X-LINKAGE OF A HUMAN GENETIC LOCUS THAT CORRECTS THE DNA SYNTHESIS LESION IN *tsC1AGOH* MOUSE CELLS[1]

RICHARD E. GILES[2] AND FRANK H. RUDDLE[3]

Department of Biology, Yale University, New Haven, Connecticut 06520

Manuscript received December 19, 1978
Revised copy received March 30, 1979

ABSTRACT

GM 126 human diploid fibroblasts were fused with a heat-sensitive mouse cell mutant defective in DNA synthesis, and primary hybrids were selected at permissive and nonpermissive temperatures in HAT medium. Primary hybrids, primary hybrid clones back-selected in 8-azaguanine at the permissive temperature, and subclones of heat-resistant primary hybrids isolated under nonselective conditions or after 8-azaguanine treatment were tested for heat sensitivity, the expression of 26 human enzymes assigned to 19 different human chromosomes, and the presence of human chromosomes. Only the human *X* chromosome and *X*-linked marker enzymes exhibited a clear pattern of concordant segregation with the heat-resistant phenotype. On the basis of these observations, we have defined the human genetic locus that corrects the heat-sensitive lesion in *tsC1AGOH* as *hrC1AGOH* and have assigned this locus to the *X* chromosome. This observation provides the first instance where two selectable markers (heat resistance and 8-azaguanine sensitivity) are found on a single human chromosome and suggests that these markers may prove to be a valuable push-pull selective system of use in determining the linear arrangement of genes on human chromosomes by somatic cell genetics.

GENETIC analysis of mammalian cells *in vitro* has been facilitated by analysis of interspecific isozyme polymorphisms in cell hybrids that exhibit preferential chromosome segregation. In man, over 100 autosomal loci and ten *X*-linked loci have been studied in somatic cell hybrids. The current status and practical value of the human gene map have been recently reviewed by McKUSICK and RUDDLE (1977). Biochemical selective systems such as the HAT system permit the selective fixation of specific human enzymes and chromosomes in hybrid cells (CREAGAN and RUDDLE 1977; RUDDLE and CREAGAN 1975). Unfortunately, only a few human chromosomes may be selectively retained in hybrids *via* biochemical selection. Genetic analysis of somatic cell hybrids in the absence of selectable markers may be complicated by the differential sensitivity of phenotype assays and the difficulty of detecting human chromosomes present in a hybrid cell population at a low frequency. Analysis of the human genome *in vitro* would be aided if it were possible to develop a collection of hybrid cell clones

[1] A preliminary report of these results was presented at the 1976 Meeting of the Genetics Society of America.
[2] Present address: Department of Genetics, Stanford University Medical Center, Stanford, California 94305.
[3] To whom reprint requests should be sent.

each of which had a different human chromosome present at a high frequency due to selective fixation. Another limiting factor in the genetic analysis of the human genome *in vitro* is the possibility that many human gene products of interest may not be conveniently distinguishable from homologous rodent gene products or selectable by biochemical or immunological means. Utilization of temperature-sensitive (ts) rodent cell mutants may alleviate some of these difficulties.

A variety of ts conditional lethal mammalian cell mutants have been isolated, including mutants defective in DNA synthesis (THOMPSON *et al.* 1970; THOMPSON *et al.* 1971), cell division (MING, CHANG and BASERGA 1976), rRNA maturation (MEISS and BASILICO 1972; TONIOLO, MEISS and BASILICO 1973) and aminoacyl tRNA synthetase activity (THOMPSON, HARKINS and STANNERS 1973; THOMPSON, LOFGREN and ADAIR 1977). All of the ts mammalian cell mutants isolated to date have behaved as recessives when tested in intraspecific cell fusion experiments (BASILICO 1977). Interspecific hybrids produced between a ts rodent cell and a human cell should retain the human chromosome(s) carrying the genetic information necessary for correction of the heat-sensitive (hs) lesion when the hybrids are propagated at the nonpermissive temperature (npT) normally lethal for the rodent parental cells. Hybrid clones isolated at the permissive temperature (pT) that have segregated the human chromosome(s) correcting the heat sensitive lesion would be expected to exhibit a heat-sensitive phenotype. In view of the wide variety of biochemical functions affected in both prokaryotic and eukaryotic ts mutants, it should be possible to map a large number of human gene loci by studying the segregation of human chromosomes, isozyme markers, and temperature sensitivity in human × ts rodent cell hybrids.

We have analyzed hybrid cell clones obtained by fusing *tsC1AGOH* mouse L-cells. which are defective in DNA synthesis at 38.5°, with human fibroblasts. Only the human *X*-linked markers *G6PD* and *HPRT* and the human *X* chromosome segregated concordantly with the heat-resistant phenotype. On the basis of these observations, we have defined the human genetic locus that corrects the heat-sensitive lesion in *tsC1AGOH* as *hrC1AGOH* and have assigned this locus to the human *X* chromosome.

MATERIALS AND METHODS

Cells and culture conditions: *tsC1AGOH* is a derivative of the *tsC1* mutant defective in DNA synthesis described by THOMPSON *et al.* (1971) and was generously provided by DRS. THOMPSON and BAKER. *tsC1AGOH* is resistant to 8-azaguanine (AG), sensitive to HAT medium, deficient in HPRT activity, and resistant to 3mM ouabain. No heat-resistant revertants of *tsC1AGOH* were found in five separate experiments employing a total of 1.7×10^7 cells. GM 126 human male fibroblasts carrying a balanced translocation (*1:15*) (PRESCOTT *et al.* 1975) were obtained from the Institute for Medical Research, Camden, New Jersey. The standard culture medium (DF medium) consisted of Dulbecco-Vogt Modified Eagles Medium (GIBCO H-21HG) supplemented with 10% fetal calf serum. Medium containing AG at a level of 40 μg per ml was used to select against cells expressing HPRT activity (DF + AG medium). HAT medium (DF + hypoxanthine 10^{-4}M + aminopterin 4×10^{-7}M + thymidine 1.6×10^{-5}M) was used to select hybrids expressing HPRT activity. Cultures were incubated at either 34° (pT) or 38.5° (npT). Temperature was continuously monitored with a temperature recorder (Honeywell

Model 112, probe sensitivity 0.06°). The gradient in the chamber varied from 0.3° to 0.7° depending on the loading of the incubator. Temperature variation at a single point was less than 0.2°. Hybrid cell clones were isolated by fusing parental cells with β-propiolactone-inactivated Sendai virus (GILES and RUDDLE 1973a) as described by GILES and RUDDLE (1973b).

Isozyme analysis: Hybrid cell clones were screened for the expression of human enzymes by analysis of the isozyme pattern after electrophoresis of cell extracts in a vertical starch gel slab as described by RUDDLE and NICHOLS (1971) and NICHOLS and RUDDLE (1973). The enzyme

TABLE 1

Isozyme repertoire

Enzyme	Abbr.	EC No.	Human chromosome	Reference
Phosphoglucomutase-1	PGM-1	2.7.5.1	*1*	NICHOLS and RUDDLE (1973)
Peptidase-C	PEP-C	3.4.11.1	*1*	NICHOLS and RUDDLE (1973)
Isocitrate dehydrogenase-1	IDH-1	1.1.1.42	*2*	NICHOLS and RUDDLE (1973)
Malate dehydrogenase (NAD)	MDH-1	1.1.1.37	*2*	NICHOLS and RUDDLE (1973)
Ribokinase	RK	2.7.1.15	*2*	GILES (1978a)
Hexosaminidase-B	HEX-B	3.2.1.30	*5*	GILES (1978a)
Malic enzyme (NAPD)	ME-1	1.1.1.40	*6*	NICHOLS and RUDDLE (1973)
Superoxide dismutase, mitochondrial	SOD-2	1.15.1.1	*6*	NICHOLS and RUDDLE (1973)
Uridine phosphorylase	UP	2.4.2.3	*7*	DENNY *et al.* (1978)
Glutathione reductase	GSR	1.6.4.2	*8*	NICHOLS and RUDDLE (1975)
Adenylate kinase, red cell form	AK-1	2.7.4.3	*9*	GILES (1978a)
Glutamate oxaloacetate transaminase cytoplasmic	GOT-1	2.6.1.1	*10*	NICHOLS and RUDDLE (1973)
Adenosine kinase	ADK	2.7.1.20	*10*	KLOBUTCHER *et al.* (1976)
Lactate dehydrogenase-A	LDH-A	1.1.1.27	*11*	NICHOLS and RUDDLE (1973)
Lactate dehydrogenase-B activity*	LDH-B	1.1.1.27	*12*	NICHOLS and RUDDLE (1973)
Peptidase-B	PEP-B	3.4.11.1	*12*	NICHOLS and RUDDLE (1973)
Triose phosphate isomerase	TPI	5.3.1.1	*12*	GILES (1978a)
Nucleoside phosphorylase (purine)	NP	2.4.2.1	*14*	NICHOLS and RUDDLE (1973)
Mannose phosphate isomerase	MPI	5.3.1.8	*15*	NICHOLS and RUDDLE (1973)
Pyruvate kinase-3	PK-3	2.7.1.40	*15*	GILES (1978a)
Adenine phosphoribosyl transferase	APRT	2.4.2.7	*16*	NICHOLS and RUDDLE (1973)
Galactokinase	GK	2.7.1.6	*17*	NICHOLS *et al.* (1974)
Peptidase-A	PEP-A	3.4.11.1	*18*	NICHOLS and RUDDLE (1973)
Phosphohexase isomerase	PHI	5.3.1.9	*19*	NICHOLS and RUDDLE (1973)
Adenosine deaminase	ADA	3.5.4.4	*20*	NICHOLS and RUDDLE (1973)
Superoxide dismutase, cytoplasmic	SOD-1	1.15.1.1	*21*	NICHOLS and RUDDLE (1973)
Phosphoglycerate kinase	PGK	2.7.2.3	*X*	NICHOLS and RUDDLE (1973)
Glucose-6-phosphate dehydrogenase	G6PD	1.1.1.49	*X*	NICHOLS and RUDDLE (1973)
Hypoxanthine-guanine phosphoribosyl transferase	HPRT	2.4.2.8	*X*	NICHOLS and RUDDLE (1973)

* LDH-B. Human and mouse B_4 homopolymers do not separate electrophoretically. Mouse cells in culture do not express detectable B_4 homopolymer activity, although a small amount of activity is occasionally present in the A_3B region of the gel. Human peripheral blood leucocytes and human cells in culture express all five forms of LDH. Interspecific cell hybrids were scored positive for human LDH-B whenever discrete bands of LDH activity were observed that migrated anodically to the most anodic band exhibited by the rodent control. The LDH-B locus in man has been assigned to chromosome *12* using HM hybrids produced with human cells heterozygous for a variant form of LDH-B (CHEN *et al.* 1973) and by studies demonstrating synteny of LDH-B activity and human PEP-B and the location of the human PEP-B locus on chromosome *12* (CHEN *et al.* 1973; RUDDLE *et al.* 1970).

activities examined are listed in Table 1. Cell extracts were prepared in one of two buffer solutions, either CMF-DPBS (GIBCO #419) pH 7.2, or 10 mM Tris-HCl pH 7.5, 0.1 mM dithiothreitol, 1 mM $MgCl_2$, 20 mM KCl, 10% v/v glycerol, and 0.5% v/v NP40. 1 to 5 $\times$ 10^7 cells per ml were resuspended in extraction buffer on ice and sonicated using two five-second bursts at 50 watts from a Biosonic II sonicator (20kc) equipped with a BP II-40T probe. The crude extracts were clarified by centrifugation at 30,000 $\times$ g for one hr at 4°. Thirty μl of extract were loaded into gel sample wells. Assay procedures employed in isozyme analysis are described in the references cited in Table 1.

Cytogenetic procedures: Metaphase chromosome spreads were prepared by a conventional procedure (FRIEND *et al.* 1976a). Giemsa bands (KOZAK, LAWRENCE and RUDDLE 1977) and Hoechst 33258 fluorescence (KUCHERLAPATI *et al.* 1975) were visualized on the same metaphase chromosome spread by standard protocols. Alkaline Giemsa staining was performed using the method of FRIEND *et al.* (1976a,b). The "minimum frequency estimate" for a specific human chromosome in a clone was calculated by counting the number of cells that exhibited one or more unambiguous examples of the specific chromosome and dividing this number by the total number of cells examined. The "maximum frequency estimate" for a specific chromosome in a clone was calculated by counting the number of cells that exhibited one or more unambiguous examples of the specific chromosome and the number of cells which contained one or more chromosomes similar to the specific chromosome, but not unambiguously identifiable as the specific chromosome and dividing this subtotal by the total number of cells examined. Identification of a human chromosome in a hybrid cell may be ambiguous because of overlaps, gross similarities to rearranged rodent chromosomes, presence of interspecific translocation chromosomes, or cytological artifacts.

RESULTS

Chromosomes of tsC1AGOH: The distribution of the number of chromosomes per cell for *tsC1AGOH* is bimodal. Of the cells examined, 70% had a total number of chromosomes ranging from 41 to 49 (mean 44 $\pm$ 2.0, $N = 19$), while the remaining 30% of the cells exhibited total numbers of chromosomes ranging from 76 to 87 (mean 84 $\pm$ 3.6, $N = 8$). The former category is referred to as 1S (S = stem cell) and the later category is referred to as 2S. The mean number of biarmed chromosomes per cell was 19 $\pm$ 1.1 ($N = 19$) for 1S cells and 39 $\pm$ 3.2 ($N = 8$) for 2S cells. When *tsC1AGOH* chromosome preparations were stained with Hoechst 33258 stain to detect the characteristic strong centromeric fluorescence exhibited by mouse chromosomes, a mean of 5 $\pm$ 1.1 ($N = 19$) nonfluorescent or weakly fluorescent (nwf) chromosomes was observed for 1S cells, and a mean number of nwf chromosomes of 8 $\pm$ 1.3 ($N = 8$) was observed for 2S cells. None of the nwf chromosomes observed in *tsC1AGOH* exhibited a Giemsa banding pattern similar to that of any human chromosome.

Outline of experiments: The origin of primary (1°), secondary (2°) and tertiary (3°) hybrids and the segregation of the heat-resistant (hr) and the heat-sensitive (hs) phenotypes are presented schematically in Figure 1. *tsC1AGOH* cells were fused with GM 126 human fibroblasts and primary hybrids were isolated at both the npT and pT using HAT selection (series 41npT and 41pT). Ouabain was added at 3 mM for seven days during the initial drug selection to kill human fibroblasts. Primary hybrid cell clones were characterized for the expression of human isozymes to confirm the hybrid nature of the primary clones and to screen for the selective fixation of human chromosomes at the npT. Two independently derived primary series 41npT clones were shifted

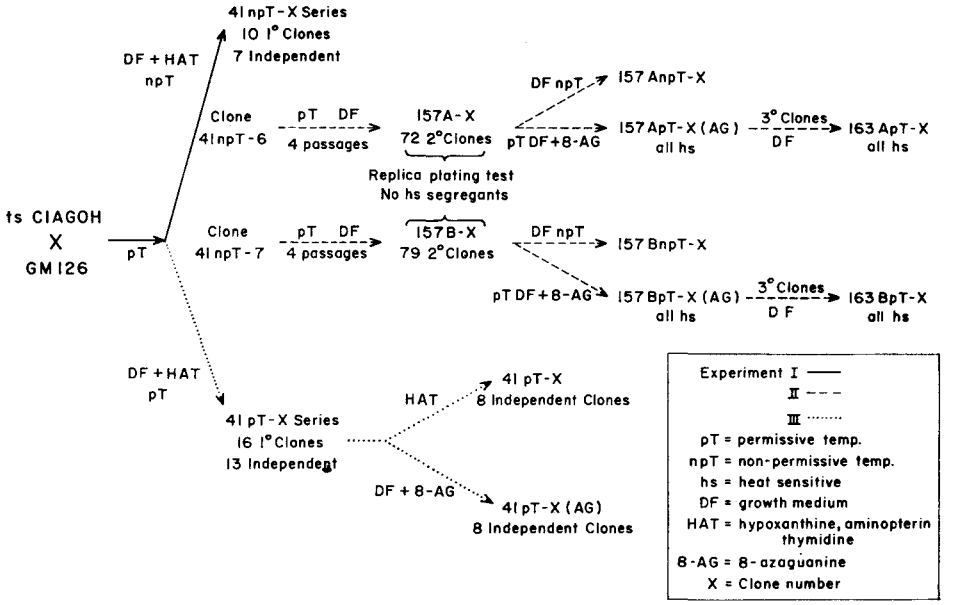

FIGURE 1.—Origin of series 41 primary hybrids and subclone series 157 and 163.

to the pT and nonselective medium to permit segregation of the human chromosome correcting the mouse cell heat-sensitive lesion. No heat-sensitive segregants were found among 151 subclones. Several representative subclones from series 157A and 157B were shifted back to the npT and maintained continuously at the npT in DF medium (series 157AnpT and series 157BnpT). Series 157A and 157B subclones maintained at the pT (series 157ApT and series 157BpT) were grown in DF + AG medium and retested for the heat-resistant phenotype. All the subclones grown in DF + AG medium exhibited a heat-sensitive phenotype. Subclones isolated from heat-sensitive segregants in nonselective medium (series 163ApT and series 163BpT) continued to exhibit a heat-sensitive phenotype. Eight independently derived 1° hybrid cell clones that had never been exposed to the npT (series 41pT) were tested for the expression of the heat-resistant phenotype and the expression of human isozymes after growth in HAT or DF + AG media at the pT. The results of these experiments are described in detail below.

Expression of human isozymes in series 41 clones: The pattern of human isozyme expression in series 41npT hybrids is summarized in Table 2. All the hybrids expressed human G6PD (see Figure 2) and HPRT as expected. All 41pT hybrids also expressed G6PD and HPRT (data not shown). Examination of the expression of human isozymes in heat-resistant series 41npT clones indicated that expression of the heat-resistant phenotype was not associated with expression of human PGM1, 1DH1, MDH1, ME1, SOD2, AK1, GOT1, MPI, PK3, PEP-A, PHI, or SOD1. From these observations it was inferred that the presence of human chromosome *1, 2, 6, 9, 10, 15, 18, 19* or *21* was not required

TABLE 2

*Expresssion of human isozymes in series 41npT human × mouse primary hybrid cell clones**

Isozyme presence:		+	—	Percent Concordance§			+	—	Percent Concordance
	Isozyme					Isozyme			
(*A1*)†	PGM-1	0	9(7)‡	0	(*C12*)	TPI	0	1	0
	PEP-C	0	2(2)	0	(*D13*)	ES-D	1	0	100
(*A2*)	IDH-1	0	10(7)	0	(*D14*)	NP	7(5)	1	88
	MDH-1	0	10(7)	0	(*D15*)	MPI	6(5)	4(3)	60
(*B5*)	HEX-B	0	1	0		PK-3	0	3(3)	0
(*C6*)	ME-1	2(2)	8(5)	20	(*E16*)	APRT	7(5)	3(3)	70
	SOD-2	0	8(5)	0	(*E17*)	GK	5(4)	2(2)	71
(*C8*)	GSR	1	0	100	(*E18*)	PEP-A	5(4)	4(4)	56
(*C9*)	AK-1	0	8(7)	0	(*F19*)	PHI	4(2)	6(6)	40
(*C10*)	GOT-1	3(3)	7(5)	30	(*F20*)	ADA	6(4)	3(3)	67
	ADK	0	1	0	(*G21*)	SOD-1	4(4)	5(2)	44
(*C11*)	LDH-A	7(4)	3(3)	70	(*X*)	HPRT	10(7)	0	100
(*C12*)	LDH-B Act.	8(5)	1	89		G6PD	9(7)	0	100
	PEP-B	8(6)	1	89					

* Ten clones were isolated at the npT; seven of these clones were operationally of independent origin.
† Human chromosome to which the specified isozyme has been assigned.
‡ Number in ( ) is the number of independent clones with the indicated phenotype.
§ Percent of heat-resistant clones positive for the indicated isozyme.

for expression of the heat-resistant phenotype. The number of clones tested was too small to permit any inference about the relationship between expression of the isozyme and expression of the heat-resistant phenotype for HEX-B, GSR, ADK, TPI and ES-D. A substantial fraction of the 41npT series clones were positive for the expression of human LDH-A, LDH-B, NP, APRT, GK, and ADA. Since spurious discordant segregation of syntenic markers may be observed due to chromosome rearrangements, a small number of discordant clones, particularly potentially nonindependent discordant clones, should not be considered as definitive evidence for excluding a syntenic association. In the case of APRT, 3 independent series 41npT clones failed to express activity of human mobility. The observation of three independent clones exhibiting discordant segregation of human APRT and the heat-resistant phenotype indicates that retention of chromosome *16* is not required for correction of the heat-sensitive lesion. A similar situation is noted for segregation of human LDH-A (chromosome *11*) and human ADA (chromosome *20*) and the heat-resistant phenotype.

Plating efficiency of series 41npT clones: Correction of the heat-sensitive lesion in *tsC1AGOH* by fusion with human cells was evaluated quantitatively by measuring the plating efficiency at the npT relative to the plating efficiency at the pT. The relative plating efficiency npT/pT (absolute plating efficiency at the npT ÷ absolute plating efficiency at the pT) exhibited by eight heat-resistant hybrids was compared to the values exhibited by *tsC1AGOH* and a wild-type L-cell derivative, A9. No heat-resistant revertants were detected in *tsC1AGOH* cell populations (1.7×10^7 cells) tested in five experiments.

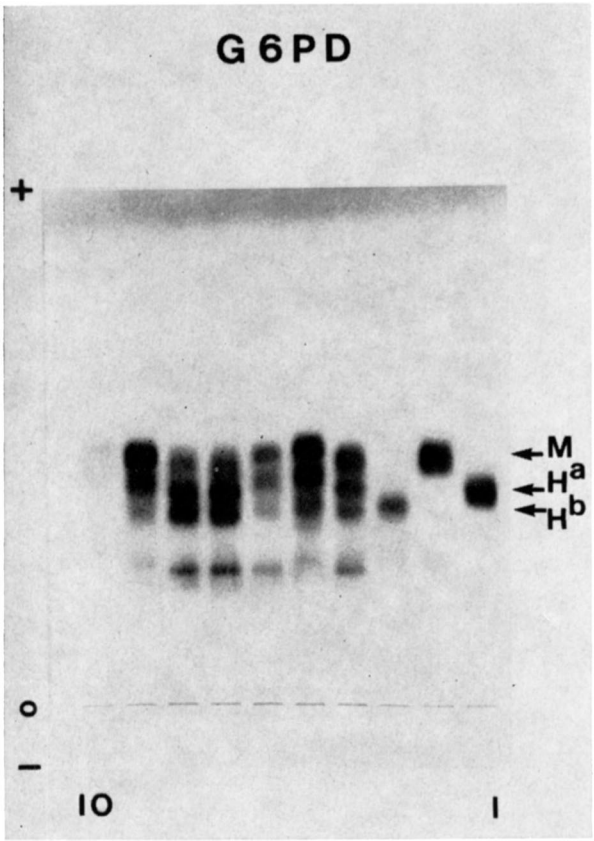

FIGURE 2.—Expression of G6PD in Series 41npT clones. Channel: (1) KB (human control, G6PD form A); (2) tsC1AGOH; (3) Human peripheral blood leucocytes (G6PD form B); (4) 4-41npT-7; (5) 3-41npT-1B; (6) 3-41npT-3; (7) 3-41npT-1D; (8) 3-41npT-2; (9) 3-41npT-4; (10) 3-41npT-1C.

M = Mouse, H^a = Human G6PD form A, and H^b = Human G6PD form B. Note the interspecific heteropolymeric form of G6PD with a mobility intermediate between that of M and H^b G6PD.

tsC1AGOH cells exhibited absolute plating efficiencies at the pT of 0.35 ± 0.15 SD, $N = 3$ and 0.08 ± 0.03, $N = 3$ in two different experiments. A9 cells exhibited a relative plating efficiency npT/pT of 0.65. All 41npT clones exhibited clear heat-resistant phenotypes with relative plating efficiencies npT/pT ≥ 0.48. Several hybrids exhibited a low absolute plating efficiency (2.5% to 20%) compared to values generally reported for L-cells. The reduced absolute plating efficiency exhibited by some hybrids suggests that restoration of a completely wild-type phenotype has not occurred in all instances. It is possible that the human chromosome complement retained by a particular hybrid may adversely affect plating efficiency through mechanisms unrelated to the heat-sensitive lesion in *tsC1AGOH*.

Isolation of heat sensitive segregants: The absence of any heat-sensitive segre-

gants in 151 subclones isolated from primary heat-resistant hybrids after growth under nonselective conditions suggested that loss of the human chromosome conferring the heat-resistant phenotype did not occur at a high frequency. Prior to transfer to nonselective conditions the primary hybrids were grown at the npT in HAT medium for six to eight passages. This strong selection might have favored cells exhibiting a stabilized chromosome complement. Heat-resistant series 157 subclones were back-selected in DF + AG medium and tested for expression of the heat-resistant phenotype, for two reasons: (1) it was considered possible that the human *X* chromosome was responsible for correction of the heat sensitive defect, and (2) selection for hybrids that had segregated the *X* chromosome might selectively enrich for cells that had lost other human chromosomes. All the heat-resistant hybrid subclones back-selected in DF + AG medium exhibited a heat-sensitive phenotype, although some of the heat-sensitive segregants retained a low background of heat-resistant cells in the population ($<4.6 \times 10^{-6}$ to 1.42×10^{-3}). The residual heat-resistant cells could have been: (1) cells with an intact *X* chromosome that express HPRT, but escaped killing in the presence of a large number of actively metabolizing AG-resistant cells, (2) cells containing a rearranged *X* chromosome that were deficient in HPRT activity due to deletion or gene inactivation, but that retained the portion of the *X* chromosome necessary for correction of the heat-sensitive lesion, (3) permeability variants that did not take up AG, or (4) cells containing an altered HPRT that no longer reacted with AG. Isolation of tertiary subclones from heat-sensitive segregants (see Figure 1), series 163ApT from 157ApT-1 (AG3) and series 163BpT from 157BpT-1(AG3), resulted in the elimination of residual heat-resistant cells in all but one instance. Seven series 163 subclones failed to form any colonies at the npT when 2.8 to 9.3×10^5 cells were inoculated. One series 163 subclone exhibited an absolute plating efficiency at the npT of 5.4×10^{-5}. The concordant segregation of AG resistance and heat sensitivity in these subclones of heat-resistant, HAT-resistant primary hybrids provides strong evidence that the human *X* chromosome is responsible for the heat-resistant phenotype. Growth and morphology of heat-resistant and heat-sensitive hybrids at the pT and npT are illustrated in Figure 3.

Segregation of human isozymes and heat resistance in series 157 and 163 subclones: The segregation of 24 human isozymes assigned to 17 different human chromosomes is presented in Table 3 for heat-resistant and sensitive subclones. These data confirm the conclusion reached on the basis of human isozyme expression in series 41npT hybrids that retention of human chromosome *1*, *2*, *6*, *10*, *11*, *15*, *16*, *18*, *19*, *20*, or *21* is not required for expression of the heat-resistant phenotype. These data also demonstrate that there is no association between expression of HEX-B, LDH B, PEP-B, ES-D, NP or GK and expression of heat resistance leading to the inference that retention of human chromosome *5*, *12*, *13*, *14*, or *17* is not associated with correction of the heat sensitive lesion in *tsC1AGOH*. Only the *X*-linked markers, *G6PD* and *HPRT*, segregated concordantly with heat resistance.

Expression of heat resistance and human isozymes in series 41pT clones grown

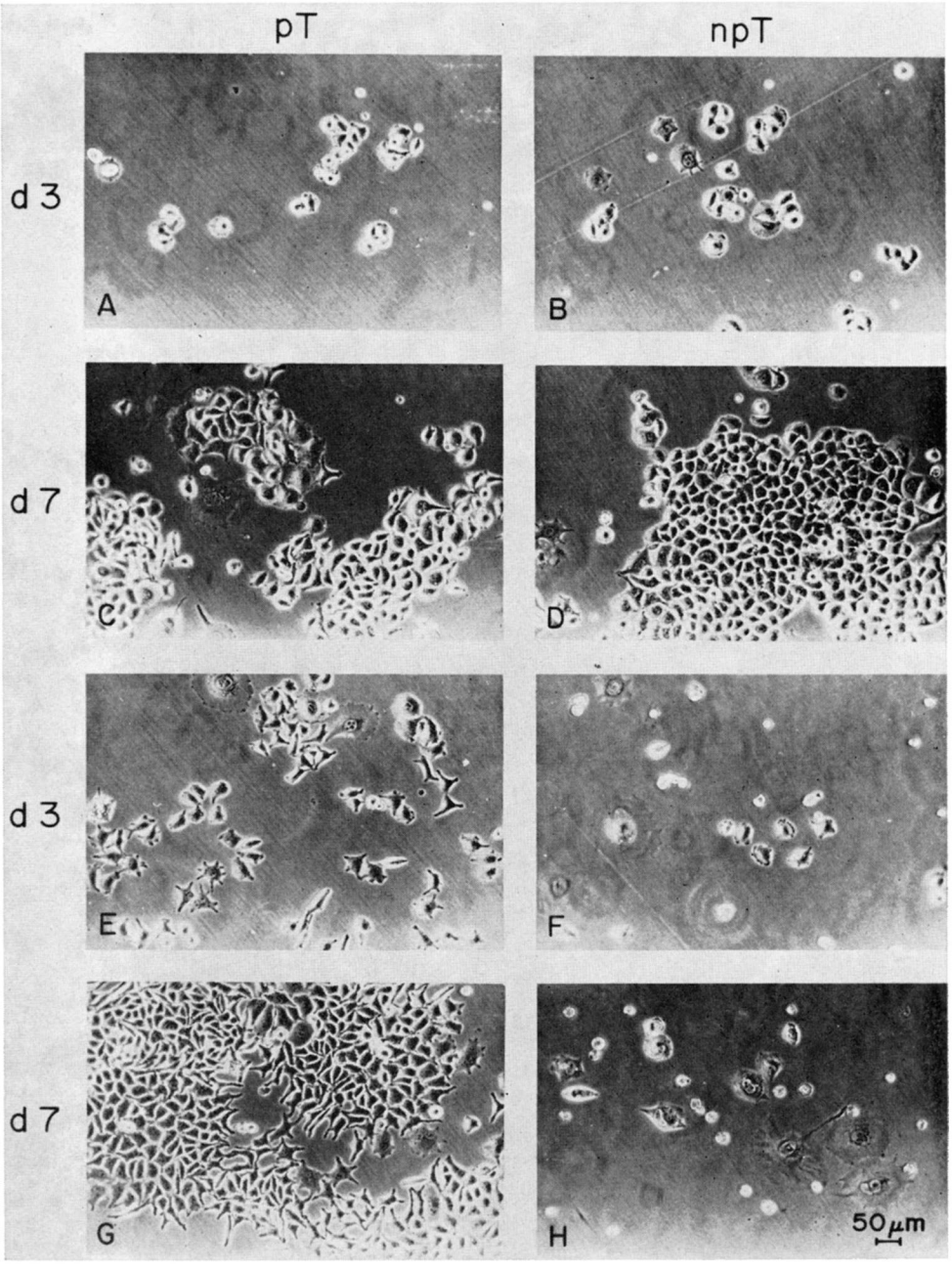

FIGURE 3.—Growth and morphology of a heat-resistant hybrid and a heat-sensitive hybrid at the pT and npT. Panels A-D, 4–157BnpT–1 seeded at 4.22×10^2 cells per cm^2; heat resistant. Panels E-H, 11–157BpT–1 (AG4) seeded at 5.27×10^2 cells per cm^2; heat sensitive. d = day. DF medium.

TABLE 3

Segregation of human isozymes and heat resistance in series 157 and 163 subclones

Heat resistance*: / Isozyme presence*:		+ / +	— / +	+ / —	— / —	Percent Concordance‡
	Isozyme					
(*A1*)†	PGM-1	2	3	6	5	44
	PEP-C	1	3	7	5	38
(*A2*)	IDH-1	0	0	8	8	50
	MDH-1	0	0	8	8	50
	RK	0	0	8	4	33
(*B5*)	HEX-B	0	0	8	6	43
(*C6*)	ME-1	0	0	8	7	47
	SOD-2	0	0	8	8	50
(*C10*)	GOT-1	0	1	7	7	47
	ADK	0	0	8	6	43
(*C11*)	LDH-A	6	4	2	3	60
(*C12*)	LDH-B	5	7	3	1	38
	PEP-B	5	7	3	1	38
(*D13*)	ES-D	0	0	8	7	47
(*D14*)	NP	7	7	1	1	50
(*D15*)	MPI	0	0	8	8	50
(*E16*)	APRT	0	0	8	8	50
(*E17*)	GK	0	2	8	5	33
(*E18*)	PEP-A	1	2	7	6	44
(*F19*)	PHI	0	0	8	8	50
(*F20*)	ADA	0	0	8	5	38
(*G21*)	SOD-1	0	0	8	8	50
(*X*)	HPRT	8	0	0	5	100
	G6PD	8	0	0	8	100

* + = hr phenotype; — = hs phenotype. + = isozyme present; — = isozyme absent.
† Human chromosome to which the specified isozyme has been assigned.
‡ Percent of clones heat resistant and positive for the isozyme or heat sensitive and negative for the isozyme.

in HAT or back-selected in DF + AG medium: 41pT series clones that had never been exposed to the npT were split into two groups. One group of clones was maintained in HAT medium (41pT-X), while parallel cultures were propagated in DF + AG medium [41pT-X(AG), see Figure 1]. After growth in DF + AG medium for three to eight passages, four clones exhibited reductions in relative plating efficiency npT/pT ranging from a minimum of 425-fold to a maximum of 2.75×10^5-fold when compared to the relative plating efficiency npT/pT displayed by the same clone maintained in HAT medium. Two additional DF + AG back-selected clones were obviously heat sensitive on morphological criteria, but were not tested for quantitative relative plating efficiency npT/pT.

Two clones displayed discordant segregation of heat sensitivity and AG resistance or heat resistance and HAT resistance. 41pT-2A(AG) continued to exhibit the ability to grow at the npT after growth in DF + AG for up to ten passages, although it became sensitive to HAT medium and lost the ability to

express human HPRT and G6PD. The "minimum frequency estimate" for the *X* chromosome in 41pT-2A(AG) was zero and the "maximum frequency estimate" was 0.12 after ten passages in DF + AG. 41pT-9 initially exhibited a heat-resistant phenotype but acquired a heat-sensitive phenotype by passage 12. At passage ten, 41pT-9 expressed both human G6PD and human HPRT. The "minimum frequency estimate" for the *X* chromosome was 0.62 and the "maximum frequency estimate" was 0.81 at passage 16. Twenty-four percent of the *X* chromosomes observed in 41pT-9 appeared abnormally short. 41pT-2A(AG) and 41pT-9 were the only hybrids that exhibited discordant segregation of the heat-resistant phenotype and the X-linked markers out of a total of 42 hybrid cells lines that were tested in this study.

Discordant segregation of syntenic isozyme markers assigned to either the human *X* chromosome (SINISCALCO 1970; GERALD and BROWN 1974; Goss and HARRIS 1975) or to human autosomes (SHOWS 1972; HAMERTON *et al.* 1975; POVEY *et al.* 1976) has been observed in several laboratories at frequencies ranging from 3.6% (POVEY *et al.* 1976) to 20% (GERALD and BROWN 1974). The frequency of discordant segregation of heat resistance and human *X* chromosome markers observed in this study (2/42 = 0.048) is within this range. Cytogenetic analysis of human-rodent hybrids has demonstrated structurally rearranged human chromosomes, including interspecific translocation chromosomes (FRIEND *et al.* 1976a; FRIEND, CHEN and RUDDLE 1976b; HAMERTON *et al.* 1975). It has been suggested that propagation of hybrid cells in HAT medium may facilitate the dissociation of syntenic markers (GERALD and BROWN 1974). In view of the fact that cytogenetic studies described below demonstrated that only the *X* chromosome segregated concordantly with heat resistance, we feel that the two discordant clones probably arose through *X* chromosome rearrangements leading to the segregation of the heat-resistance locus and the *HPRT* and *G6PD* loci.

The segregation of the heat-resistant phenotype and 26 human isozymes assigned to 19 different chromosomes is presented in Table 4. All of the DF+AG back-selected clones were negative for the expression of human G6PD and human HPRT. All of the HAT-selected clones were positive for human G6PD and human HPRT. The *X*-linked markers exhibited the highest frequency of concordant segregation with the heat-resistant phenotype (88%). No autosomal isozyme listed in Table 4 segregated concordantly with the heat-resistant phenotype. A relatively large fraction of the clones tested (75%) were either positive for GK and heat resistant or negative for GK and heat sensitive. However, the data from series 157 clones and series 163 clones (Table 3) clearly eliminated any association of expression of GK and correction of the heat-sensitive lesion. The data compiled in Table 4 confirm the absence of association between retention of human chromosome *1, 2, 5, 6, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20* or *21* and correction of the heat sensitive defect in *tsC1AGOH*. GSR (chromosome *8*) also segregated discordantly with the heat-resistant phenotype.

Segregation of human chromosomes in heat-resistant and heat-sensitive clones: The objectives of the cytogenetic study were: (1) to confirm the conclusions

TABLE 4

Segregation of human isozymes and heat resistance in series 41pT-X clones and 41pT-X(AG) back-selected clones

Heat resistance: Isozyme presence:		+ +	− +	+ −	− −	Percent Concordance
	Isozyme					
(*A1*)*	PGM-1	0	1	8	7	44
	PEP-C	2	1	6	7	56
(*A2*)	IDH-1	0	0	8	8	50
	MDH-1	0	0	8	8	50
(*B5*)	HEX-B	1	2	7	5	38
(*C6*)	ME-1	1	1	7	7	50
(*C8*)	GSR	2	1	6	5	50
(*C9*)	AK-1	0	0	7	8	53
(*C10*)	GOT-1	1	1	3	4	56
	ADK	3	1	4	5	67
(*C11*)	LDH-A	2	3	6	4	40
(*C12*)	LDH-B	5	5	3	3	50
	PEP-B	3	5	5	3	38
	TPI	2	1	1	1	60
(*D13*)	ES-D	3	1	4	6	64
(*D14*)	NP	6	6	2	3	50
(*D15*)	MPI	2	0	6	8	62
	PK-3	0	0	4	4	50
(*E16*)	APRT	4	3	4	5	56
(*E17*)	GK	2	0	2	4	75
(*E18*)	PEP-A	4	1	4	7	69
(*F19*)	PHI	4	2	4	6	62
(*F20*)	ADA	1	0	6	7	57
(*G21*)	SOD-1	5	3	3	5	62
(*X*)	HPRT	7	1	1	7	88
	G6PD	7	1	1	7	88

* Human chromosome to which the specified isozyme has been assigned.
All HAT-selected clones were positive for human HPRT and G6PD and AG back-selected clones were negative for these two enzymes.

about chromosome segregation and expression of heat resistance inferred from the pattern of isozyme segregation, and (2) to test for concordant segregation of human chromosome *3*, *4*, *7* or *22* and the heat-resistant phenotype. Three heat-resistant hybrids, 41pT-8, 157AnpT-2 and 157BnpT-1, and three heat-sensitive hybrids, 41pT-8 (AG), 163ApT-1 and 163BpT-4, were selected for chromosome analysis. The heat-resistant hybrids exhibited absolute plating efficiencies ± the standard deviation (N=3) at the npT of 0.061 ± 0.010, 0.065 ± 0.024 and 0.025 ± 0.006, respectively. The heat-sensitive hybrids exhibited absolute plating efficiencies at the npT of $(4.95 \pm 0.38) \times 10^{-5}$, $<1.06 \times 10^{-6}$ and $<5.25 \times 10^{-7}$, respectively.

The mean number of chromosomes and the mean number of human chromosomes per cell in these six hybrid clones are presented in Table 5. Both the heat-resistant and heat-sensitive primary hybrid clones exhibited a greater mean

TABLE 5

Chromosome numbers in heat-resistant and heat-sensitive hybrids

Hybrid/phenotype	Mean total number of chromosomes ± SD	N*	Mean number of human chromosomes ± SD
11–41pT-8 /hr	88 ± 6.1	11	14.3 ± 5.2
5–157AnpT-2 /hr	85 ± 4.8	15	3.6 ± 2.0
7–157BnpT-1 /hr	81 ± 2.4	14	2.4 ± 1.2
15–41pT-8(AG8)/hs	87 ±10.0	15	11.7 ± 4.3
3–163ApT-1 /hs	74 ± 2.2	19	5.5 ± 1.5
3–163BpT-4 /hs	81 ± 2.4	18	2.1 ± 1.1

* N = number of cells analyzed.

number of human chromosomes than did the secondary (series 157) or tertiary (series 163) clones. Subtracting the mean number of human chromosomes from the mean number of total chromosomes yields a figure for each of the hybrid clones that falls near or within the 2S number range of *tsC1AGOH*. Conceptually, these hybrids could have resulted from either the fusion of two 1S *tsC1AGOH* cells with a human cell or the fusion of one 2S *tsC1AGOH* cell with a human cell. There does not appear to be any gross correlation of chromosome number with heat resistance or sensitivity.

Each human chromosome was tested for concordant segregation with the heat-resistant phenotype, using both the "minimum frequency estimate" and the "maximum frequency estimate." A frequency of 10% or greater was chosen as the level of discrimination for scoring a human chromosome present (+). In view of the great difference in plating efficiency at the npT between heat-resistant hybrid clones (0.025 to 0.39) and *tsC1AGOH* ($<5.88 \times 10^{-8}$) and the nonleaky expression of the heat-sensitive phenotype at high cell density (data not shown), it was felt that the human chromosome correcting the heat-sensitive lesion in *tsC1AGOH* would be retained at a substantially higher frequency than 10%. The absolute plating efficiency of a hybrid at the npT provides an estimate of the frequency of cells carrying the human chromosome with the heat-resistance locus. Clearly, the frequency of cells carrying the human locus conferring heat resistance is negligible in all the heat-sensitive clones selected for chromosome analysis.

From the results of the cytogenetic analysis of heat-resistant and heat-sensitive hybrids presented in Table 6, it is clear that there was no association of retention of human chromosome *3*, *4*, *7* or *22* and expression of heat resistance. Furthermore, these data exclude association of any other human autosome and expression of the heat-resistant phenotype, confirming the conclusions reached on the basis of the pattern of human isozyme segregation and the pattern of expression of heat resistance. The minimum/maximum chromosome frequency estimate data, which were used to construct Table 6, are presented for each clone in Table 7, together with the human isozyme expression data. A karyotype of a heat-resistance hybrid is shown in Figure 4, and a karyotype of a heat-sensitive cell is shown in Figure 5.

TABLE 6

Segregation of human chromosomes and heat resistance in human × tsC1AGOH *hybrids*

Heat resistance: / Chromosome*:	+ / +	− / +	+ / −	− / −	Percent Concordance	+ / +	− / +	+ / −	− / −	Percent Concordance
Chromosome										
1	0	1	3	2	33	0	1	3	2	33
2	0	0	3	3	50	0	0	3	3	50
3	2	3	1	0	33	2	3	1	0	33
4	1	1	2	2	50	1	1	2	2	50
5	1	1	2	2	50	1	1	2	2	50
6	0	0	3	3	50	1	0	2	3	67
7	1	1	2	2	50	1	1	2	2	50
8	0	1	3	2	33	1	1	2	2	50
9	0	0	3	3	50	0	0	3	3	50
10	0	1	3	2	33	0	1	3	2	33
11	1	3	2	0	17	1	3	2	0	17
12	1	2	2	1	33	2	2	1	1	50
13	1	0	2	3	67	1	1	2	2	50
14	2	3	1	0	33	2	3	1	0	33
15	0	2	3	1	17	2	3	1	0	33
16	1	1	2	2	50	1	1	2	2	50
17	1	1	2	2	50	1	1	2	2	50
18	1	2	2	1	33	1	2	2	1	33
19	1	1	2	2	50	1	1	2	2	50
20	1	1	2	2	50	1	1	2	2	50
21	1	1	2	2	50	1	1	2	2	50
22	2	2	1	1	50	2	2	1	1	50
X	3	0	0	3	100	3	0	0	3	100

* Chromosomes were scored as present (+) if found in 10% or more of the cells examined. The first five columns were tabulated using the minimum frequency estimate and the next five columns were tabulated using the maximum frequency estimate listed in Table 7.

It might be hypothesized that the balanced translocation present in the human parental cell [46,*XY*,t(*1*;*15*) (*1*qter→*1*p36::*15*q1→*15*qter;*15*pter→*15*q1::*1*p36→*1*pter)] could obscure an association between either chromosome *1* or *15* and expression of the heat-resistant phenotype. The following considerations, taken together, eliminate this hypothesis. Observation of heat-resistant hybrids negative for human PEP-C or MPI eliminated the possibility that the heat-resistant locus was associated with the large translocation chromosome. The presence of an intact human chromosome *1* in the heat-sensitive hybrid 163ApT-1 (PGM1 +, PEP-C +, MPI −) eliminates the possibility that the heat-resistance locus is on chromosome *1* (see Figure 5). Isozyme markers are unavailable for the small translocation chromosome, and this chromosome cannot be reliably identified cytologically in interspecific hybrids because of its very small size and lack of distinctive morphological features. Since selection with DF + AG medium resulted in loss of heat resistance, the heat-resistance locus must be located on either the *X* chromosome or an autosome that is also selectively eliminated by growth in DF + AG. The analysis of human-rodent hybrids in a large number

TABLE 7

Segregation of human isozymes and human chromosomes in heat-resistant and heat-sensitive hybrid cell clones

Chromosome* or isozyme†	Heat resistant 11–41–pT-8	5–157AnpT-2	7–157BnpT-1	15–41pT-8(AG8)	Heat sensitive 3–163ApT-1	3–163BpT-4
A1	0/0*	0/0	0/0	0/0	0.36/0.79	0/0
PGM-1	—	+	—	—	+	—
PEP-C	—	—	—	—	+	—
A2	0/0	0/0	0/0	0/0	0/0	0/0
IDH-1	—	—	—	—	—	—
MDH-1	—	—	—	—	—	
RK		—	—		—	
A3	0/0.18	0.40/0.54	0.50/0.64	0.27/0.53	0.79/0.89	0.22/0.50
B4	0.27/0.27	0/0	0/0	0.13/0.27	0/0	0/0
B5	0.27/0.45	0/0	0/0	0.13/0.20	0/0	0/0
HEX-B	+	—	—	+	—	
C6	0/0.27	0/0	0/0	0/0	0/0	0/0
ME-1	—	—	—	—	—	
SOD-2	—	—	—	—	—	—
C7	0.18/0.27	0/0	0/0	0.27/0.40	0/0	0/0
UP	+	—	—		—	
C8	0/0.45	0/0	0/0	0.20/0.47	0/0	0/0
GSR	+			+		—
C9	0/0	0/0	0/0	0/0	0/0	0/0
AK-1	—			—	—	
C10	0/0.09	0/0	0/0	0.20/0.20	0/0	0/0
GOT-1	—	—	—	—	—	
ADK	—	—	—	—	—	—
C11	0.91/0.91	0/0.07	0/0	1.00/1.00	0.95/0.95	0.78/0.89
LDH-A	+	+‡	—	+	+	
C12	0.45/0.55	0/0.13	0/0	0.47/0.47	0.32/0.63	0/0
LDH-B act.	+	—	—	+	+	+
TPI					+	
PEP-B	+	—	—	+	+	+
D13	0.18/0.18	0/0	0/0	0/0.13	0/0	0/0
ES-D	+	—	—	+	—	
D14	0.45/0.55	0/0	0.36/0.71	0.33/0.33	0.21/0.37	0.28/0.39
NP	+	—	+	+	+	+
D15	0.09/0.36	0/0	0.07/0.14	0.27/0.27	0.21/0.26	0.06/0.17
MPI	—	—	—	—	—	—
PK3	—		—	—		
E16	0.18/0.27	0/0	0/0	0.20/0.27	0/0	0/0
APRT	+	—	—	+	—	—

TABLE 7—Continued

Chromosome* or isozyme†	Heat resistant 11-41-pT-8	5-157AnpT-2	7-157BnpT-1	Heat sensitive 15-41pT-8(AG8)	3-163ApT-1	3-163BpT-4
E17	0.45/0.45	0/0	0/0	0.80/0.87	0/0.05	0.06/0.06
GK		—	—		+§	—
E18	0.27/0.36	0/0.07	0/0	0.13/0.13	0.53/0.79	0/0.06
PEP-A	—	—	—	—	+	—
F19	0.54/0.73	0/0	0/0	0.27/0.27	0/0	0/0
PHI	+	—	—	+	—	—
F20	0.18/0.18	0/0	0/0	0.33/0.60	0/0	0/0
ADA	—	—	—	—		—
G21	0.64/0.73	0/0	0/0	0.87/0.87	0/0	0/0
SOD-1	+	—	—		—	—
G22	0.73/0.73	0.73/0.73	0/0	0.67/0.80	0.53/0.58	0/0
X	0.82/1.0	0.40/0.73	0.57/0.79	0/0	0/0	0/0
HPRT	+	+	+	—	—	—
G6PD	+	+	+	—	—	—

* Minimum frequency estimate/maximum frequency estimate based on cytogenetic analysis of 11, 15, 14, 15, 19, and 18 cells, respectively, for each of the clones listed above.
† + = present, and — = absent.
‡ Cytogenetic analysis demonstrated a mouse-human translocation chromosome carrying a portion of human chromosome *11*.
§ Cytogenetic analysis indicated a possible rearranged human chromosome *17* (iso *17*q).

of laboratories has shown that only the *X* chromosome is specifically eliminated during back-selection with AG.

On the basis of the experimental results described above, we define the human genetic locus correcting the heat sensitive lesion in *tsC1AGOH* as *hrC1AGOH* and assign this locus to the human *X* chromosome.

DISCUSSION

Gene assignment using ts rodent cells: We have defined the human genetic locus assigned to the *X* chromosome in this study in terms of the heat-sensitive mouse cell mutant used in this gene assignment because the specific biochemical lesion responsible for generating the heat-sensitive DNA synthesis phenotype is not known at the present time. Biochemical characterization of *tsC1* by SHEININ and GUTTMAN (1977) has demonstrated three phases in the inhibition of DNA synthesis at the npT: (1) an initial phase of rapid inactivation of semi-conservative DNA synthesis, (2) a second phase characterized by a low level of semi-conservative DNA synthesis, and (3) a third phase of nonconservative DNA synthesis. Mitochondrial DNA synthesis after shift to the npT is initially unaffected at a time when a substantial inhibition of nuclear DNA synthesis has occurred (SHENIN and GUTTMAN 1977; SHEININ, DARAGH and DUBSKY 1977). An eventual decline in mitochondrial DNA synthesis proportional to the decreased nuclear DNA synthesis was noted. *tsC1* is unable to support the replica-

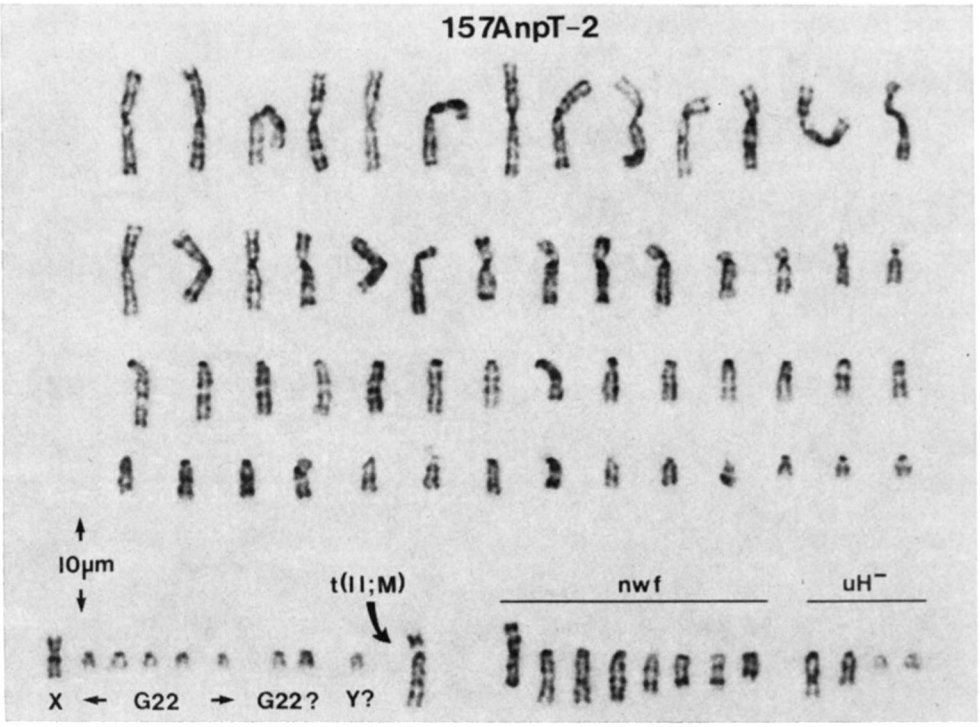

FIGURE 4.—Karyotype of a heat-resistant hybrid. nwf = non- or weakly fluorescent centromere when stained with Hoechst stain (similar to chromosomes exhibited by the rodent parent). uH⁻ = unidentified Hoechst negative (nwf-type Hoechst staining, but not similar to chromosomes exhibited by the rodent parent or the standard human karyotype). Note the human *X* chromosome. Unlabelled chromosomes are mouse chromosomes.

tion of polyoma virus at the npT in contrast to another ts L-cell mutant defective in DNA synthesis, *tsA1S9*, which exhibits a phenotype somewhat different from that of *tsC1* (SHEININ and GUTTMAN 1977; SHEININ 1976a,b). Further biochemical analysis of the heat-sensitive lesion in *tsC1* may permit redesignation of the *hrC1AGOH* locus in terms of a specific enzymatic activity. Genetic and biochemical analysis of ts mammalian cell *dna*⁻ mutants should eventually lead to an understanding of mammalian DNA synthesis comparable to our understanding of prokaryotic DNA synthesis. However, a knowledge of the biochemical nature of the heat-sensitive lesion in any ts mutant is not required in order to exploit the properties of these conditional lethal mutations for purposes of genetic manipulations or gene assignments.

Since the initial report of the assignment of *hrC1AGOH* to the human *X* chromosome appeared (GILES and RUDDLE 1976), similar results have been independently described by JHA and OZER (1977a,b) for segregation of heat resistance and thioguanine sensitivity in interspecific cell hybrids produced with a ts *dna*⁻ mutant, *ts2*, isolated from Balb/3T3 mouse cells by SLATER and OZER (1976). Complementation tests performed between several independently isolated heat

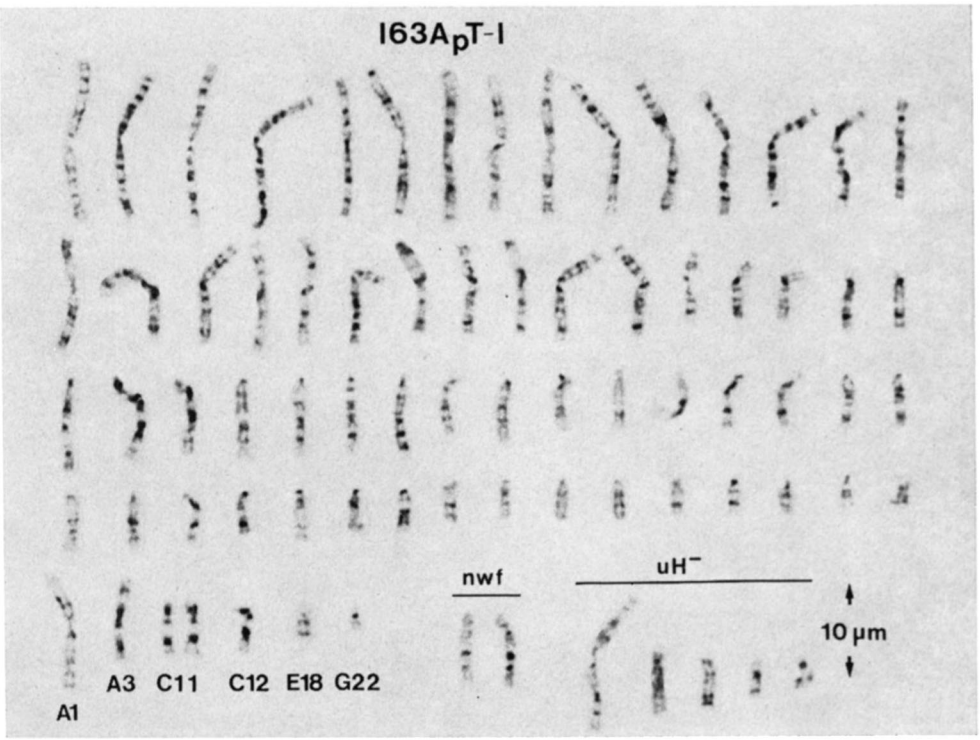

FIGURE 5.—Karyotype of a heat-sensitive hybrid. nwf and uH⁻, see legend to Figure 4. Note the absence of the human *X* chromosome. Unlabeled chromosomes are mouse chromosomes.

sensitive *dna*⁻ Balb/3T3 mutants, *ts2* and *tsC1* failed to demonstrate complementation (OZER, personal communication). These results suggest that these mutants are all altered at the same genetic locus. In the case of *tsC1*, from which *tsC1AGOH* was derived, and *ts2*, the intraspecific complementation test data are consistent with the observed concordant segregation of heat resistance and the human *X* chromosome in interspecific hybrids. Preferential isolation of *X*-linked recessive heat-sensitive mutations may occur because of hemizygosity in the progenitor cell.

Regional gene localization in human chromosomes: Regional localization of human genes *via* analysis of somatic cell hybrids was first reported by RICCIUTI and RUDDLE (1973), who assigned the genes for PGK, HPRT and G6PD to the long arm of the *X* chromosome by studying the segregation of human isozymes and chromosomes in human-mouse hybrids produced with a human cell carrying a balanced *X;14* translocation. This translocation mapping approach has been utilized for the regional localization of several other human genes. Translocation mapping is limited by the resolution of chromosome banding procedures and the availability of appropriate translocations, and it does not yield quantitative information on intergene distances. Goss and HARRIS (1975) have utilized HAT-selected hybrids produced by fusing HPRT⁻ rodent cells with X-irradiated human

cells to determine gene order and to estimate the relative distances between PGK, α-galactosidase, HPRT and G6PD on the *X* chromosome. The use of a single selectable marker in this experiment required the use of high doses of X rays (1,000–4,000 rads) in order to obtain a usable level of dissociation of X-linked markers in a random sample of hybrid clones selected for isozyme analysis. EVANS (1974) has noted that about ten single-strand breaks per rad are produced in X-irradiated mammalian cells and cites data from several investigators demonstrating the induction of dicentric chromosomes at frequencies ranging from 5% to 50% over a dose range of approximately 50 to 300 rads.

The presence of two selectable markers on the human *X* chromosome (*HPRT* and *hrC1AGOH*) in series 41 hybrids and derivatives provides a unique experimental system for obtaining quantitative estimates of the distances between genes on the *X* chromosome. It should be possible to select for *X*-chromosome rearrangements and segregation of rearrangement products resulting in separation of the *hrC1AGOH* and *HPRT* loci by growing irradiated human × *tsC1AGOH* hybrids initially containing a single active intact human *X* chromosome at the npT in DF + AG medium. Hybrids that retain the intact human *X* chromosome or a fragment of the *X* chromosome containing the *HPRT* locus will be eliminated because of sensitivity to AG. Hybrids that have lost the human *X* chromosome will be killed because they are heat sensitive. Only those hybrid cells that have lost the portion of the human *X* chromosome carrying the *HPRT* locus, but retained the portion carrying the *hrC1AGOH* locus will be capable of growing at the npT in DF + AG medium.

This procedure should markedly enhance the determination of relative distances between syntenic genes using the Goss and HARRIS (1975) approach since: (1) clones carrying translocation products of the *X* chromosome may be directly selected in DF + AG at the npT, permitting the use of much lower X-ray dosages and avoiding problems associated with the production of large numbers of complicated chromosome aberrations, and (2) employment of hybrid clones initially containing only the *X* chromosome would facilitate cytogenetic analysis of translocation-containing clones providing the opportunity to obtain quantitatitve data on isozyme segregation frequency and cytogenetic localization in a single experiment. *tsC1AGOH* should be a useful parental cell input in the determination of gene assignments and gene localization using balanced human *X*-autosome translocations and the experimental approach of RICCIUTI and RUDDLE, (1973) since it should be possible to specifically select for each translocation chromosome in hybrids in cases where the breakpoint on the *X* chromosome separates the *hrC1AGOH* and *HPRT* loci.

Assignment of the *hrC1AGOH* locus to the human *X* chromosome does not result in the ability to selectively "fix" a previously unselectable human chromosome in human-mouse hybrids. However, the *hrC1AGOH* gene assignment does permit selection for the *X* chromosome in the absence of HAT medium, which has been suggested as a factor in the dissociation of syntenic markers (GERALD and BROWN 1974) and should facilitate regional chromosome mapping and genetic manipulation of segments of the *X* chromosome. A number of other

studies have utilized ts rodent cell mutants to assign human genetic loci. MING, CHANG and BASERGA (1976) have assigned the *tsAF8* locus to human chromosome *3* by analyzing the human chromosome complement of human-hamster hybrids produced with a Syrian hamster cell-cycle mutant. As previously noted, JHA and OZER (1977a) have demonstrated concordant segregation of heat resistance and thioguanine sensitivity in human-mouse hybrids produced with *ts2* cells. We have assigned the *hrO25C1* locus to human chromosome *5* using a heat-sensitive Chinese hamster ovary cell mutant deficient in leucyl-tRNA synthetase activity (GILES *et al.* 1977) and the *hrTA3ts1C1* locus to human chromosome *3* using a heat sensitive growth mutant derived from TA3 cells (GILES 1978b). These studies demonstrate the utility of temperature-sensitive conditional lethal mutations as tools for the genetic analysis of mammalian cells.

We are indebted to ELIZABETH NICHOLS for skillful assistance in the isozyme studies, to JEANNE LAWRENCE for expert assistance in the cytogenetic analysis, and to MAE REGER and MARIE SINISCALCHI for preparation of the manuscript.

This research has been supported by Public Health Service Grant GM09966.

LITERATURE CITED

BASILICO, C., 1977 Temperature sensitive mutations in animal cells. Adv. Can. Res. **24**: 223–266.

CHEN, T. R., F. A. MCMORRIS, R. P. CREAGAN, F. RICCIUTI, J. TISCHFIELD and F. H. RUDDLE, 1973 Assignment of the genes for malate oxido-reductase decarboxylating to chromosome *6* and peptidase B and lactate dehydrogenase B to chromosome *12* in man. Am. J. Human Genet. **25**: 200–207.

CREAGAN, R. P. and F. H. RUDDLE, 1977 New approaches to human gene mapping by somatic cell genetics. pp. 90–143. In: *Molecular Structure of Human Chromosomes.* Edited by J. J. YUNIS, Academic Press, New York.

DENNY, R. M., E. A. NICHOLS and F. H. RUDDLE, 1978 Assignment of a gene for uridine phosphorylase to chromosome 7. Cytogenet. Cell Genet. **22**: 195–199.

EVANS, H. J., 1974 Effects of ionizing radiation on mammalian chromosomes. pp. 191–237. In: *Chromosomes and Cancer.* Edited by J. GERMAN. Wiley, New York.

FRIEND, K. K., B. P. DORMAN, R. S. KUCHERLAPATI and F. H. RUDDLE, 1976a Detection of interspecific translocations in mouse-human hybrids by alkaline Giemsa staining. Exp. Cell Res. **99**: 31–36.

FRIEND, K. K., S. CHEN and F. H. RUDDLE, 1976b Differential staining of interspecific chromosomes in somatic cell hybrids by alkaline Giemsa stain. Somatic Cell Genet. **2**: 183–188.

GERALD, P. S. and J. A. BROWN, 1974 Report of the Committee on the genetic constitution of the *X* chromosome. Cytogenet. Cell Genet. **13**: 29–34.

GILES, R. E., 1978a Utilization of Temperature Sensitive Rodent Cell Mutants for the Selective Fixation of Human Chromosomes in Human × Rodent Hybrid Cells. Ph.D. Dissertation, Yale University. ——, 1978b Isolation of a temperature sensitive mutant TA3ts1C1 and assignment of the hrTA3ts1C1 locus to human chromosome *3*. In Vitro **14**: 348.

GILES, R. E. and F. H. RUDDLE, 1973a Production of Sendai virus for cell fusion. In Vitro **9**: 103–107. ——, 1973b Production and characterization of proliferating somatic cell hybrids. pp. 475–500. In: *Tissue Culture: Methods and Applications.* Edited by P. F. KRUSE and M. K. PATTERSON, Academic Press, New York. ——, 1976 Replacement of a mouse cell heat sensitive function by the human *X* chromosome in interspecific cell hybrids. Genetics **83**: s26.

GILES, R. E., N. SHIMIZU, E. NICHOLS, J. LAWRENCE and F. H. RUDDLE, 1977 Correction of a heat sensitive lesion associated with reduced leucyl-tRNA synthetase activity in Chinese hamster cells by fusion with human leucocytes. J. Cell Biol. **75:** 387a.

GOSS, S. J. and H. HARRIS, 1975 New method for mapping genes in human chromosomes. Nature **255:** 608–684.

HAMERTON, J. L., T. MOHANDAS, P. J. MCALPINE and G. C. DOUGLAS, 1975 Localization of human gene loci using spontaneous chromosome rearrangements in human-Chinese hamster somatic cell hybrids. Am. J. Human Genet. **27:** 595–608.

JHA, K. K. and H. L. OZER, 1977a *X*-chromosome location of a human gene(s) correcting the temperature-sensitive defect in DNA synthesis in ts-2 Balb/3T3 mouse cells. Abstracts Ann. Meet. Am. Soc. Microbiol., K25, p. 190. ——, 1977b Genetic studies with a mutant mouse cell, ts 2 Balb/3T3, with a temperature sensitive defect in DNA synthesis. Genetics **86:** s32.

KLOBUTCHER, L. A., E. A. NICHOLS, R. S. KUCHERLAPATI and F. H. RUDDLE, 1976 Assignment of the gene for human adenosine kinase to chromosome *10* using a somatic cell hybrid clone panel. Cytogenet. Cell Genet. **16:** 171–174.

KOZAK, C. A., J. B. LAWRENCE and F. H. RUDDLE, 1977 A sequential staining technique for the chromosomal analysis of interspecific mouse/hamster and mouse/human somatic cell hybrids. Exp. Cell Res. **105:** 109–117.

KUCHERLAPATI, R. S., I. HILWIG, A. GROPP and F. H. RUDDLE, 1975 Mammalian chromosome identification in interspecific hybrid cells using Hoechst 33258. Humangenetik **27:** 9–14.

MCKUSICK, V. A. and F. H. RUDDLE, 1977 The status of the gene map of the human chromosomes. Science **196:** 390–405.

MEISS, H. K. and C. BASILICO, 1972 Temperature sensitive mutants of BHK 21 cells. Nature New Biol. **239:** 66–68.

MING, P.-M., H. L. CHANG and R. L. BASERGA, 1976 Release by human chromosome 3 of the block at G-1 of the cell cycle in hybrids between tsAF8 hamster and human cells. Proc. Natl. Acad. Sci. U.S. **73:** 2052–2055.

NICHOLS, E. A., S. M. ELSEVIER and F. H. RUDDLE, 1974 A new electrophoretic technique for mouse, human, and Chinese hamster galactokinase. Cytogenet. Cell Genet. **13:** 275–278.

NICHOLS, E. A. and F. H. RUDDLE, 1973 A review of enzyme polymorphism, linkage, and electrophoretic conditions for mouse and somatic cell hybrids in starch gels. J. Histochem. Cytochem. **21:** 1066–1081. ——, 1975 Polymorphism and linkage of glutathione reductase in *Mus musculus*. Biochem. Genet. **13:** 323–329.

POVEY, S., C. A. SLAUGHTER, D. E. WILSON, I. P. GORMLEY, K. E. BUCKTON, P. PERRY and M. BOBROW, 1976 Evidence for the assignment of the loci *AK1*, *AK3*, and *ACONS* to chromosome *9* in man. Ann. Human Genet. **39:** 413–422.

PRESCOTT, G. H., B. K. MCCAW, B. E. TOLBY, F. HECHT, R. C. MILLER, A. E. GREENE and L. L. CORIELL, 1975 A (*1;15*) translocation, balanced, 46 chromosomes. Repository identification No. GM 126. Cytogenet. Cell Genet. **14:** 84.

RICCIUTI, F. C. and F. H. RUDDLE, 1973 Assignment of three loci (PGK, HGPRT, and G6PD) to the long arm of the human *X* chromosome by somatic cell genetics. Genetics **74:** 661–678.

RUDDLE, F. H., V. M. CHAPMAN, T. R. CHEN, and R .F. KLEBE, 1970 Linkage between human lactate dehydrogenase A and B and peptidase B. Nature **227:** 251–257.

RUDDLE, F. H. and R. P. CREAGAN, 1975 Parasexual approaches to the genetics of man. Ann. Rev. Genet. **9:** 407–486.

RUDDLE, F. H. and E. A. NICHOLS, 1971 Starch gel electrophoretic phenotypes of mouse × human somatic cell hybrids and mouse isozyme polymorphisms. In Vitro **7:** 120–131.

SHEININ, R., 1976a Preliminary characterization of the temperature sensitive defect in DNA replication in a mutant mouse L-cell. Cell **7**: 49–57. ——, 1976b Polyoma and cell DNA synthesis in mouse L cells temperature sensitive for the replication of cell DNA. J. Virology **17**: 692–704.

SHEININ, R., P. DARRAGH and M. DUBSKY, 1977 Mitochondrial DNA synthesis in mouse L.-cells temperature sensitive in nuclear DNA replication. Can. J. Biochem. **55**: 543–547.

SHEININ, R. and S. GUTTMAN, 1977 Semi-conservative and non-conservative replication of DNA in temperature-sensitive mouse L-cells. Biochim. et Biophys. Acta **479**: 105–118.

SHOWS, T. B., 1972 Genetics of human-mouse somatic cell hybrids: Linkage of human genes for isocitrate dehydrogenase and malate dehydrogenase. Biochem. Genet. **7**: 193–204.

SINISCALCO, M., 1970 Somatic cell hybrids as tools for genetic studies in man. Symposia Internat. Soc. Cell Biol. **9**: 205–231.

SLATER, M. L. and H. L. OZER, 1976 Temperature sensitive mutants of Balb/3T3 cells, II: Description of a mutant affected in cellular and polyoma virus DNA synthesis. Cell **7**: 289–295.

THOMPSON, L. H., J. L. HARKINS and C. P. STANNERS, 1973 A mammalian cell mutant with a temperature sensitive leucyl-transfer RNA synthetase. Proc. Natl. Acad. Sci. U.S. **70**: 3094–3098.

THOMPSON, L. H., D. J. LOFGREN and G. M. ADAIR, 1977 CHO cell mutants for arginyl-, asparagyl-, glutaminyl-, histidyl-, and methionyl transfer RNA synthetases: Identification and initial characterization. Cell **11**: 157–168.

THOMPSON, L. H., R. MANKOVITZ, R. M. BAKER, J. E. TILL, L. SIMINOVITCH and G. F. WHITMORE, 1970 Isolation of temperature sensitive mutants of L-cells. Proc. Natl. Acad. Sci. U.S. **66**: 377–384.

THOMPSON, L. H., R. MANKOVITZ, R. M. BAKER, J. A. WRIGHT, J. E. TILL, L. SIMINOVITCH and G. F. WHITMORE, 1971 Selective and non-selective isolation of temperature-sensitive mutants of mouse L-cells and their characterization. J. Cell Physiol. **78**: 431–440.

TONIOLO, D., H. K. MEISS and C. BASILICO, 1973 A temperature sensitive mutation affecting 28S ribosomal RNA production in mammalian cells. Proc. Natl. Acad. Sci. U.S. **70**: 1273–1277.

Corresponding editor: S. WOLFF