THE LINKAGE OF GENES FOR THE HUMAN INTERFERON-INDUCED ANTIVIRAL PROTEIN AND INDOPHENOL OXIDASE-B TRAITS TO CHROMOSOME G-21

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Mouse-human somatic cell hybrids have been used in a number of studies to demonstrate that particular human genes are syntenic and that they are linked to a specific human chromosome. The reason for using mouse-human cell hybrids lies in the preferential loss of human chromosomes, that certain mouse and human phenotypes are distinguishable from each other, and that the human chromosomes are identifiable from the mouse chromosomes in the hybrid cells (1, 2).

When two or more human phenotypes are always present together in a hybrid cell population, never one without the other, and if both are lost together, then one can infer that they are syntenic (present on the same chromosome). This is also referred to as concordant segregation. However, when two or more human phenotypes in the hybrid cell population are present, sometimes one without the other, then they are asyntenic. This is referred to as discordant segregation. Furthermore, if the presence of one or more human phenotypes always correlates with the presence of a particular human chromosome, and if the phenotype(s) is (are) lost together with the loss of that particular chromosome, then one can infer that the particular gene(s) is (are) linked to that chromosome.

In this paper, using the technique of mouse-human somatic cell hybridization and the rationale just described, we have been able to demonstrate the concordant segregation of the human dimeric form of indophenol oxidase (IPO-B)[1] and antiviral protein (AVP) induced by human interferon. Thus, we have demonstrated the two human functions are syntenic. Furthermore, we have been able to provide evidence which indicates that both functions are linked to the human G-21 chromosome.

Materials and Methods

Cell Hybridization and Culture Methods.—Hybrid cell populations were produced by mixing mouse and human cells together, in either a 1:4 or 1:10 human to mouse cell ratio at a total cell density of 4×10^6 cells/25 cm^2 Falcon (Oxnard, Calif.) tissue culture flask (3). Cell fusion

[1] *Abbreviations used in this paper:* APRT, adenine phosphoribosyltransferase; AVP, antiviral protein; CPE, cytopathic effect; DVME, Dulbecco-Vogt modified Eagle's minimal medium; IPO-B, indophenol oxidase-b; MOI, multiplicity of infection; NDV, Newcastle disease virus; VSV, vesicular stomatitis virus.

was stimulated by the addition of β-propiolactone-inactivated (1 ml) Sendai virus at concentrations of 1,000 hemagglutination units/ml (3, 4). In the case where both parent cells were fibroblasts, fusion was performed with the cells in monolayer. In those hybrids which were formed from a human leukocyte parent and a mouse fibroblast, fusion was performed with the cells in suspension (5).

The following three hybrid series were analyzed in this study. Wa hybrids were formed between mouse A9 cells deficient in adenine phosphoribosyltransferase (APRT) and the normal human female fibroblastic cell strain WI-38. JBA hybrids were formed between A9 cells deficient in APRT and human leukocytes, JB, which carry a heterozygous $1\frac{4}{22}$ centric fusion.[2] J hybrids were formed between mouse RAG cells which are deficient in hypoxanthine-guanine phosphoribosyltransferase (HGPRT) and normal human leukocytes (5). The Wa and JBA hybrid series were isolated in alanosine, adenine hybrid selection medium which selects those cells which have APRT activity, usually due to the presence of the human gene for APRT (6).[2] Alanosine-adenine medium was made by adding alanosine (the gift of Dr. Piero Sensi, Lepetit S.p.A.) at a concentration of 7 μg/ml and adenine at 5×10^{-5} M in Dulbecco-Vogt modified Eagle's minimal medium (DVME) from Grand Island Biological Co. (Grand Island, N. Y.) supplemented with 10% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 100 μg/ml kanamycin. In the case of the Wa hybrids, the selective system operated only against the mouse parent. The hybrids could be distinguished from the human parent, however, by their different morphology (Fig. 1) and their more rapid, non-contact-inhibited growth. The J hybrids were isolated with the HAT[3] selective system of Littlefield (7) using DVME supplemented with calf serum as above. It should be noted that in the cases where the human parent is a leukocyte, one need only select against the mouse parent as normal leukocytes do not adhere to the surface of the flask and can be washed away from the attached cells.

Hybrid clones were isolated, one colony per bottle, with stainless steel rings using the method of Puck et al. (8). These procedures help ensure the independence of each hybrid cell strain, the importance of which has been discussed elsewhere (1, 2). Secondary hybrid colonies were isolated by plating low cell numbers of primary hybrid strains and picking colonies as above. The Wa V subclones were isolated in medium containing 2-6-diaminopurine and 2-fluoroadenine and thus lacked APRT activity. The details of this back selection are described by Tischfield and Ruddle[2] and are not relevant to the present paper.

Gel Assays of Enzymes.—Hybrid clones were examined for the human forms of the following 22 enzymes by starch gel (9) and acrylamide gel electrophoresis.[4] For each gel identification of the enzyme, cell extract from 5×10^7 cells was used. The 22 enzymes are: adenosine deaminase (EC 3.5.4.4), adenine phosphoribosyltransferase (EC 2.4.2.7), glutamate oxaloacetate transaminase (EC 2.6.1.1), glucose-6-phosphate dehydrogenase (EC 1.1.1.49), glucose phosphate isomerase (EC 5.3.1.9), hypoxanthine-guanine phosphoribosyltransferase (EC 2.4.2.8), isocitrate dehydrogenase (EC 1.1.1.42), indophenol oxidase-A [tetrameric form], indophenol oxidase-B [dimeric form], lactate dehydrogenase-A (EC 1.1.1.27), lactate dehydrogenase-B (EC 1.1.1.27), malate oxidoreductase decarboxylating (EC 1.1.1.40), malate oxidoreductase (EC 1.1.1.37), mannose phosphate isomerase (EC 5.3.1.8), nucleoside phosphorylase (EC 2.4.2.1), peptidase-A, peptidase-B, peptidase-C, peptidase-D, phosphoglycerate kinase (EC 2.7.2.3), phosphoglucomutase-1 (EC 2.7.5.1), and thymidine kinase (2.7.1.21).

For the starch gel assays of indophenol oxidase, the gels were stained after completion of

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[2] Tischfield, J., and F. H. Ruddle. Human linkage assignment of adenine phosphoribosyltransferase by means of somatic cell hybridization. Manuscript in preparation.

[3] 10^{-4} M hypoxanthine, 10^{-5} M aminopterin, 4×10^{-5} M thymidine, and 10^{-5} M glycine.

[4] Tischfield, J., and F. H. Ruddle. A new electrophoretic-autoradiographic method for visualization of phosphotransferases. Submitted to *Anal. Biochem.*

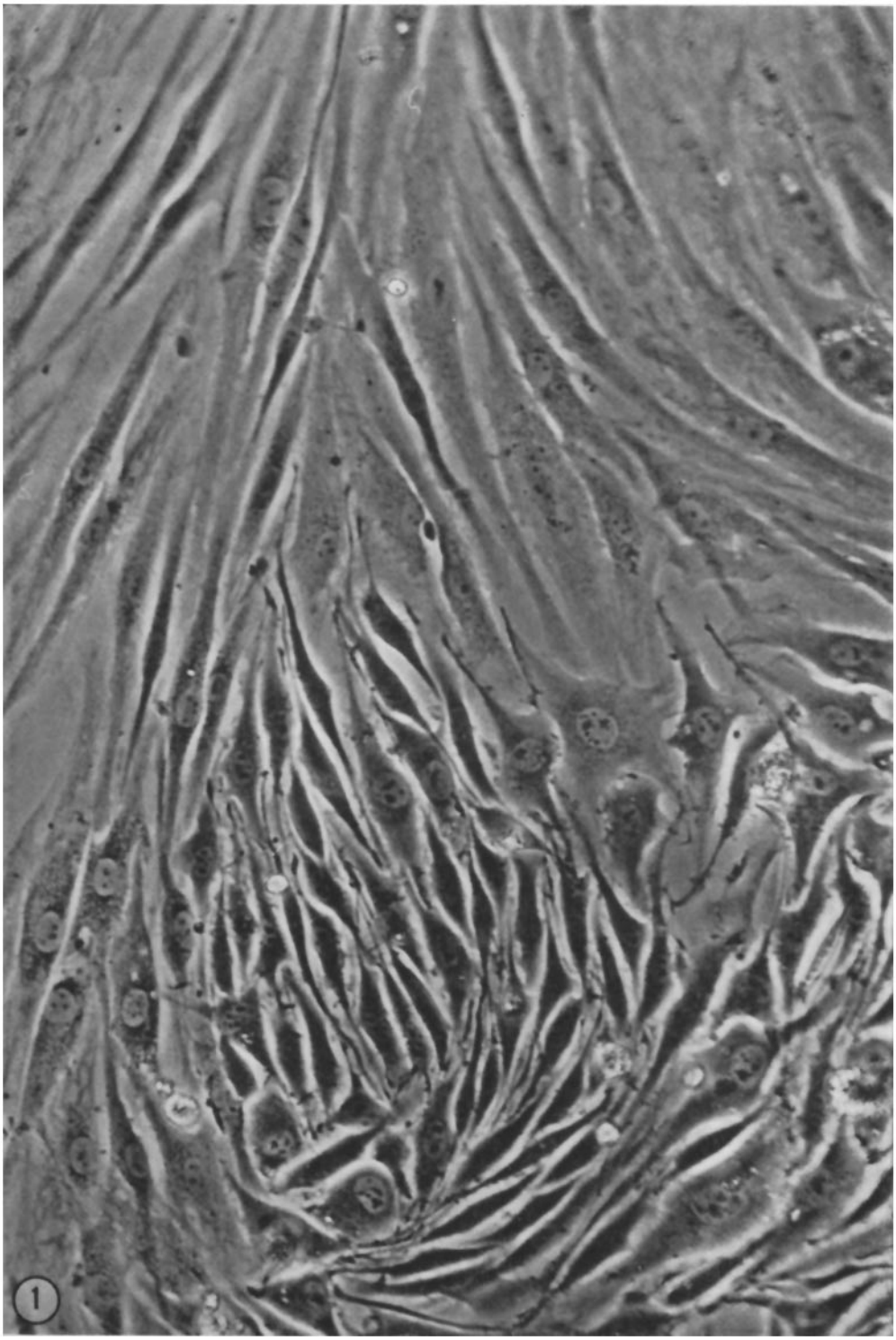


FIG. 1. Mouse-human somatic cell hybrids of WI-38 and A9 cells against a background of WI-38 fibroblasts.

electrophoresis with a mixture composed of 5.8×10^{-4} M MTT tetrazolium (Sigma Chemical Co., St. Louis, Mo.), 1.54×10^{-4} M phenazine methosulfate (Sigma Chemical Co.), and 8.3×10^{-2} M Tris HCl (Sigma Chemical Co.) buffer pH 8.0. Exposure of the stained gel to light resulted in the appearance of light bands in a bluish background (Fig. 3). The reaction causing these light bands to appear is catalyzed by the indophenol oxidase activity of the cell extracts. This enzyme oxidizes the tetrazolium dye in the presence of phenazine methosulfate to a colorless form. Although indophenol oxidase activity is widely distributed in human tissues, little is known of its physiological function in vivo. Two forms of the oxidase are analyzed in our study the A (tetrameric) and B (dimeric) forms. Both forms of the enzyme are also known to catalyze the Nadi reaction and the oxidation of dichlorophenolindophenol (10).

Chromosome Analysis.—The human chromosomes were identified in metaphase cells prepared to reveal quinacrine banding, Giemsa banding (11), and constitutive heterochromatin (12, 13). To facilitate the identification of chromosomes, metaphases that were photographed with ultraviolet illumination for quinacrine mustard banding were also photographed using dark-field illumination in the visible spectrum. This resulted in a photograph which clearly showed chromosome size and morphology and which facilitated chromosome identification particularly of the small human chromosomes G-21 and G-22. Of the metaphase cells analyzed in this study, the majority were prepared as karyotypes.

Interferon.—We are grateful to Dr. J. Valenta of Smith Kline & French Laboratories (Philadelphia, Pa.) for a gift of unfractionated human leukocyte interferon. This material was previously acid treated at pH 2.0 for 72 h. It was then purified on a Sephadex G-150 column (2.5 × 94 cm) and eluted at a ve of about 342 ml. Fractions from 350 to 385 ml were pooled and dialyzed for 2 days at 4°C against one change of 2 liters DVME without calf serum. Heat-inactivated calf serum was added to the pooled interferon fraction to a final concentration of 10%. Unless otherwise stated, purified interferon was used throughout this study. Similarly, mouse serum interferon induced by Newcastle disease virus, Herts strain, was purified on a Sephadex G-75 column (2.5 × 45 cm). The partially purified interferons were standardized with a interferon preparation previously standardized to the internal standards for human and murine interferon.

Semi-Microdye-Binding Assay for Antiviral State.—Our test for the antiviral resistance induced by interferon is an adaptation of Armstrong's (14) dye-binding assay for interferon. For the assay described here, 0.2 ml of suspended cells (4×10^4) was placed in each microwell of a microtest plate (Linbro Chemical Co., New Haven, Conn.). The microtest plates have 96 flat-bottomed wells of 6 mm diameter each. The plates were used for assay 24 h after seeding. Fluids from the wells were decanted by vigorous inversion and wiped dry with sterile napkins. One set of triplicate wells was used for each concentration of interferon used to induce the antiviral state. The volume of partially purified interferon of human or murine origin added to the microwells was 0.2 ml. For each cell line induced for antiviral resistance, a set of triplicate microwells serves as viral control and another set serves as cell control. After incubating the cells overnight with interferon, the microplates were decanted vigorously, wiped dry with sterile napkins, and challenged with vesicular stomatitis virus (VSV) at a multiplicity of infection (MOI) of 2 except where otherwise indicated. The cell controls which were not treated with interferon were replenished with 0.2 ml of fresh medium per well whereas the viral controls were challenged with VSV. After the viral challenge, the cultures were incubated for another 24 h at which time the viral controls showed 90–100% cytopathic effect (CPE) as observed microscopically and incubation was terminated. Occasionally, a longer incubation was required. The plates were decanted and washed twice with 0.85% NaCl before staining in 0.5% methylrosaniline chloride, 5% formalin (vol/vol), and 5% ethanol (vol/vol) in 0.85% NaCl. The dye was removed by copious washing with tap water and the plates were left to air dry. For quantitative assay the dye was extracted

by 2-methoxyethanol and read at 550 nm. The amount of antiviral resistance in the interferon-treated cells is expressed as a percent of the dye extracted from the cell control minus the dye extracted from the viral control:

$$\frac{\text{OD interferon treated} - \text{OD viral control}}{\text{OD cell control} - \text{OD viral control}} \times 100\%.$$

Species Specificity of Mouse and Human Interferon.—Sets of triplicate microwells of mouse 8-azaguanine-resistant A9 cells, WI-38, and an 8-azaguanine-resistant mouse kidney adenocarcinoma cells (RAG) were incubated with 0.2 ml of mouse (100 U/ml) and human (1,000 U/ml interferon overnight. The microwells were decanted the next day and challenged with VSV. The effect of the viral challenge was read the following day and the results are expressed in Table I. The table shows that a strict species specificity barrier exists between mouse and human interferon. The hybrid cell line Wa V (WI-38 × A9) was protected by both human and mouse interferon.

The Effect of Actinomycin D on the Induction of Antiviral Expression by Interferon.—Actinomycin D (Merck Sharp & Dohme, West Point, Pa.), an irreversible inhibitor of DNA-depend-

TABLE I

Species Specificity of Murine and Human Interferon

Cell line	Protection of control	
	Mouse interferon*	Human interferon‡
	%	%
WI-38 (human)	0	94
A9 (mouse)	102	0
RAG (mouse)	118	0
Wa V (mouse × human)	105	112

* 100 U/ml.

‡ 1,000 U/ml.

ent RNA synthesis was previously shown by Taylor (15) to inhibit the synthesis of an antiviral protein in cell cultures induced with interferon. Sets of triplicate microwells of mouse human hybrids and parental cells from which these hybrids originated were incubated with 0.2 ml of medium containing mouse or human interferon at concentrations indicated in Table II. Actinomycin D was added to the wells such that the final concentration was 1.2 μg/ml. It was added either simultaneously to the wells with interferon or 11 h after the addition of interferon. The concentration of actinomycin D used in our system inhibited the uptake of [^{14}C]uridine (1 μCi/ml, 60 mm culture dish) by 83 and 91% in WI-38 and A9 cells, respectively, after the cells were exposed to the inhibitor for 1 h. The cells were then challenged with VSV at an MOI of 5 at h 12. The incubation with virus was terminated at h 30. The timing of this experiment is important since actinomycin D causes minor CPE in A9 cells and a few of the hybrid cells at 36 h and beyond.

The effect of actinomycin D on the induction of antiviral resistance is shown in Table II. When the cultures were exposed to actinomycin D simultaneously with interferon, the cultures were not resistant to a viral challenge. However, this effect was greatly reduced when actinomycin D was added 11 h after the cells were exposed to interferon. The result suggests the induction of antiviral resistance by interferon in the hybrid cells is at the transcriptional level. Furthermore, the data are consistent with Taylor's observation that interferon induces an antiviral protein which is responsible for the antiviral status of the cell (15).

TABLE II

The Effect of Actinomycin D on Antiviral State Induced by Interferon

Cell line	Type	Actinomycin D added at	Protection of control*	
			Mouse interferon	Human interferon
		h	%	%
WI-38	Human	0	N.D.	0.5
A9	Mouse	0	0	N.D.
Wa V	Hybrid	0	1.1	0.8
Wa III	Hybrid	0	0	N.D.
$J_{10}H_9$	Hybrid	0	0	0
WI-38	Human	11	N.D.	N.D.
A9	Mouse	11	98	N.D.
Wa V	Hybrid	11	88	93
Wa III	Hybrid	11	121	N.D.
$J_{10}H_9$	Hybrid	11	72	89

N.D. = not done.

* Control sets of triplicate wells were protected with homologous interferon but not treated with actinomycin D, and the antiviral resistance in these sets were assigned an arbitrary figure of 100. The concentrations of mouse and human interferon used was 100 U and 1,000 U/ml, respectively, and were added to the cells at h 0.

RESULTS

A total of 13 mouse-human cell hybrids derived from 13 independent cell fusions of mouse and human cells were analyzed for protection by human and mouse interferon, and as far as possible, complete chromosomal and enzyme analyses were done on these cells simultaneously.

The test for antiviral resistance induced by heterologous interferon in these mouse-human cell hybrids shows that the parental cells from which the hybrids were derived were protected only by homologous interferon (Table III). Human leukocytes have previously been shown to be protected by human interferon (16). Treatment of the hybrid clones with mouse interferon induced an antiviral state in all the hybrids tested, whereas, treatment of the hybrids with human interferon induced an antiviral state in only three of the hybrid clones belonging to the Wa series (WI-38 × A9) and all of the hybrids of the J series (human lymphocyte × RAG) and JBA (human lymphocyte × A9). However, six hybrid clones belonging to the Wa series did not respond to treatment with human interferon. This loss of sensitivity to protection by human interferon could be indicative of the loss of a specific human gene responsible for the expression of the human antiviral protein. Alternatively, it could suggest the loss of interferon receptor sites, but this is unlikely since all the hybrid cells tested were sensitive to mouse interferon, and furthermore, they were equally susceptible to VSV challenge.

In testing for the presence or absence of the 22 enzymes examined, the dimeric form of the human indophenol oxidase was found to be the only enzyme which

TABLE III

Protection of Somatic Cell Hybrids by Interferon against VSV Challenge

Cell line	U of interferon/ml	Protection of control by	
		Human interferon	Mouse interferon
		%	%
WI-38 × A9			
Wa I	1,000	57	69
	100	42	98
Wa II	1,000	41	93
	100	29	112
Wa III	1,000	0	76
	100	0	87
Wa IV	1,000	0	83
	100	2	101
Wa V	1,000	132	93
	100	104	100
	10	39	83
Wa VI	500	3	100
	100	0	104
Wa VII	1,000	0	77
	100	1	89
Wa VIII	1,000	4	94
	100	0	102
Wa IX	1,000	6	94
	100	0	94
WI-38	1,000	112	1.8
	100	77	N.D.
A9	1,000	0.8	98
Human leukocyte × A9			
JBA-1	1,000	89	87
	100	12	N.D.
Human leukocyte × RAG			
J_3S	200	N.D.	65
	20	62	43
$J_{10}H_9$	200	99	106
	20	98	111
$J_{10}H_7$	200	104	96
	20	98	96

N.D. = not done.

segregated concordantly with protection by human interferon (Table IV). Chromosomal analysis was done for 12 of the 13 primary clones. The number of metaphases examined for the chromosomal analysis is also indicated. Chromosome G-21 was the only chromosome which was detected in all clones which were pro-

TABLE IV

The Segregation of Antiviral Protein (AVP) Indophenol Oxidase (IPO-B) and Human Chromosome G-21 in Primary Clones

Hybrid clone	no. of metaphases analyzed	no. of metaphases showing G-21	IPO-B	AVP
Wa Ia	23	13	+	+
Wa IIa	32	21	+	+
Wa III	50	0	−	−
Wa IVa	29	0	−	−
Wa V	59	31	+	+
Wa VIa	58	0	−	−
Wa VIIa	48	0	−	−
Wa VIIIa	31	0	−	−
Wa IXa	27	0	−	−
JBA-1	55	42	+	+
J_8S	10	10	+	+
$J_{10}H_9$	27	13	+	+
$J_{10}H_7$	N.D.	N.D.	+	+

+, indicates phenotype present; −, indicates phenotype absent.

tected by human interferon and which contained the human form of indophenol oxidase (Table IV). Table IV also shows an average frequency of 63% for chromosome G-21 in those clones which retained both the AVP and IPO-B human phenotypes. Among the clones not showing the two phenotypes the frequency of G-21 chromosome was 0%.

From the chromosomal analysis of those clones which showed both phenotypes (AVP and IPO-B), it was determined that Wa V contained the smallest number of human chromosomes. It seemed reasonable that Wa V was our best candidate for providing further evidence for or against the synteny of IPO to AVP and the linkage of both phenotypes to chromosome G-21. Wa V cells were, therefore, subjected to a back selection procedure using 1,6-diaminopurine and 2-fluoroadenine resulting in further loss of human chromosomes in these cells.[2] This back selection method, selects for those cells which have lost APRT and, therefore, continue to grow in the presence of 2,6-diaminopurine and 2-fluoroadenine. A total of nine subclones were isolated after a period of a month by this procedure. These subclones were exposed to mouse and human interferon. The results in Table V show all the nine subclones were protected by mouse interferon. All nine subclones but one (Wa V R4c) were protected by human interferon. The eight that were protected by human interferon also expressed the dimeric form of human indophenol oxidase (Table VI). However, the subclone (Wa V R4c) which lost the AVP phenotype also lost IPO-B phenotype. A complete chromosomal analysis of four of the nine subclones isolated indicates the clones which retained both human phenotypes had an average frequency of 68.7% cells retaining the G-21 chromosome and 0% in the case of Wa V R4c which had lost both phenotypes (Table VI).

TABLE V

Protection of Wa V Subclones by Interferon against VSV Challenge

Cell type	U of interferon/ml	Protection by	
		Human interferon	Mouse interferon
		%	%
Wa V 2Ra	1,000	91	
	100	92	86
Wa V 2Rb	1,000	87	
	100	66	97
Wa V 4Ra	1,000	68	
	100	57	104
Wa V 4Rb	1,000	77	
	100	65	78
Wa V 4Rc	1,000	0	
	100	0	98
Wa V 4Rd	1,000	38	
	100	44	69
Wa V 5Ra	1,000	86	
	100	77	77
Wa V 5Rb	1,000	46	
	100	28	89
Wa V 5Rc	1,000	72	
	100	41	101

TABLE VI

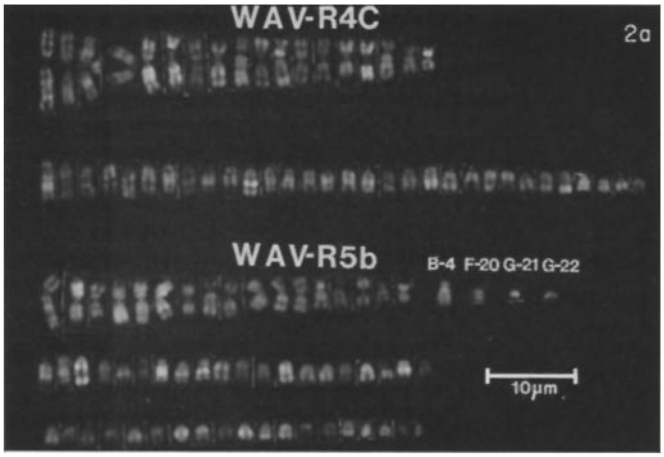
The Segregation of Antiviral Protein, Indophenol Oxidase, and Human Chromosome G-21 in Subclones of Wa V

Subclones	no. of metaphases analyzed	no. of metaphases showing G-21	IPO-B	AVP
Wa V R4c	69	0	−	−
Wa V R4d	27	19	+	+
Wa V R5b	25	18	+	+
Wa V R2b	25	16	+	+
Wa V R2a	N.D.	N.D.	+	+
Wa V R4a	N.D.	N.D.	+	+
Wa V R4b	N.D.	N.D.	+	+
Wa V R5a	N.D.	N.D.	+	+
Wa V R5c	N.D.	N.D.	+	+

N.D. = not done.

+, indicates phenotype present; −, indicates phenotype absent.

The loss of the G-21 chromosome in Wa V R4c is clearly shown in the representative metaphases of the Wa V subclones (Figs. 2 *a* and *b*). Correspondingly, the loss of IPO-B phenotype from Wa V R4c is clearly indicated in the starch gel stained for IPO-B (Fig. 3). The simultaneous loss of AVP, IPO-B human pheno-



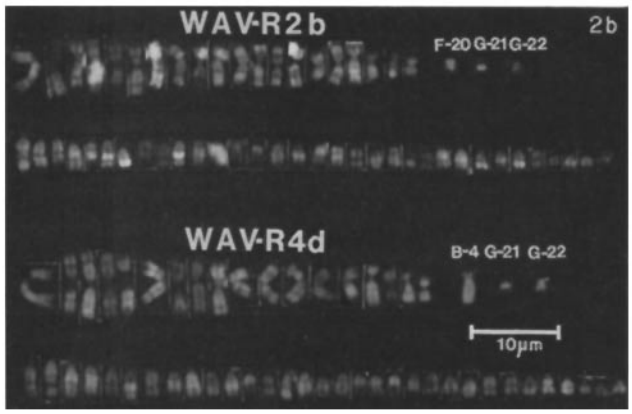


FIG. 2. (*a*) Karyotypes of Wa V R4c and R5b; (*b*) karyotypes of Wa V R4d and R2b.

type, and genotype G-21 in subclone Wa V R4c support the synteny of the human genes for IPO-B and AVP and their linkage to the G-21 chromosome.

It is also possible to strengthen the proposed assignments by the demonstration of negative correlations between the IPO-B and AVP phenotypes and chromosome other than 21. Such negative correlations exist between the two phenotypes and all the chromosomes except 21 (Table VII). These negative results in addition to the positive correlation strongly support the suggested chromosome assignment.

DISCUSSION

Previous to this study, little was known of the genetics of the interferon system except that the structural gene for the synthesis of interferon is located on a locus separate from that coding for the synthesis of antiviral protein (17–19).

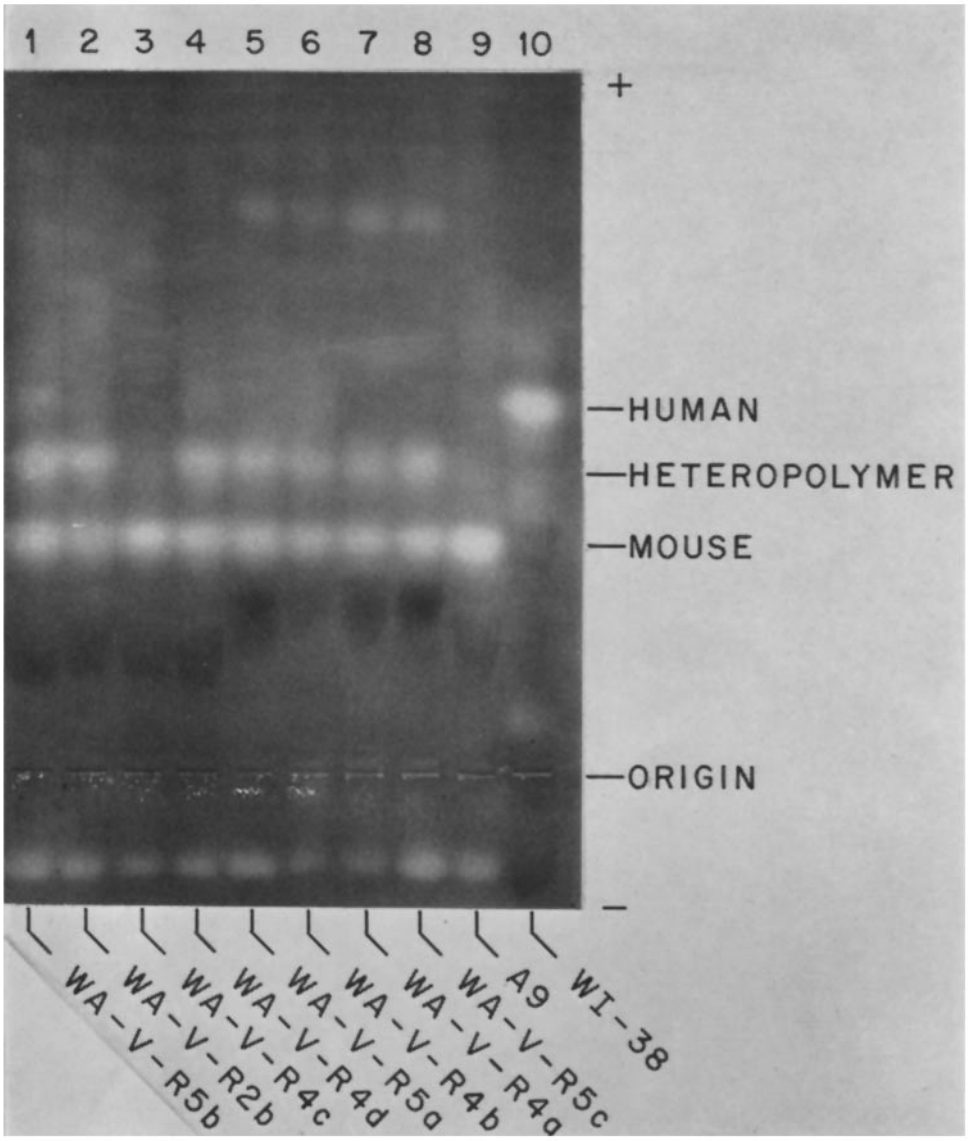


FIG. 3. Zymogram showing IPO-B activities of Wa V subclones.

Using monkey-mouse hybrid cells, Cassingena et al. (19) suggested that the genome governing the synthesis of simian interferon-induced AVP possibly is located in one of the VSST (very very small subtelocentric of simian origin) or on a VLSM chromosomes (very large submetacentric of simian or murine origin).

TABLE VII

Negative Correlation of Indophenol Oxidase and Antiviral Protein with Chromosomes Other Than G-21

Human chromosome	Human phenotype IPO-B and AVP / Human chromosome			
	+/+	−/−	+/−	−/+
1	3	6	6	1
2	1	6	8	1
3	2	6	7	1
4	7	7	2	0
5	0	7	9	0
6	0	6	9	1
7	3	6	6	1
8	3	6	6	1
9	0	7	9	0
10	3	6	6	1
11	3	6	6	1
12	3	5	6	2
13	1	6	8	1
14	1	4	8	3
15	0	7	9	0
16	4	3	5	4
17	2	7	7	0
18	4	7	5	0
19	2	7	7	0
20	5	6	4	1
21	9	7	0	0
22	5	3	4	4
X	4	5	5	2

+ = present; − = absent.

In our present study, we also examined the ability of hybrid clones of the Wa series to synthesized interferon. Primary clones of the Wa series were exposed to inducers of interferon such as Newcastle disease virus (NDV) at an MOI of 1 and to poly I·poly C (100 and 200 μg/ml from P. L. Biochemicals, Milwaukee, Wis.) for 1 h. However, none of the hybrid clones of the Wa series was shown to produce any human interferon although all of them produced between 8 and 24 U of mouse interferon from each culture dish in response to NDV stimulation. In view of the observation that three of the Wa primary clones are protected by human interferon (Table III), the data are in agreement with the hypothesis that the genes for interferon synthesis and AVP synthesis are located in different chromosomes.

From our present study, we have traced the concordant segregation of AVP and IPO-B in the 13 primary mouse-human hybrids and nine subclones (Tables IV and VI). The simultaneous disappearance of AVP and IPO-B in one of the nine subclones of Wa V (Table VI) provides further evidence for the synteny of

the two phenotypes. Additional evidence in support of this hypothesis comes from the observation that there were strong negative correlations between the two human phenotypes and all of the human chromosomes except for chromosome B_4 for which there is a weaker negative correlation (Table VII). One primary clone $J_{10}H_9$ and one subclone Wa V R2b, however, rule out the possibility of linkage to B_4 since in a total of 52 metaphases examined, none had a B_4 chromosome; whereas, 56% of the cells examined possessed a human G-21 (Tables IV and VI).

The assignment of AVP to chromosome G-21, we believe, represents the first mapping of a genetic factor regulating an inducible phenotype in mammalian cells. The evidence suggesting that AVP is an inducible phenotype comes from the use of actinomycin D in our system. Exposure of those hybrid cells (Table II) which were protected by human interferon to actinomycin D at the time of interferon addition eliminated the protection. However, once the antiviral state is induced in these cells by human interferon, actinomycin D was no longer effective.

SUMMARY

13 independent mouse-human somatic cell hybrid clones derived from β-propiolactone-inactivated Sendai stimulated cell fusion of human cells with mouse cells were tested for their sensitivities to human and mouse interferon. All of them were protected by mouse interferon and only six of the clones were protected by both human and mouse interferon. Only the six that were protected by human interferon were shown to express the human dimeric form of indophenol oxidase. Complete chromosomal analysis of the clones indicated human chromosome G-21 to be the only human chromosome in common for the six clones which had both phenotypes present. Nine subclones were derived from one of the clones expressing both phenotypes. Eight of the nine subclones were shown to retain both phenotypes, whereas one subclone lost both. Chromosomal analysis of the subclones indicated the loss of chromosome G-21 from the subclone which lost both phenotypes. It is apparent from these findings that the gene(s) for indophenol oxidase (IPO-B) and the gene(s) for the antiviral protein are syntenic and that they are linked to human chromosome G-21.

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