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Genetics

Genetics of somatic mammalian cells: Genetic, immunologic, and biochemical analysis with Chinese hamster cell hybrids containing selected human chromosomes*

(cell hybridization/cell surface antigens/immunogenetic analysis)

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ABSTRACT **Through hybridization of specific Chinese hamster cell auxotrophs with human cells and selection in media lacking the nutritional supplements required by the former cells, a series of stable hybrid clones can be prepared. These hybrids have genomes consisting of a common part—the complete or almost complete set of Chinese hamster chromosomes, plus a variable part—one or a few human chromosomes. The identity of the human chromosomes can be varied by utilizing different Chinese hamster auxotrophs and the appropriate selective media. The human chromosomes present can be determined by a combination of cytogenetic analysis with chromosome banding and testing for specific human marker genes. Hybrids containing single human chromosomes 11 and 12 and the combination of both 11 and 12 are described. The system appears to lend itself to various studies such as identification of human cell surface antigens, determination of their chromosomal loci, measurement of their distribution among cells of normal human tissues, study of interrelations among syntenic and asyntenic genes, and mutational analysis of the human genome.**

In previous studies, a series of human–Chinese hamster cell hybrids was produced by fusion of specific Chinese hamster mutant auxotrophs with human cells and growth in selective media lacking the nutrients required by the Chinese hamster auxotroph (1–5). The clones developing under these selective conditions must retain the human chromosomes complementing the deficiency of the Chinese hamster cell mutant employed. By using a variety of such Chinese hamster mutants, hybrids can be prepared containing different complements of human chromosomes. These hybrids tend to lose human chromosomes rapidly, so that in appropriate selective media, as few as one or two human chromosomes have been retained in particular hybrid clones. While the initial presence of a selective medium is usually necessary to ensure retention of a particular human chromosome, hybrid clones are sometimes obtained with stable chromosomal constitution after a short period of growth in culture, so that thereafter the clones can be grown for long periods even in nonselective media without further loss of human chromosomes (2, 5).

It was also shown that antisera to tissue culture cells can be produced in the rabbit and other animals which are active against cell surface antigens and produce lethal effects in the presence of complement (2, 5–7). When this lethal action is measured by means of single cell survival curves, the amount of antiserum needed to produce killing is extremely low—0.1% or less of standard antisera usually sufficing to kill 99% or more of the cell population used for immunization. Under these conditions, antisera to human and Chinese hamster cells, respectively, are highly specific and exhibit no cross-reactivity. Thus, by restricting the concentrations of antisera to less than 1% in the measurement of cell killing potency, it is possible to classify all of the reactions between cells and antisera so far studied as either + or 0, the former designating a reaction in which more than 99% of the plated cells are killed by 0.1% of the given antiserum while the latter means that no significant killing is observed by the presence of 0.1% of the antiserum in question (6).

The human–Chinese hamster ovary (CHO) cell hybrids produced were studied for their susceptibility to killing by specific antisera prepared against particular human cells. Since under standard conditions these antisera have no effect on CHO antigens, they react only to the human antigens present on the surface of the hybrid cell. Two classes of genetically determined antigenic markers were previously identified and named A_L and B_L (5). These two markers were demonstrated to act as well-behaved genetic loci and to possess different patterns of distribution among somatic cells of different adult human tissues. By measurement of the association between A_L and other human markers, the A_L characteristic was shown to be syntenic with lactate dehydrogenase A (LDH-A) gene activity (2, 5), which has been demonstrated to be on human chromosome 11 (8). A_L was also shown to be resolvable into at least two separate loci named a_1 and a_2, each of which produces a characteristic cell surface antigenic activity (6).

If to these immunologic and biochemical operations, cytogenetic analysis with banding could be added so that all of the human chromosomes retained in such hybrids could be precisely identified, a highly versatile system for genetic–biochemical analysis would be achieved. The present paper describes the application of cytogenetic analysis to this system and indicates the range of studies for which these hybrid cells now appear useful.

MATERIALS AND METHODS

Cell Cultures. All cell stocks, including the hybrid clones, were routinely cultured in F12 medium supplemented with 8% fetal calf serum. The preparation of hybrid A_L^+ clones by fusion of CHO-K1 auxotrophs with various human cells has been described previously (2, 6). One particular subclone named J1, derived by three successive subclonings from a primary hybrid clone of a cross between the Chinese hamster auxotrophic mutant gly^-A (9) and the normal human amniotic fluid fibroblast (2) was selected for intensive study. The original hybrid clone contained a chromosome number

Abbreviations: CHO, Chinese hamster ovary; LDH, lactate dehydrogenase.

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ranging between 23 and 32 and displayed the human gly$^+$A and LDH-B markers in addition to the A_L and LDH-A characteristics. The subclone isolated after growth in glycine-containing medium possesses 20 chromosomes, has lost the first two and retained the last two markers, and has revealed no further changes in phenotype after 3 years of continuous cultivation. The cell surface antigen markers were stable after long term growth in the absence of the selective conditions used in their clonal isolation, a necessary condition for their being considered as true genetic markers.

Mutagenesis. Mutagenesis experiments were carried out, using the standard, previously described procedures (11). The J1 clone and subclones derived from it were treated with mutagens like ethylmethanesulfonate (100–300 μg/ml) or ICR-191 (2 μg/ml), an acridine mustard kindly supplied by Dr. H. J. Creech. The survivors were subjected to selection and A_L^- subclones were isolated which survived treatment with antisera lethal to A_L^+ cells (6).

Assays for the Activity of a_1 and a_2. The detailed methodologies employed were described previously (5, 6). Cells were assayed for a_1 and a_2 antigens using single cell plating in the presence of specific antiserum plus normal rabbit serum as a source of complement. Rabbit anti-human erythrocyte serum was used to test for a_1; rabbit anti-HeLa serum adsorbed with human erythrocytes was used to test for a_2. Survival curves were determined for each clone using a range of concentrations for each antiserum. Specific adsorptions were used to confirm antigen assignments.

Isozyme Analysis. The presence of the following human isozymes was assayed using either Cellogel, acrylamide gel, or acrylamide slab electrophoresis: 6-phosphogluconate dehydrogenase (PGD) (EC 1.1.1.43); phosphoglucomutase-1 (PGM_1) (EC 2.7.5.1); malate dehydrogenase (cytoplasmic) (MDH-1) (EC 1.1.1.37); malic enzyme (cytoplasmic) (ME-1) (EC 1.1.1.40); superoxide dismutase-1,2 (SOD-1,2) (EC 1.15.1.1); glutamate oxaloacetate transaminase (GOT) (EC 2.6.1.1); lactate dehydrogenase-A,B (LDH-A,B) (EC 1.1.1.27); nucleoside phosphorylase (NP) (EC 2.4.2.1); phosphohexose isomerase (PHI) (EC 5.3.1.9); adenosine deaminase (ADA) (EC 3.5.4.4); glucose-6-phosphate dehydrogenase (G6PD) (EC 1.1.1.49); galactose-1-phosphate uridylyltransferase (Gal-1-PT) (EC 2.7.7.12); hexosaminidase-A,B (Hex-A,B) (EC 3.2.1.30); glutathione reductase (GR) (EC 1.6.4.2); adenine phosphoribosyltransferase (APRT) (EC 2.4.2.7); peptidase-A,B (Pep-A,B) (EC 3.4.13.-); galactokinase (GaK) (EC 2.7.1.6); isocitrate dehydrogenase (cytoplasmic) (IDH-1) (EC 1.1.1.42); triose phosphate isomerase (TPI) (EC 5.3.1.1).

The methods used for most of these isozymes have been described previously (2, 3, 5). The following 10 enzymes were assayed by methods described in the accompanying references: Gal-1-PT (12, 13), Hex-A,B (14), GR (15), APRT (16), GaK (17), TPI (15), Pep-B (15), IDH-1 (18). In every case, there was no difficulty in distinguishing between the Chinese hamster and the human enzyme.

Chromosome Banding Analysis. The procedure used for revealing bands in the metaphase chromosomes was a modification of that described previously (19). Cells grown in 60 mm plates were arrested with Colcemid for 1 hr. The cells were harvested and treated with 0.075 M KCl for 10 min at 37° and then fixed in three changes of methanol:acetic acid (3:1). Air-dried slides were prepared by depositing cells onto chilled slides previously stored at −4°. The 0.25% trypsin stock solution was prepared fresh using 2× crystallized, salt-free trypsin (ICN Pharmaceuticals, Inc., Cleveland, Ohio).

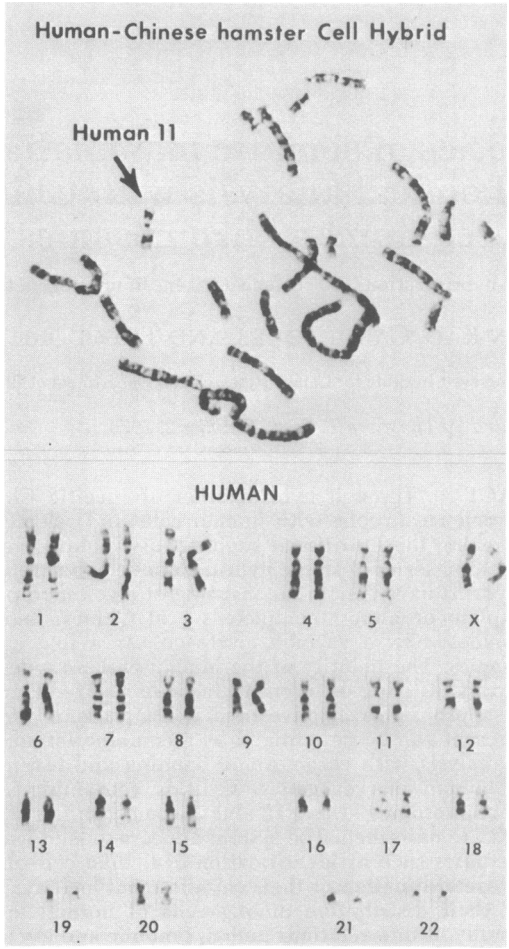

FIG. 1. Comparison of the banded chromosomal constitution of the normal human cell (bottom) and the A_L^+ human–CHO hybrid clone, J1, which contains the CHO chromosomes plus human chromosome 11 (top).

Chromosome slides that had been stored for at least 2 days were immersed in the 1:1 dilution of the stock trypsin for 5–10 sec in ice buckets, then washed and stained with Giemsa. The chromosomes were photographed using Kodak High Contrast Film with blue and green filters.

EXPERIMENTAL RESULTS

1. Cytogenetic Demonstration That the J1 Clone of the A_L^+ Hybrid Contains Chromosome 11 As Its Only Human Chromosome. The A_L^+ hybrid, clone J1, has been shown to possess all (or almost all) of the chromosomes of the CHO-K1 cell; the human LDH-A isozyme; and the specific A_L human antigens a_1 and a_2 which produce cell lethality in the presence of complement and the appropriate specific antiserum (2, 5–7).

In Fig. 1 are presented the banded karyotypes of a normal human cell and of the A_L^+ J1 clone. Analysis of clones from such cultures revealed the presence of a single unique chromosome which displays a banding pattern corresponding to that of human chromosome 11. Thus human chromosome 11 is the only necessary human chromosome for the expression of the A_L^+ immunologic activity.

2. Confirmatory Isozyme Analysis Showing Absence of 17 Other Human Chromosomes. Assays of 23 human isozyme markers in the J1 clone yielded negative reactions for all of these except LDH-A (Table 1), in accordance with the

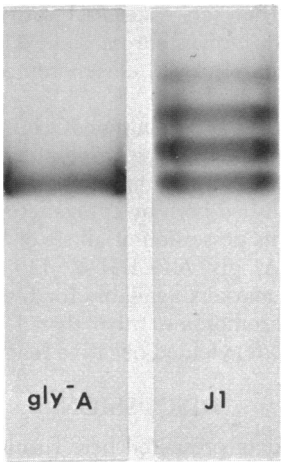

FIG. 2. Comparison of the zymograms for LDH-A activity in the parental CHO auxotroph gly⁻A and the J1 human–CHO cell hybrid. The human *LDH-A* gene is expressed in the hybrid and its product forms heteropolymers with the molecules of CHO LDH-A.

cytogenetic findings. A typical zymogram showing positive reaction of the human LDH-A in the J1 clone is presented in Fig. 2. The experiments on isozyme analysis exclude the presence of all the human chromosomes except 4, 7, 9, 13, 22, Y (and, of course, 11), from the J1 clone.

3. Demonstration That When Spontaneous Loss of Human Chromosome 11 from J1 Hybrid Cells Occurs, All of Its Markers Are Lost Together. Clones which have suffered loss of A_L^+ marker have been found to arise spontaneously in J1 cells at low but definite frequencies when cells are plated in large numbers in the presence of antisera against the A_L antigens. Such A_L^- clones were found to have lost a_1, a_2 and LDH-A. The chromosomal constitution of such a clone, J1-1, is shown in Fig. 3. It is evident that human chromosome 11 has been lost in its entirety. The incidence of spontaneous loss of human chromosome 11 is somewhat variable in different experiments, ranging in the neighborhood of 10^{-4}, or less.

Table 1. Isozyme analysis of the J1 clone

Human isozyme marker	Human chromosome assignment (20)	Presence of human isozyme marker
PGD, PGM_1	1	—
MDH-1, IDH-1	2	—
Gal-1-PT	3	—
Hex-B	5	—
ME-1, SOD-2	6	—
GR	8	—
GOT	10	—
LDH-A	11	+
LDH-B, TP1, Pep-B	12	—
NP	14	—
Hex-A	15	—
APRT	16	—
GaK	17	—
Pep-A	18	—
PHI	19	—
ADA	20	—
SOD-1	21	—
G6PD	X	—

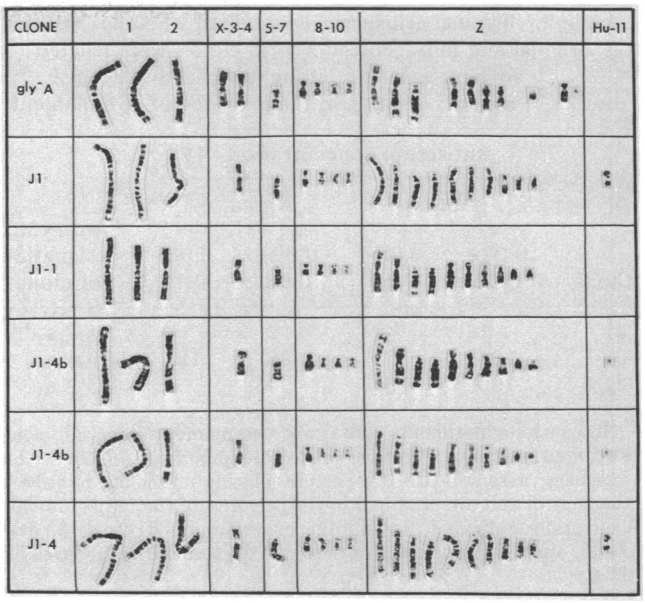

FIG. 3. Banded karyotypes of some of the clones described in Table 3:

gly⁻A: The parental CHO auxotrophic mutant used in the initial fusion contains 20 chromosomes.

J1: A typical J1 cell containing 19 CHO chromosomes plus a single human chromosome 11. This hybrid clone expresses the component markers associated with human A_L and human LDH-A. Its karyotype, as compared with that of the gly⁻A, appears to have lost one CHO chromosome of the X-3-4 group and also to have two altered chromosomes of the Z group. Karyotypic changes of this magnitude may occur spontaneously in the CHO chromosomes after long term culture with repeated re-cloning.

J1-1: A subclone of J1 which has spontaneously lost human chromosome 11. The remaining 19 CHO chromosomes are identical to those in J1. The human A_L immunogenetic characteristics and LDH-A isozyme were lost simultaneously.

J1-4b: The chromosome complement of the J1-4b clone which was mutagenized and lost the human A_L and LDH-A markers. The 19 CHO chromosomes are present with no apparent change, but instead of human chromosome 11, a centromeric fragment is present which is presumed to be from the missing human chromosome 11. Two typical cells are shown.

J1-4: The chromosomes of the J1-4 clone which has lost the a_1 but not a_2 marker of the A_L complex, and has also retained human LDH-A. The human chromosome 11 appears unchanged.

4. Demonstration That Mutagenesis and Selection Can Produce Clones Retaining Either Some or None of the Markers of Human Chromosome 11. By treatment of the A_L^+ hybrid cell with mutagenic agents, it should be possible to increase the frequency of genetic changes within localized regions of human chromosome 11. The desired mutant forms can be obtained by growth in the presence of selective antisera and analyzed by immunologic, biochemical, and cytogenetic procedures. Table 2 exhibits typical responses of the various clones and subclones to a standard antibody titration. Table 3 presents the properties of typical clones isolated in such mutagenesis experiments.

Clones J1-1, J1-5, J1-6, J1-2, and J1-4a described in Table 2 appear to be examples of marker disappearance due to loss of the complete human chromosome 11. Clones J1-3 and J1-4, however, have lost only the a_1 marker, but retain a_2. No change in the appearance of human chromosome 11 is evident cytologically (Fig. 3). Therefore, the loss of the a_1 marker in these clones may represent point mutations rather than gross chromosomal deletions. Clone J1-4b has lost both of the human immunologic markers and the human LDH-A

Table 2. Typical cell survivals obtained when the various original and mutagenized hybrid clones were treated with antisera against a_1 alone (anti a_1) and against both $a_1 + a_2$ (anti $a_1 + a_2$), in the presence of complement

	Antiserum concentration (%)				
	Anti a_1		Anti $a_1 + a_2$		
Clone	0.02–0.03	0.08–0.10	0.02–0.03	0.08–0.10	Antigenic designation of clone
J1	1	1	2	0	$a_1^+ a_2^+$
J1-1	124	130	128	124	$a_1^- a_2^-$
J1-4	90	105	0	0	$a_1^- a_2^+$

In control experiments containing complement alone, all clones exhibited plating efficiencies in the neighborhood of 100%. The numbers presented are the percent plating efficiency relative to the control and are obtained in the presence of the antiserum concentration indicated. In similar experiments, J1-2, J1-5, J1-6, J1-4a, and J1-4b behave identically to J1-1, and J1-3 behaves like J1-4.

isozyme marker but retains a centromeric fragment of what is presumed to be the original chromosome 11 (Fig. 3). The existence of such a clone affords presumptive evidence suggesting that none of the four markers of chromosome 11 here studied is located in the centromeric region.

5. Stable Hybrids with Other Human Chromosomes. We have previously demonstrated incorporation of human chromosome 12 in a human–Chinese hamster cell hybrid on the basis of synteny between the human LDH-B and the gene complementing the Chinese hamster auxotroph, gly^-A, which is deficient in serine hydroxymethyl transferase (EC 2.1.2.1) (3). Cytogenetic analysis of a series of such hybrids has revealed some of these to contain only a single human chromosome which in every case has been identified as 12 on the basis of its banding pattern (Fig. 4) and by the possession of three other syntenic marker genes for human chromosome 12, namely *LDH-B*, *TPI*, and *Pep-B*. Subclones which by cytogenetic analysis appear to have spontaneously lost this chromosome also have simultaneously lost all four of the human gene markers associated with it. In contrast to the J1 hybrid with human chromosome 11, the hybrid containing 12 as its only human chromosome displays none of the A_L antigens.

In analysis of still other human–CHO hybrids, a stable clone was found possessing the two human chromosomes 11 and 12 and no other human chromosome. The identification has been accomplished both by cytogenetic analysis (Fig. 4), and by virtue of its possession of all six of the human markers: A_L^+, LDH-A, gly^+A, LDH-B, TPI, and Pep-B. All human isozymic markers available for testing markers carried on human chromosomes other than 11 and 12 (see *Materials and Methods*) yielded negative reactions.

DISCUSSION

The cytogenetic data presented here furnish needed confirmation of the validity of the system consisting of a series of stable hybrid clones containing the CHO-K1 chromosomes plus single or small numbers of specific human chromosomes. As of this time, four different hybrids of this kind have been prepared in this laboratory, containing human chromosomes 11, 12, 11 and 12, and one containing a single human chromosome not yet completely identified but presumed to be 4 or 5 (4). Other such hybrids can be prepared and analyzed. Since 25 different auxotrophic mutants of the CHO-K1 culture have been prepared and others can be sought by means of mutagenesis and the bromodeoxyuridine–"near visible" light technique (10, 11), many different members of this hybrid cell series should become available. Presumably, additional hybrids containing particular combinations of human chromosomes can be obtained by use of multiple auxotrophic CHO mutants (a number of which have already been prepared) cultivated after hybridization in media with multiple nutritional deficiencies.

An advantageous feature of the hybrids described is their chromosomal stability. Thus, the A_L^+ hybrid with human chromosome 11, the hybrid containing only 12, and that containing both 11 and 12 have been cultivated continuously for long periods without change in any properties despite the fact that no selective medium yet exists for retention of chromosome 11.

Table 3. Immunologic, isozymic, and cytogenetic characterization of typical clones isolated with and without mutagenesis from the J1 clones of the A_L^+ hybrid in the presence of various antisera containing antibodies to a_1 obtained by rabbit immunization with human erythrocytes or HeLa cells

Clone or sub-clone designation	Mutagenic agent used	Use of anti-a_1 serum in clone isolation	Total chromosome no.	Presence of human LDH-A isozyme	Presence of human antigens associated with chromosome 11		Presence of human chromosome 11 by cytogenetic analysis
					a_1	a_2	
J1 (parental A_L^+ hybrid)	None	—	20	+	+	+	+
J1-1	None	+	19	—	—	—	—
J1-5	EMS	+	19	—	—	—	—
J1-6	EMS	+	19	—	—	—	—
J1-2	ICR-191	+	19	—	—	—	—
J1-3	EMS	+	20	+	—	+	+
J1-4	EMS	+	20	+	—	+	+
J1-4a	EMS	+	19	—	—	—	—
J1-4b	EMS	+	19 + fragment	—	—	—	Centromeric fragment

EMS, ethylmethanesulfonate.

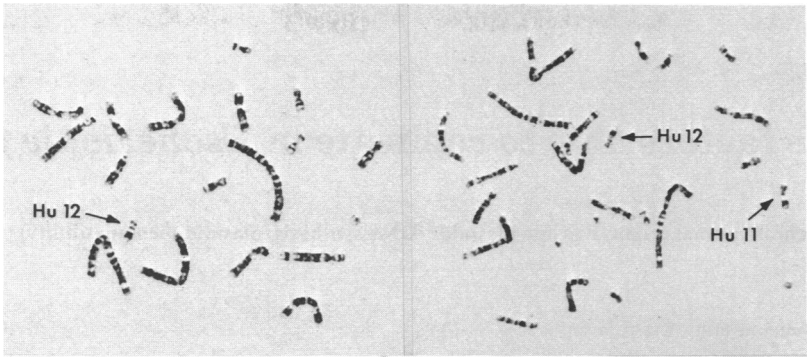

FIG. 4. Banded metaphase chromosomes of the hybrid clone containing chromosome 12 as the single human chromosome (left), and of the hybrid clone containing both human chromosomes 11 and 12, as the only two human components (right).

The hybrids with single human chromosomes furnish a system in which all the human genes that can be identified are, by definition, syntenic. Thus, genetic loci for human proteins identified by electrophoretic, enzymatic, and immunologic methods in such cells can be assigned to the retained human chromosome. Even more interesting is the fact that they permit search for mechanisms by which syntenic genes are activated or inactivated simultaneously.

Identification of families of cell surface antigens produced by many human chromosomes when present alone in such a hybrid cell, may now be possible by use of the methodologies which have identified the A_L character and established its relationship to human chromosome 11. At least one other family of cell surface antigenic loci, named B_L, has already been isolated (5). Each such family of antigens would appear resolvable into component syntenic antigenic markers by use of methods like those which have been successful in the A_L case. This step involves obtaining antisera differentially active against some but not all of the products of the individual genetic loci. Description of how such antisera can be obtained has been presented for the A_L family of antigens (6).

Since antisera against cell surface antigenic markers permit isolation of forward mutational phenotypes, the system offers promise for quantitation of mutagenic action. Moreover, human chromosomes can be secured in the hemizygous state in these hybrids, so that mutational analysis of human genes without the complications of diploidy is possible.

The hybrid system described here makes possible exploration of tissue cell surface antigens in normal and pathological situations. Since antisera can be individually prepared against each of the component antigens identified in the family of antigens associated with a specific chromosome, these can be used as reagents for adsorption by the cells of specific human tissues. The quantitative and reproducible nature of the single cell survival curves obtained when a standard hybrid cell culture is treated with a standard antiserum permits measurement of the degree of adsorption of specific killing activities from standard antisera by cells from various normal and pathologic tissues.

Finally, the existence of these hybrids may make possible isolation of DNA from different specific single human chromosomes. By attaching density labels to the DNA of the cell hybrids and then subjecting it to molecular hybridization with radioactively labeled DNA from a human cell culture, doubly-labeled double-stranded DNA separated from such a mixture should represent the DNA from the particular human chromosome present in the original hybrid.

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