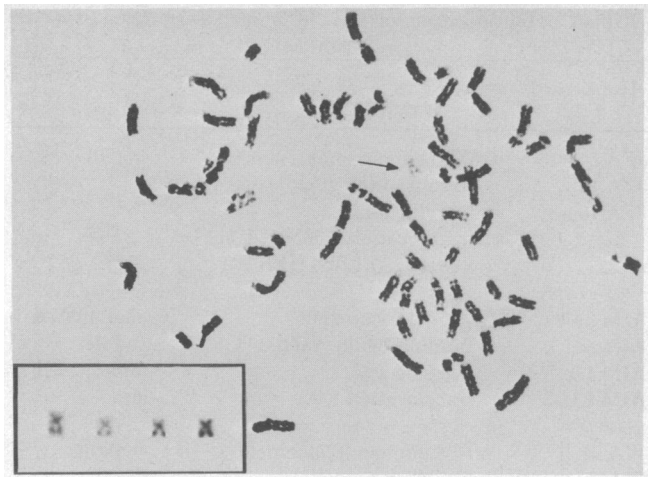

FIG. 3. Alkaline Giemsa-stained chromosome preparation of the human microcell hybrid cell line TMSIII-2a showing a single human chromosome (arrow). (*Inset*) Giemsa-trypsin banding pattern of the single human chromosome 17 in TMSIII-2a.

Table 2 lists the hybrid cell lines assayed for human type I procollagen along with their human chromosome constitutions and their expression of human isozymes. Also included in Table 2 is the presence or absence of human type I procollagen in each hybrid cell line. Only chromosome 17 showed a strong correlation with type I procollagen production, having complete concordance and no discordant cell lines. One cell line, JFA 14b, initially gave a positive reaction for type I procollagen production and had chromosomes 5, 17, and 18. Later studies with this cell line demonstrated a loss of type I procollagen production concomitant with the loss of human chromosome 17 from this cell line.

Two additional cell lines were assayed for human type I procollagen. Chromosome analysis of the human microcell hybrid cell line TMSIII-2a by the alkaline Giemsa differential staining procedure revealed a single human submetacentric chromosome in 82% of the cells (Fig. 3), with 4% of the population apparently possessing two copies of this chromosome. Sequential Giemsa banding-Hoechst centromeric staining (32) identified only human chromosome 17 in this cell line (Fig. 3 *inset*), again with a small percentage of the cells possessing two copies of human chromosome 17. The cell line WL II 24a-2a is a highly segregated clone derived from the fusion of human fetal lung fibroblasts (WI-38) with mouse A9 (HPRT$^-$) cells (33). The only human chromosome present in this cell line is the long arm of chromosome 17 which has been translocated onto a mouse chromosome. Isozyme analysis of TMSIII-2a and WL II 24a-2a revealed only human GalK, a chromosome 17 marker enzyme. Both TMSIII-2a and WLII 24a-2a were positive for human type I procollagen production.

Cell lines ILII-5, TMSIII-2a, and WL II 24a-2a were grown in 5-bromodeoxyuridine at 30 μg/ml in order to select for cells that had lost thymidine kinase activity. The thymidine kinase gene has been assigned to human chromosome 17 (16). After back-selection, no human chromosome material could be detected by alkaline Giemsa staining in the TMSIII-2a and WL II 24a-2a cell lines (25 karyotypes each). In addition, no human

Table 2. Human chromosomes and isozymes present in the human–mouse hybrid cell panel

Human chromosome	Cell lines											
	AIM 8a	AIM 11a	AIM 23a	JFA 14a-13	JFA 14b	JFA 16a-8	WA Ia	WA IIa	WA V	AIM 3a-07	ANOM 594	IL II-5
1			1									
	−Pgm-1	−Pgm-1	+Pgm-1	−Pgm-1	−Pgm-1	−Pgm-1	−Pgm-1	−Pgm-1	−Pgm-1	−Pgm-1	−Pgm-1	−Pgm-1
	−Pep-c	−Pep-c	−Pep-c	−Pep-c	−Pep-c	−Pep-c	−Pep-c	−Pep-c	−Pep-c	−Pep-c	−Pep-c	−Pep-c
2			2	2		2					2	
	−Idh-1	−Idh-1	+Idh-1	+Idh-1	−Idh-1	+Idh-1	−Idh-1	−Idh-1	−Idh-1	−Idh-1	+Idh-1	−Idh-1
	−Mdh-1	−Mdh-1	−Mdh-1	+Mdh-1	−Mdh-1	+Mdh-1	−Mdh-1	−Mdh-1	−Mdh-1	−Mdh-1	nd	−Mdh-1
3		3	3					3				
4							4	4	4		4	
5				5	5	5				5		
	−Hex-b	−Hex-b	−Hex-b	+Hex-b	+Hex-b	+Hex-b	−Hex-b	−Hex-b	−Hex-b	+Hex-b	−Hex-b	−Hex-b
6						6						
	−Me-1	−Me-1	−Me-1	−Me-1	−Me-1	+Me-1	−Me-1	−Me-1	−Me-1	−Me-1	−Me-1	−Me-1
7	7	7					7					7
8												
9	−Ak-1	−Ak-1	−Ak-1	−Ak-1	−Ak-1	−Ak-1	−Ak-1	−Ak-1	−Ak-1	−Ak-1	−Ak-1	−Ak-1
10			10							10		
	−Got-1	−Got-1	+Got-1	−Got-1	−Got-1	−Got-1	−Got-1	−Got-1	−Got-1	+Got-1	−Got-1	−Got-1
	−AdoK	−AdoK	+AdoK	−AdoK	−AdoK	−AdoK	−AdoK	−AdoK	−AdoK	nd	nd	−AdoK
11	11					11						
	+Ldh-a	−Ldh-a	nd	−Ldh-a	−Ldh-a	+Ldh-a	−Ldh-a	−Ldh-a	−Ldh-a	−Ldh-a	−Ldh-a	−Ldh-a
12	12		12					12		12		12
	+Pep-b	−Pep-b	+Pep-b	−Pep-b	−Pep-b	−Pep-b	−Pep-b	+Pep-b	−Pep-b	+Pep-b	−Pep-b	+Pep-b
	+Ldh-b	−Ldh-b	+Ldh-b	−Ldh-b	−Ldh-b	−Ldh-b	−Ldh-b	+Ldh-b	−Ldh-b	+Ldh-b	−Ldh-b	+nd
13	13					13						
	+Esd	−Esd	−Esd	−Esd	−Esd	+Esd	−Esd	−Esd	−Esd	−Esd	−Esd	−Esd
14	14	14						14		14	14	
	+NP	+NP	−NP	−NP	−NP	−NP	−NP	+NP	−NP	+NP	+NP	−NP
15	15		15									
	+Mpi	−Mpi	+Mpi	−Mpi	−Mpi	−Mpi	−Mpi	−Mpi	−Mpi	−Mpi	−Mpi	nd
	nd	−Hex-a	+Hex-a	−Hex-a	−Hex-a	−Hex-a	−Hex-a	−Hex-a	−Hex-a	nd	−Hex-a	−Hex-a
16				16					16		16	
	−Aprt	−Aprt	−Aprt	+Aprt	−Aprt	−Aprt	−Aprt	−Aprt	+Aprt	−Aprt	+Aprt	nd
17	17			17		17						17
	+GalK	−GalK	−GalK	+GalK	−GalK	+GalK	−GalK	−GalK	−GalK	−GalK	−GalK	+GalK
18	18			18	18							
	+Pep-a	−Pep-a	−Pep-a	+Pep-a	+Pep-a	−Pep-a	−Pep-a	−Pep-a	−Pep-a	−Pep-a	−Pep-a	−Pep-a
19	19		19				19				19	19
	+Gpi	−Gpi	+Gpi	−Gpi	−Gpi	−Gpi	+Gpi	−Gpi	−Gpi	−Gpi	+Gpi	+Gpi
20	20					20	20		20			
	+Ada	−Ada	−Ada	−Ada	−Ada	+Ada	+Ada	−Ada	+Ada	−Ada	−Ada	−Ada
21	21		21					21	21	21	21	21
	+Sod-1	−Sod-1	+Sod-1	−Sod-1	−Sod-1	−Sod-1	−Sod-1	+Sod-1	+Sod-1	+Sod-1	+Sod-1	+Sod-1
22									22			
X	X	X	X							X	X	
	+Hprt	+Hprt	+Hprt	−Hprt	−Hprt	−Hprt	−Hprt	−Hprt	−Hprt	+Hprt	−Hprt	−Hprt
	+Gpd	+Gpd	+Gpd	−Gpd	−Gpd	−Gpd	−Gpd	−Gpd	−Gpd	+Gpd	+Gpd	−Gpd
Human type I procollagen*	+	−	−	+	−	+	−	−	−	−	−	+

Presence of human chromosomes and isozymes whose genes have been assigned to human chromosomes (15, 16) are indicated. Human chromosomes are scored positive if present in >15% of the cells analyzed. Isozyme abbreviations: Pgm-1, phosphoglucomutase 1 (EC 2.7.5.1); Pep-c, peptidase C (EC 3.4.11.1); Idh-1, isocitrate dehydrogenase, cytoplasmic (EC 1.1.1.42); Mdh-1, malate dehydrogenase, cytoplasmic (EC 1.1.1.37); Hex-b, hexosaminidase B (EC 3.2.1.30); Me-1, malic enzyme, cytoplasmic (EC 1.1.1.40); Ak-1, adenylate kinase, erythrocyte form (EC 2.7.4.3); Got-1, glutamate oxaloacetate transaminase, cytoplasmic (EC 2.6.1.1); AdoK, adenosine kinase (EC 2.7.1.20); Ldh-a, lactate dehydrogenase A (EC 1.1.1.27); Pep-b, peptidase B (EC 3.4.11.1); Ldh-b, lactate dehydrogenase B (EC 1.1.1.27); Esd, esterase D (EC 3.1.1.—); NP, purine nucleoside phosphorylase (EC 2.4.2.1); Mpi, mannosephosphate isomerase (EC 5.3.1.8); Hex-a, hexosaminidase A (EC 3.2.1.30); Aprt, adenine phosphoribosyltransferase (EC 2.4.2.7.); GalK, galactokinase (EC 2.7.1.6); Pep-a, peptidase A (EC 3.4.11.1); Gpi, glucosephosphate isomerase (EC 5.3.1.9); Ada, adenosine deaminase (EC 3.5.4.4); Sod-1, superoxide dismutase, cytoplasmic (EC 1.15.1.1); Hprt, hypoxanthine phosphoribosyltransferase (EC 2.4.2.8); Gpd, glucose-6-phosphate dehydrogenase (EC 1.1.1.49).

* + indicates presence and − indicates absence.

E group chromosomes could be found in the IL-II-5 back-selectant. Both TMSIII-2a and IL-II-5 back-selectants no longer expressed the human form of the GalK isozyme (WL II 24a-2a back-selectant was not tested for GalK isozyme). When these cell lines were assayed for human type I procollagen production, they were found to be negative.

DISCUSSION

Although somatic cell hybridization has been used to study various cellular metabolic functions, there exists only one report of its use to study collagen biosynthesis. Green *et al.* (34) hybridized two fibroblast lines, NCTC 2472 and NCTC 2555, that made considerable amounts of collagen with another fibroblast, 3T6, that made little collagen. They observed that, among the sets of hybrid clones from the crosses, the hybrids produced collagen at a rate intermediate between the rates of the parent lines. However, there were no indications as to the type of collagen synthesized or the karyology of the hybrids.

The present study's use of a specific antibody produced against purified human type I procollagen to examine a series of human–mouse hybrid cell lines has provided evidence for the assignment of the type I procollagen gene to human chromosome 17. The assignment is strengthened by the analysis of the human microcell hybrid cell line containing only human chromosome 17 and producing human type I procollagen. The type I procollagen gene can be assigned more specifically to the long arm of human chromosome 17, because the cell line containing only the long arm of human chromosome 17 was also positive for type I procollagen production. The possibility does exist that our antiprocollagen antibody was produced to only human pro α1 or pro α2 chains and that our gene assignment is to either pro α chain rather than to the procollagen molecule itself. This possibility would necessitate that "hybrid" procollagen molecules are being formed between mouse and human pro α chains. Alternatively, both the pro α1 and pro α2 proteins may be coded by genes located on chromosome 17.

It is also of interest that no abnormalities involving human chromosome 17 have been reported that affect collagen production or connective tissue in general. It is probable that any chromosomal abnormalities involving type I procollagen production (or the synthesis of an abnormal type I procollagen) would be a very early lethal disorder, in view of the important role of type I collagen in the structure of many organs.

Preliminary studies (C. V. Sundar Raj, F. H. Ruddle, and R. L. Church, unpublished data) have indicated that human type III procollagen and procollagen peptidase segregate independently from type I procollagen and, therefore, the synthesis of each of these proteins must be controlled by separate genes. Further work using somatic cell hybrids will aid in defining the genes involved in the multistep process of collagen biosynthesis as well as provide a means of examining disorders involving connective tissue.

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