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Assignment of Three Human Genes to Chromosomes (LDH-A to 11, TK to 17, and IDH to 20) and Evidence for Translocation between Human and Mouse Chromosomes in Somatic Cell Hybrids

(thymidine kinase/lactate dehydrogenase A/isocitrate dehydrogenase/C-11, E-17, and F-20 chromosomes)

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ABSTRACT **Independently derived man–mouse somatic cell hybrids and their derivative subclones show a positive correlation between the expression of human lactate dehydrogenase A subunits and the occurrence of the human C-11 chromosome. Data are also presented that confirm the previously reported linkage of the thymidine kinase locus to the E-17 chromosome. A translocation of the E-17 chromosome provides presumptive evidence for the assignment of the thymidine kinase locus to the long arm segment of the E-17 chromosome. This translocation also provides evidence for translocation between man and mouse chromosomes in somatic cell hybrids. A presumptive association between the human phenotype for isocitrate dehydrogenase and the human F group is also described. Identification of specific human chromosomes was achieved by the application of several new cytological techniques: measurement of chromosome arm length, *in situ* annealing with mouse satellite complementary RNA, constitutive heterochromatin staining with Giemsa, and quinacrine mustard fluorochromatic staining.**

In a previous report, we described enzyme phenotypes in somatic hybrids between mouse and human cells (1). Two independently derived hybrid populations [21b and 24a] displayed both mouse and human phenotypes for the A subunit of the enzyme lactate dehydrogenase (LDH-A; EC 1.1.1.27). Karyotype analyses of these populations revealed the presence of human chromosomes from groups C, E, and F at high frequencies (2). There were no more than five intact human chromosomes present in these human–mouse hybrids.

Since it has been reported that human chromosomes are preferentially lost from human–mouse cell hybrids (3, 4), we attempted to reveal latent heterogeneities of chromosome and enzyme complements by cloning. By comparing enzyme and chromosome composition of segregating clones, we hoped to localize the gene for human LDH-A to a specific human chromosome. We have applied various new chromosome analytical techniques in this attempt. Evidence is presented indicating that a human C-11 chromosome is responsible for human LDH-A production, and that chromosome E-17 codes for thymidine kinase (TK; EC 2.7.1.21) in confirmation of recent reports by Miller *et al.* (5) and Ruddle and Chen (6). New data are presented that delimit the *TK* locus to the long arm of chromosome E-17. Preliminary information on the role of F-group chromosomes in hybrids is also presented.

Abbreviations: TK, thymidine kinase; LDH, lactate dehydrogenase; HAT, hypoxanthine–aminopterin–thymidine; c-RNA, RNA complementary to mouse satellite DNA.

MATERIALS AND METHODS

Cell lines and culture methods

The mouse cells used in the establishment of hybrids were LM(TK$^-$), a permanent cell line isolated by Kit *et al.* (7) from L cells. These cells are resistant to 30 μg/ml (about 0.1 mM) of 5-bromodeoxyuridine (BrdU), since they lack the enzyme thymidine kinase. The population used in the present study had a modal chromosome number of 48, with a modal biarmed chromosome number of 12 (2, 8). The human cell population was WI-38, a diploid strain of human (female) embryonic lung fibroblasts isolated by Hayflick (9). These cells were supplied by the South Jersey Medical Research Foundation, Camden, N.J. WI-38 was used during its 26th–30th cell doubling.

Hybrid selection techniques and culture and cloning methods have been described (1). Basically, the technique involves selecting colonies of rapidly-growing cells in medium containing hypoxanthine, aminopterin, and thymidine (HAT). LM(TK$^-$) mouse cells cannot grow in aminopterin because they lack thymidine kinase. Human cells grow slowly in HAT and possess a distinctive fibroblastic morphology. Hybrid cells retain the human form of thymidine kinase and have a growth rate and morphology intermediate between the parental cells. Previous reports have established the location of the human TK locus on an E-group chromosome (1, 10). Samples of clonally derived populations were analyzed within 3- to 5-cell generations for enzyme and chromosome analysis. Hybrids were maintained in HAT medium unless otherwise stated.

Enzyme studies

Enzyme study methods, including extraction and electrophoresis procedures, have been given in detail (1, 11, 12).

Chromosome studies

Preparation of Metaphase Spreads. Cells were accumulated in metaphase by addition of colcemid (0.5–2.0 μg/ml final concentration) to logarithmic-phase cultures for periods of 3–8 hr. Metaphase cells were collected by hypotonic trypsin-EDTA treatment (13) for about 10 min at 37°C, then fixed and flattened by air drying according to the method of Hungerford (14) with slight modifications (15 and 16).

Chromosomes were visualized in all clones by staining with 1.5% aceto-orcein (Gurr's), treatment with absolute alcohol plus about 4% HCl, rinsing twice with absolute alcohol, clearing twice with xylene, and mounting in Permount (Fisher). Zeiss photomicroscopes were used for scanning and photographing with ×40 and ×100 oil planapochromat lenses. Kodak high-contrast copy and tri-X films were used.

Mouse Satellite DNA·RNA In Situ Annealing. In situ annealing procedures were essentially those described by Gall and Pardue (17). RNA complementary to mouse satellite DNA (c-RNA) was prepared *in vitro* by the use of *E. coli* RNA polymerase, purified mouse satellite DNA as template, and nucleotides having a high specific activity of tritium label. Air-dried metaphase chromosomes were treated with acid and RNase for the removal of histone and RNA, then their DNA was denatured by treatment with 0.07 N NaOH. Localization of the c-RNA to specific chromosome regions was revealed by autoradiography. Generally, c-RNA specifically labeled mouse centromeric regions. No crossreaction occurred with any of the human chromosomes.

Differential Constitutive Heterochromatin Staining. A modification of the DNA·c-RNA *in situ* annealing technique by use of Giemsa stain has been used to differentiate constitutive heterochromatin. This method was developed by Pardue and Gall (18) and Arrighi and Hsu (19), and its application to chromosome analysis of somatic cell hybrids has been reported recently by Chen and Ruddle (8). We have shown that this staining reaction is specific for mouse chromatin rich in satellite DNA. A similar staining reaction in human chromosomes is most probably specific for a class or classes of highly redundant human DNA (6, 8).

Quinacrine Mustard Staining. Air-dried chromosome preparations were prepared for fluorescence microscopy by the following procedure. Slides were treated 15 min in 95% ethanol, 5 min in 70% ethanol, and no more than 2 min in distilled water. Aqueous quinacrine mustard solution (0.01 or 0.05%) sufficient to cover the slide was placed on the preparation, a coverslip was lowered into place, and the slides were inverted and incubated at room temperature for 5 min. The slides were then rinsed in running tap water (with the coverslip removed) for 5 min; after a brief rinse in distilled water, the slides were mounted in phosphate buffer [0.2 M (pH 5.5), adjusted with citric acid] and sealed with Kronig's cement. Such preparations may be stored in the cold for several days, but are best analyzed immediately. This procedure is a slight modification of the technique originally described by Caspersson *et al.* (20).

Analysis of Metaphase Spreads. In all instances, photographic records and idiograms were made for each cell analyzed. Individual chromosomes were scored in each idiogram. A total of 972 cells was analyzed in this study.

RESULTS

As part of a survey of 11 independently derived human-mouse hybrids, two populations [21b and 24a] were found to have both mouse and human LDH-A. These populations were subsequently subcloned and analyzed for enzyme and chromosome complements. Nine clones derived from hybrid 21b were analyzed for LDH expression. All of these retained human as well as mouse LDH-A. Of 10 clones derived from hybrid 24a, only seven retained human LDH-A expression. Four of the 21b clones were further analyzed: three possessed human isocitrate dehydrogenase phenotypes. Human LDH-A-positive and -negative hybrid phenotypic patterns have been published (1). Murine LDH-5 (mouse homopolymer A) migrates anodally, whereas human LDH-5 migrates to a more cathodal position. The electrophoretic mobilities of mouse and human LDH-1 are similar (1, 12). In LDH-A-positive clones, hybrid isozymes (man–mouse heteropolymers) are apparent between the mouse and human LDH-5 bands. Such hybrid bands did not appear in extracts from mixed mouse and human populations; therefore, this result cannot be attributed to hybrid isozyme formation during the extraction procedure.

Karyotype analysis with orcein-stained material was performed on four of the 21b clones. In a total of 73 cells analyzed, a discernible human C-group chromosome was found in 44 cells, an E-group chromosome was present in 69 cells, and an F chromosome was found in 24 cells (Table 1). Karyotype analysis was performed on three positive and three negative LDH-A clones of 24a. In negative clones, there were no identifiable human C-group chromosomes in 54 cells examined, although a member of the human E group was discernible in 33 cells. In LDH-A-positive clones of 24a, 53 of 55 karyotypes contained a chromosome that resembled a human C-group member, and an E chromosome was present in 29 cells (Table 1). These judgments were based on arm ratio and total length.

Constitutive heterochromatin staining

Of four clones analyzed by the constitutive heterochromatin staining technique, three were positive and one was negative for LDH-A expression. The data for individual clones are presented in Table 2. A human C-11 chromosome (8) was recognized in 21 of 42 cells analyzed from LDH-A-positive clones, and an E chromosome was present in 20 of 42 cells. A representative karyotype of cells stained with heterochromatin is shown in Fig. 1. In the case of orcein-stained

TABLE 1. *Human biochemical phenotypes and human chromosome constitution. Chromosome identification is based on morphology and arm length measurements*

Cell line	Human phenotypes			Human chromosome constitutions		
	TK	LDH-A	IDH	E	C	F
21b	+	+	?*	10/12	8/12	6/12
21b-1-A	+	+	+	16/17	10/17	11/17
21b-4-A	+	+	+	19/20	14/20	8/30
21b-4-E	+	+	+	17/19	14/19	5/19
21b-6-A	+	+	−	17/17	6/17	0/17
24a	+	+	−	16/20	4/20	0/20
24a-2-A	+	−	nt†	0/15	0/15	0/15
24a-3-A	+	+	?	7/14	14/14	1/14
24a-5-B	+	−	−	14/16	0/16	0/16
24a-5-C	+	+	nt	1/16	14/16	0/16
24a-7-A	+	−	nt	19/23	0/23	0/23
24a-7-C	+	+	−	7/25	25/25	0/25

* ?, enzyme analysis ambiguous.
† nt, not tested.

TABLE 2. *Human biochemical phenotypes and human chromosome constitution. Chromosome identification is based on c-RNA annealing (C-RNA), constitutive heterochromatin (H), and quinacrine mustard fluorescence (QM)*

Cell line	Human LDH-A	TK	Method	Total no. of cells	C_{11}	E_{17}	E_T	Other chromosomes
21b-1-A	+	+	H	14	9	12		4F, 1G, $1C_9$
			GM	7	7	6		$4E_{16}$, 1C
21b-4-A	+	+	C-RNA	6	6	5		2F
21b-4-E	+	+	QM	5	3	5		1B, $1E_{16}$, $1C_7$
24a-3-A	+	+	H	10	8	3		$3E_{16}$, 6Q, 1(?)
			C-RNA	32	19	13		$5E_{18}$, $1A_2$, 1Tr
			QM	6	3	1		2C?, $2E_{18}$, 1Tr, 1?
24a-4-A	+	+	QM	4	—	—		3?
24a-4-C	+	+	QM	18	8	—		7B, $4C_{12}$, $1E_{16}$, 1F
25a-5-C	+	+	H	18	4	—		13B, $1C_{12}$, $1E_{16}$, 7Tr, 8?
			C-RNA	27	—	—		17B, 1F, 3Tr, 15?
			QM	15	—	1	2	8B, $1C_{12}$, $1E_{18}$, 19Tr
24a-2-A	−	+	H	25	—	5	24	3B
	−	+	C-RNA	18	—	1	4	$1A_1$, $2A_2$, 2B, $3C_{12}$
	−	+	QM	44	—	—	41	7B, 1?
(BrdU)	−	−	QM	34	—	—	5	8B, 2?
24a-5-B	−	+	C-RNA	17	—	15		$1E_{16}$, $1F_{18}$, 2G
24a-7-A	−	+	C-RNA	18	—	15		3F, 1G

material, chromosomes of the C type were identified. However, retrospectively, these appear to have been translocation products whose true nature was revealed by the quinacrine stains. The original unadjusted orcein data are given in Table 1. In an LDH-A-positive clone (24a-5-C, Table 2), unmodified C-11 chromosomes could not be detected by quinacrine mustard staining. Instead, a questionable C-11 chromosome is detectable at a high frequency. This chromosome bears an achromatic section indicating a possible loss of material from the distal long arm and addition to the original short arm (Fig. 2). It is possible that the rearrangement represents a pericentric inversion. *In situ* annealing provided evidence that the centromere was of human origin (see section below). Fluorochromatic staining indicates that the chromosome contains a large part of the C-11 chromosome (proximal short arm, centromere, and long arm segment), with an additional chromosome segment of unknown origin attached to the distal deleted short arm of C-11 (Fig. 2).

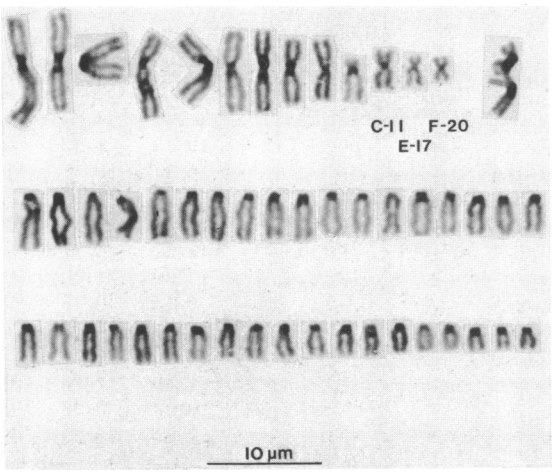

FIG. 1. Constitutive heterochromatin staining from an LDH-A-positive subclone showing the presumed C-11 chromosomes.

No C group chromosomes were observed in 25 cells of the LDH-A-negative clone analyzed. This particular negative clone (24a-2-A) also contained an extremely low count of recognizable E chromosomes. The TK-bearing E chromosome should be present in some form, since the population had been maintained in medium selective for retention of human TK. Indeed, there was a chromosome in 24 of 25 cells that has been designated as a translocated chromosome (Fig. 3). The distal segment of this chromosome, which appears to have been appended to a mouse biarmed chromosome, corresponds in length to the long arm of the human E chromosome. More will be said about this chromosome in the section on quinacrine mustard staining.

In situ mouse satellite

Annealing Studies. Autoradiographic studies using the *in situ* satellite-DNA·c-RNA annealing technique were made on three positive and three negative clones. Radioactive mouse c-RNA anneals specifically with mouse chromosomes in human–mouse cell hybrids, as evidenced by the presence of silver grains over the centromeres of mouse chromosomes and the abscence of grains over human chromosomes (see Fig. 4). In LDH-A-positive clones, 25 of 65 cells contained a presumptive C-11 chromosome. Data for individual clones are given in Table 2. One of the positive clones (24a-5-C) contained no recognizable C-11 chromosome, but did possess a unique chromosome that was not labeled by mouse c-RNA. We interpret this chromosome to be a modified C-11 chromosome. This interpretation is supported by observations made on quinacrine mustard preparations (Fig 2).

In the LDH-A-negative clones, no C-11 chromosomes were found in a total of 53 cells examined. In one clone, 24a-2-A, the E chromosome was present in only one of 18 cells, but 4–7 putative translocated chromosomes were pres-

ent. A-1, A-2, and possibly B, C-12, F, and G chromosomes were observed in low frequencies in these clones (Table 2).

Quinacrine Mustard Staining. Seven clones, six LDH-A positive and one LDH-A negative, were analyzed by quinacrine mustard staining and fluorescence microscopy (Fig. 5). A human C-11 chromosome was discernible in 21 of 55 cells from LDH-A-positive clones (see Table 2). In two of these clones there were no distinct C-11 chromosomes. However, an increased number of non-mouse, but otherwise unidentifiable chromosomes were present. Some of the unidentifiable chromosomes were determined to be translocation products, as judged by quinacrine mustard (Fig. 2) and constitutive heterochromatin staining patterns (8). A definite E-17 chromosome was present in 13 of the 55 cells. Again, the E-17 count was low in those clones that had increased numbers of unrecognizable, non-mouse chromosomes. Similar observations have been reported by Migeon and Miller (10).

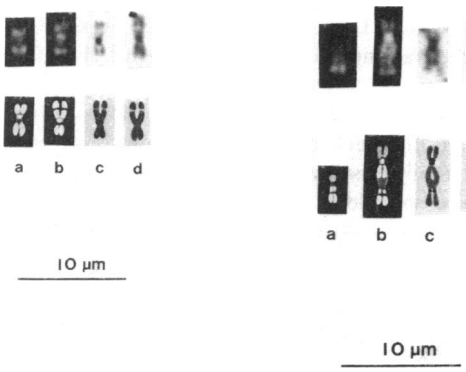

FIG. 2 (*left*). C-11 rearrangement from clone 24a-5-C (*a*) Reference human, intact C-11 chromosome stained by quinacrine mustard fluorescence. (*b*) Rearranged human C-11 chromosome stained by quinacrine mustard fluorescence. Chromosome banding pattern from top of figure to bottom is interpreted as follows: added segment; centric region with attached short arm, also possibly partially deleted, and probable deletions of the long arm with retention of proximal portion. It is possible that added segment may represent the deleted distal segment of the long arm, as in the case of a pericentric inversion. (*c*) Rearranged human C-11 chromosome stained to reveal centric heterochromatin. Centric heterochromatin size and association with the short arm is consistent with the normal C-11 chromosome pattern (see Fig. 1). (*d*) Rearranged human C-11 chromosome annealed with tritium-labeled complementary RNA (c-RNA) to mouse satellite DNA. The centric heterochromatin does not anneal with mouse satellite c-RNA, providing evidence that the centric region of the chromosome is nonmurine in origin. The single silver grain in the photograph represents either nonspecific binding to the chromosome or background.

FIG. 3 (*right*). E-17 rearrangement from clone 24a-2-A. (*a*) Reference human intact E-17 chromosome stained by quinacrine mustard fluorescence. Note that the long-arm staining pattern from the centromere to distal end of the long-arm is bright, dull, bright. This is the same pattern observed in the distal long-arm element of the translocated chromosome (see Fig. 3*b*). (*b*) Man–mouse translocated chromosome stained by quinacrine mustard fluorescence. The fluorochromatic staining pattern is consistent with the translocation of the long arm of E-17 to a mouse chromosome. (*c*) Man–mouse translocated chromosome stained to reveal centric heterochromatin. Large (*upper*) heterochromatic block corresponds to the mouse centromere. Small (*lower*) block represents human centric heterochromatin. This staining procedure provides evidence that the entire long arm of E-17 contributes to the mouse–man translocated chromosome. (*d*) Man–mouse translocated chromosome annealed with tritium-labeled complementary RNA (c-RNA) to mouse satellite DNA. The satellite c-RNA anneals to the mouse centric heterochromatin. Other grains represent nonspecific binding or background. This procedure provides strong evidence that the centromere region is murine in origin. It also indicates that the smaller heterochromatic block is nonmurine in origin.

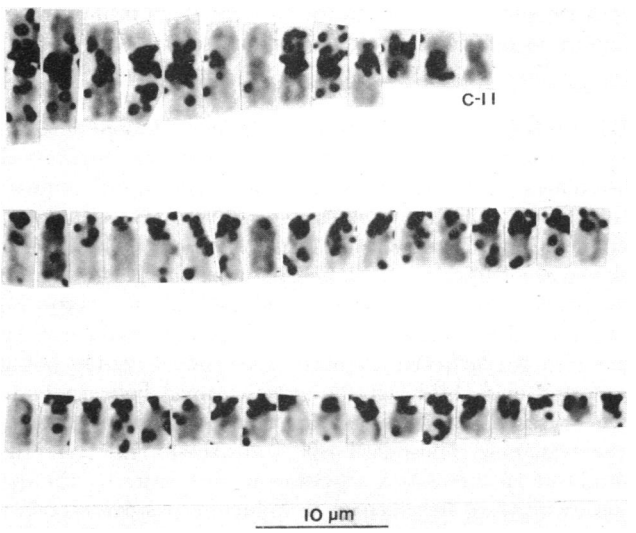

FIG. 4. Karyogram of a satellite-DNA·c-RNA *in situ* annealing preparation from an LDH-A-positive subclone showing the presumed C-11 chromosome.

In the one LDH-A-negative clone (24a-2-A) that was extensively examined, there were no discernible C-chromosomes in a total of 44 cells, nor were there any E-17 chromosomes. There were, however, 41 putative translocated E chromosomes. A sample was made of 24a-2-A cells that had been grown in the presence of BrdU. Growth in BrdU selects against TK, and should result in the loss of the human E linkage unit. If the putative translocation chromosome that is consistently present in the aminopterin (HAT medium)-grown populations does indeed involve the human E chromosome (and consequently human TK), then this chromosome should be eliminated in BrdU-grown populations. In fact, there were no translocated E chromosomes detected out of 34

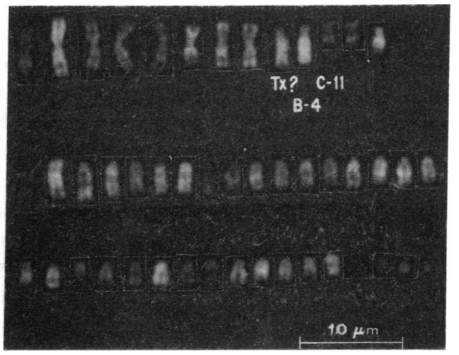

FIG. 5. Karyogram of a quinacine mustard-stained preparation from an LDH-A-positive subclone showing C-11 chromosomes. Although two C-11 chromosomes are present in this particular cell, generally only one C-11 is found per cell.

cells examined in BrdU-grown cultures. This result strongly implicates the long arm of E-17 as the site for TK.

DISCUSSION

Localization of genes to specific human chromosomes has long been a problem. The present report demonstrates the usefulness of somatic cell hybrid analysis as a new approach to this long-standing problem. Our report also exhibits the applicability of new chromosome analytical tools. We have shown the constant correlation between the presence of one particular human autosome, C-11, and a nonselected gene product—the A subunit of LDH. The data presented here are also strengthened by other independent studies not yet reported on hybrid cells (J series) formed between human leucocytes and RAG mouse line that is deficient in hypoxanthine–guanine phosphoribosyl transferase (12, 21). In a study of 16 clones and subclones in this series by the quinacrine mustard fluorescence technique, a positive correlation between human LDH-A and the C-11 chromosome was found. In no instance was a C-11 chromosome detected in six LDH-A-negative clones.

The simplest assumption would be that the human structural gene for the A subunit is located on the C-11 chromosome. However, the possibility of a regulatory gene should be considered. It could be postulated that the structural information for LDH-A is located on another human chromosome, and that an activator gene necessary for the expression of this information is associated with the presence of the C-11 chromosome. The only other human chromosome consistently present in our positive clones was E-17, which is necessary for TK production (5, 6, 8, 10). However, a positive association between C-11 and LDH-A is also observed in the J series, which does not retain the E-17 chromosome at a high frequency. It is also possible that a human D chromosome might be present and confused with the numerous acrocentric mouse chromosomes. This is rendered less likely, however, by the fact that no human D chromosomes were found consistently in hybrids with any of the analytical techniques used. Because of these considerations, we consider it most likely that the structural gene for LDH-A is located in C-11. The occurrence of C-11-translocated chromosomes may ultimately allow the assignment of the *LDH-A* locus to a subregion of the chromosome.

The quinacrine mustard staining technique allows reliable identification of homolog pairs of human chromosomes (Fig. 5). With this technique we were able to ascertain that the E-17 chromosome is the TK-bearing unit. This confirms other recent reports (5, 6, 8). The possibility of translocation between mouse and human chromosomes in the case of clone 24a-2-A is of interest. If the TK-bearing material has been translocated to mouse chromosomal material, it is most probably located in the long arm of the E chromosome, since the appended segment is of the dimensions of the long arm of the E (Fig. 3). Man–mouse translocations are important for somatic genetic linkage studies, since such an event may cause human gene fixation. The demonstration of a human–mouse chromosome exchange suggests that translocation mechanisms are not species specific. It also supports current ideas concerning the genetic functional integration of foreign DNA into eukaryotic genomes. Additional observations supporting mouse–man chromosome exchange have also been detected and will be reported elsewhere (Chen and Ruddle, unpublished data). Chromosomal rearrangement is a serious problem in chromosome mapping by cell hybrids, but with the aid of refined techniques, it can work to our advantage for finer genetic analysis.

Although the number of cells involved is small, our chromosome and enzyme data from the 21b clones point to the involvement of an F chromosome (F-20) in human isocitrate dehydrogenase (EC 1.1.1.42) production. This possible association is being examined in other independent hybrid cell populations.

Currently, some 20 biochemical markers (isoenzymes) exist that differ between men and mouse and that can be relatively easily distinguished in somatic cell hybrids (12). It should be possible in the near future to assign the majority of these markers to specific human chromosomes by use of the general approaches reported here.

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