Selective Linkage Disruption in Human-Chinese Hamster Cell Hybrids: Deletion Mapping of the *leuS*, *hexB*, *emtB*, and *chr* Genes on Human Chromosome 5

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Chinese hamster-human interspecific hybrid cells, which contain human chromosome 5 and express four genes linked on that chromosome, were subjected to selective conditions requiring them to retain one of the four linked genes, *leuS* (encoding leucyl-tRNA synthetase), but lose another, either *emtB* (encoding ribosomal protein S14) or *chr*. Cytogenetic and biochemical analyses of spontaneous segregants isolated by using these unique selective pressures have enabled us to determine the order and regional location of the *leuS*, *hexB*, *emtB*, and *chr* genes on human chromosome 5. These segregants arise primarily by terminal deletions of various portions of the long arm of chromosome 5. Our results indicate that the order of at least three of these genes is the same on human chromosome 5 and Chinese hamster chromosome 2. Thus, there appears to be extensive homology between Chinese hamster chromosome 2 and human chromosome 5, which represents an extreme example of the conservation of gene organization between very divergent mammalian species. In addition, these hybrids and selective conditions provide a very simple and quantitative means to assess the potency of various agents suspected of inducing gross chromosomal damage.

Studies involving interspecific somatic cell hybrids have provided a number of gene assignments to specific human chromosomes, endeavors which had previously relied primarily on pedigree analyses (15). Regional chromosome mapping has followed by comparing Giemsa banding patterns among hybrids whose human parental cells carried chromosomal aberrations arising spontaneously in the human population (6, 7, 16) or induced in culture by chromosome-breaking agents (9-11). Alternatively, hybrids can be constructed and subsequently studied for spontaneous or induced breaks in retained human chromosomes (1, 13).

In a previous report (4), we described a series of hybrids derived from normal human leukocytes and a Chinese hamster ovary (CHO) cell line, UCW 56, with recessive mutations in three linked genes. The Chinese hamster parent has a temperature-sensitive leucyl-tRNA synthetase (*LeuS^{Ts}*) and is resistant to normally cytotoxic levels of both emetine (*Emt^r*) and sodium chromate (*Chr^r*). These three genes, *leuS*, *emtB*, and *chr*, are linked on chromosome 2 in Chinese hamster cells (3, 19). The *leuS* gene encodes leucyl-tRNA synthetase. The *emtB* locus is one of three genes that can be altered to give rise to

the *Emt^r* phenotype (20, 21), and it encodes ribosomal protein S14 (4, 14). We have recently found that UCW 56 does have an electrophoretic alteration in this ribosomal protein (S. Dana and J. Wasmuth, unpublished results). The product of the *chr* locus appears to be involved in sulfate transport (2). Hybrids between UCW 56 and human leukocytes were selected for expression of the human leucyl-tRNA synthetase by requiring that they grow at 39°C. All of the temperature-resistant hybrids simultaneously became emetine sensitive and chromate sensitive, indicating that they retained and expressed the human *emtB* and *chr* genes, which can complement the altered Chinese hamster genes (4). Giles et al. (8) had provisionally assigned the gene for leucyl-tRNA synthetase to human chromosome 5. We found that *emtB* and *chr* were also linked on human chromosome 5 by demonstrating concordant segregation of the human *leuS*, *hexB*, *emtB*, and *chr* genes and identification of human chromosome 5 in Giemsa-banded metaphase chromosome spreads from hybrid cells (4). Hexosaminidase B (Hex B) is a marker isozyme for human chromosome 5 (15).

In this report, we describe studies in which cytogenetic analyses were applied to spontane-

ous segregants from these interspecific hybrids and selected such that they were required to retain one of these four linked human genes, *leuS*, but lose another. These segregants were isolated at 39°C, to ensure retention of the human *leuS* locus, in the presence of either emetine or chromate, to select for loss of either the human *emtB* or *chr* locus. These unique selective pressures have allowed us to specifically isolate segregants in which the coordinate segregation of the linked human markers has been disrupted. These segregants, which arise by breakage and loss of various regions of human chromosome 5, were then scored for expression of unselected markers on human chromosome 5, either *emtB* or *chr*, and *hexB*. Determination of segregation patterns and cytogenetic analysis of segregants expressing one, two, or three of the four human markers have enabled us to determine the order of the genes as well as provide regional assignments of these genes on chromosome 5. Our results indicate the relative order of the four genes on human chromosome 5 is *leuS-hexB-emtB-chr*. Interestingly, the order of three of these genes on the long arm of Chinese hamster chromosome 2 is the same: centromere-*leuS-emtB-chr* (L.-Y. Chu and J. Wasmuth, manuscript in preparation). The *hexB* gene has not been localized. Thus, the homology between Chinese hamster chromosome 2 and human chromosome 5 appears to be extensive. Our results also indicate that these hybrids provide a simple and very quantitative means to assess the potency of various suspected clathrogenic agents.

MATERIALS AND METHODS

Cell lines and cell culture. All cell lines were grown as monolayer cultures in Dulbecco modified Eagle medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 0.5 mM proline, 2% fetal bovine serum, and 8% bovine calf serum. Cultures were maintained at 33°C or 39°C in a humidified atmosphere of 5% CO₂-95% air. The CHO triple mutant, UCW 56, was derived from the CHO leucyl-tRNA synthetase (*leuRS*) mutant *tsH1* (18) as described previously (19). It is resistant to 0.3 μ M emetine and 30 μ M sodium chromate as well as being temperature sensitive due to the defective *leuRS*. UCW 56 is maintained routinely at 33°C.

The interspecific hybrids between the CHO triple mutant, UCW 56, and human peripheral leukocytes have been described before (4). These hybrid clones express the human *leuS*, *emtB*, *chr*, and *hexB* genes, and are designated by the prefix HHW. They are maintained routinely at 39°C. All segregants described in this study were derived from three independent parent hybrids: HHW 105 (containing only human chromosome 5), HHW 106 (containing two human chromosomes in addition to 5), and HHW 108 (containing one human chromosome in addition to 5) (4).

Selection of segregants. All hybrids are phenotypically wild type for *leuS*, *emtB*, and *chr*; that is, they are

temperature resistant, emetine sensitive, and chromate sensitive. From these, *Emt^r* or *Chr^r* segregants were selected at 39°C in the presence of either emetine or chromate. To do this, 24 independent subcultures (8 from each of the three hybrids) were established from inocula of 500 cells. After 19 days and one passage, 10⁶ cells from each independent culture were plated in a 100-mm culture dish at 39°C. In half of the cultures, emetine was included in the medium at a concentration of 0.3 μ M. The remaining cultures were incubated overnight at 39°C, and the following day, sodium chromate was added to a final concentration of 20 μ M. One resistant colony (*Emt^r* or *Chr^r*) per subculture was isolated and grown into mass culture at 39°C for subsequent screening of unselected markers and karyological analyses.

Determination of chromate sensitivity. For each segregant tested, growth from replicate cultures of 2×10^4 cells was followed in 0, 5, 10, 20, 40, and 80 μ M sodium chromate as described previously (4). Sensitive cells are killed at chromate concentrations above 5 μ M, whereas resistant cells grow to confluence at concentrations up to 40 μ M.

Determination of emetine sensitivity. In vivo protein synthetic rates were compared as a function of emetine concentration as detailed previously (20, 21). Rates of incorporation of [³H]lysine (L-[4,5-³H]lysine, 72.13 Ci/mmol; Amersham Corp. Arlington Heights, Ill.) into protein were measured at emetine concentration of 0, 0.1, 0.2, 0.4, 0.8, and 1.6 μ M. The concentration of emetine required to reduce the rate of protein synthesis to 50% of the rate in the absence of the drug (ID₅₀) was <0.2 μ M for sensitive cells and ≥ 0.8 μ M for resistant cells.

Hex B isozyme analysis. The presence or absence of Hex B, a marker enzyme for human chromosome 5 (15), was determined after starch gel electrophoresis of cell extracts as detailed previously (4).

Cytogenetic procedures. Differential staining (alkaline Giemsa staining) of metaphase chromosome spreads was performed as described (4, 5). Trypsin-Giemsa banding of metaphase spreads was performed as described by Worton and Duff (22). For each cell line analyzed, at least 14 Giemsa-banded metaphase spreads were photographed and examined for the status of human chromosome 5.

In cases where it was difficult to identify the partially deleted chromosome 5, a combination of Giemsa banding and differential staining was used. Metaphase chromosome preparations were Giemsa-banded as usual, photographed the same day, destained briefly in fixative (3 parts methanol, 1 part glacial acetic acid), and processed for differential staining the following day. In general, the time required in alkaline Giemsa stain for optimal differentiation was shortened. Photographed Giemsa-banded spreads were located again under the microscope after differential staining and powder blue (human) chromosomes were circled on the corresponding photographic print of the Giemsa-banded spread. This procedure allowed unequivocal identification of human chromosome 5 derivatives in photographs of Giemsa-banded chromosome spreads.

RESULTS

As discussed above, we have previously demonstrated that the human *leuS*, *emtB*, and *chr*

genes are linked on chromosome 5 (4). Interspecific hybrids between the CHO triple mutant, UCW 56, and normal human leukocytes were selected at 39°C so that the human *leuS* gene was required to complement the defective *leuS* gene in UCW 56. These hybrids also express the human form of Hex B as well as the human *emtB* and *chr* genes, rendering the cells sensitive to emetine and chromate, and retain an intact human chromosome 5 (4). When *Emt*^r segregants were selected from three such hybrids at 33°C, in 13 of 13 segregants the human *leuS* and *chr* genes segregated along with the human *emtB* locus (4). Such segregants, which lose the entire human chromosome 5, arise at a very high frequency ($\sim 10^{-3}$) in the absence of selective pressure to retain this human chromosome (4).

We wondered whether disruption of this coordinate segregation could be observed at a significant frequency if hybrids were subjected to appropriate selection conditions without the use of chromosome-breaking agents. To this end, we examined the segregation frequency for the human *emtB* gene at 39°C. At 33°C there is no selective pressure for loss or retention of the linked human *leuS* gene. At 39°C, the human *leuS* gene, and thus part of human chromosome 5, must be retained, whereas reexpression of the recessive emetine resistance mutation requires loss of the human *emtB* locus, by either chromosome breakage or gene inactivation. Two independent subclones were established from each of the three hybrids under investigation, HHW 105, HHW 106, and HHW 108. From each subclone, cells were plated at 5×10^5 cells per 100-mm dish in medium containing 0.3 μ M emetine and incubated at 39°C. After 20 days, dishes were fixed, stained, and the number of emetine-

TABLE 1. Frequency of emetine-resistant, temperature-resistant segregants from Chinese hamster-human hybrids^a

Cell line	<i>Emt</i> ^r segregants at 39°C	Frequency
HHW 105-1	5	1×10^{-5}
HHW 105-2	2	4×10^{-6}
HHW 106-1	3	6×10^{-6}
HHW 106-2	13	2.6×10^{-5}
HHW 108-1	26	5.2×10^{-5}
HHW 108-2	0	$<2 \times 10^{-6}$

^a Cells from two subclones from each of the three hybrids were distributed at a density of 5×10^5 cells per 100-mm dish at 39°C. The next day, emetine was added to the medium to a final concentration of 0.3 μ M, and cultures were returned to 39°C. Twenty days later, the number of emetine-resistant colonies was determined. There was a total of 49 *Emt*^r segregants and an average frequency of 1.6×10^{-5} .

TABLE 2. Phenotypes of 12 independent emetine-resistant segregants selected at 39°C^a

Segregant	Sensitivity to chromate ^b	Human Hex B ^c
HHW 178	S	NT
HHW 179	R	NT
HHW 180	R	NT
HHW 181	R	NT
HHW 182	R	NT
HHW 183	R	NT
HHW 207	S	Negative
HHW 208	R	Negative
HHW 209	R	Negative
HHW 210	R	Negative
HHW 211	R	Positive
HHW 212	R	Positive

^a Independent *Emt*^r segregants, selected for loss of the human *emtB* locus at 39°C, were isolated as described in the text. The segregants were then tested to determine whether they had retained the human *chr* locus (Chr^s) or lost the human *chr* locus (Chr^r), as described in the text. Six of these were also examined for the presence of human Hex B activity.

^b R, Resistant; S, sensitive.

^c NT, Not tested.

resistant colonies determined. The results are summarized in Table 1. *Emt*^r segregants arise at 39°C at an overall frequency of $\sim 10^{-5}$, about 100-fold lower than the frequency of *Emt*^r segregants at 33°C. As shown below, the vast majority of these segregants at 39°C arise via deletion of part of human chromosome 5. The frequency with which *Emt*^r segregants arise at 39°C, therefore, is a simple and quantitative determination of the frequency of spontaneous chromosome breakage.

We were encouraged that spontaneously arising noncoordinate segregants of this type would enable us to determine the order and regional location of genes on human chromosome 5 by correlating the loss of human markers with the loss of specific regions of this chromosome. In CHO cells, there is evidence that the *leuS* gene is centromere proximal to both *emtB* and *chr* on the long arm of chromosome 2 (L.-Y. Chu and J. Wasmuth, manuscript in preparation). If the relative order of these genes were the same on human chromosome 5, *Emt*^r and Chr^r segregants isolated from our hybrids at 39°C could arise by terminal deletions after chromosome breaks between the selected marker and the centromere proximal *leuS* gene. As shown below, these initial assumptions appear to have been valid. There were 24 independent segregants, 12 each emetine resistant and chromate resistant, isolated as described above and subsequently scored for the unselected marker, *Emt* or Chr. The results of these experiments are summarized in Tables 2 and 3.

TABLE 3. Phenotypes of 12 independent chromate-resistant segregants selected at 39°C^a

Segregant	Sensitivity to Emt (ID ₅₀ in μ M) ^b	Human Hex B ^c
HHW 213	R (1.21)	Negative
HHW 214	R (1.45)	NT
HHW 215	R (1.22)	NT
HHW 216	R (0.97)	Positive
HHW 217	R (0.80)	Positive
HHW 218	S (0.06)	Positive
HHW 219	S (0.07)	NT
HHW 220	S (0.10)	NT
HHW 221	R (1.14)	NT
HHW 222	R (1.10)	Positive
HHW 223	S (0.18)	Positive
HHW 224	S (0.10)	Positive

^a Independent Chr^r segregants, selected for loss of the human *chr* locus at 39°C, were isolated as described in the text. The segregants were then examined to determine whether they had retained the human *emtB* locus (Emt^r) or lost the human *emtB* locus (Emt^s), as described in the text. The concentration of emetine required to reduce the rate of in vivo protein synthesis to 50% of the rate in the absence of the drug (ID₅₀) is listed for each segregant. For the Emt^r CHO parent, UCW 56, this value is 1.1 μ M. For Emt^s CHO or human cells, this value is 0.10 μ M. Segregants with an ID₅₀ of 0.20 μ M or less were scored as Emt^r, whereas segregants with an ID₅₀ of 0.80 μ M or greater were scored as Emt^s. Seven of these segregants were also examined for the presence of human Hex B activity.

^b R, Resistant; S, sensitive.

^c NT, Not tested.

Out of 12 Emt^r segregants, 10 were also Chr^r. Only 7 out of 12 Chr^r segregants were also Emt^r. These results suggest that *chr* is most likely distal to *emtB* if one assumes that terminal deletion is more likely than interstitial deletion, which would require both breakage and reunion events. Thus, terminal deletions that remove the centromere proximal marker, *emtB*, must also remove the centromere distal marker, *chr*. However, terminal deletions that remove *chr* may or may not delete the *emtB* locus, depending upon how far toward the centromere they extend. Presumably, the two Emt^r, Chr^s segregants arose as a result of interstitial deletions.

Six Emt^r and seven Chr^r segregants were also examined for the presence of human Hex B after starch gel electrophoresis of cell extracts. As mentioned before, Hex B is a marker enzyme for human chromosome 5 (15) and has been regionally mapped by George and Francke (7) to the region 5cen $\rightarrow$ 5q13. To summarize the results presented in Tables 2 and 3 for Hex B, out of nine segregants which were both Emt^r and Chr^r, four were negative for human Hex B and five were positive for Hex B. These results suggest two things. First, the *chr*, *emtB*, and *hexB* genes

are most likely on the same arm of chromosome 5. Since these segregants are spontaneous and occur at a relatively low frequency, it seems reasonable to assume that each arises from a single deletion event. That about half of the Chr^r Emt^r segregants also lose the *hexB* locus indicates that loss of one portion of one arm can delete all three genes. Second, *hexB* is probably centromere proximal to both *chr* and *emtB*, since in about half of the segregants examined, the latter two genes are deleted without deleting *hexB*. This idea is strengthened by the results shown in Tables 2 and 3 for the Chr^r Emt^s segregants. All three of the Chr^r segregants examined that retained *emtB* also retained the *hexB* locus. Thus, terminal deletions that do not remove the centromere distal *emtB* marker cannot delete the centromere proximal *hexB* locus. One segregant, HHW 207, which lost *emtB* and *hexB* but retained *chr*, probably arose by an interstitial deletion as discussed below. As mentioned above, George and Francke (7) have localized the *hexB* locus to the centromere proximal region of the long arm of chromosome 5, specifically in the region 5cen $\rightarrow$ 5q13. Therefore, we can tentatively assume the gene order on 5q is centromere-*hexB*-*emtB*-*chr*. Since all of the segregants selected were required to retain the human *leuS* gene and 5 of the 13 segregants scored for Hex B were negative, we can also assume that *leuS* is centromere proximal to *hexB* on 5q or is on the short arm of this chromosome.

Thus, we have identified three major classes of segregants expressing one, two, or three of these four human genes linked on chromosome 5. Class I segregants lost the human *chr* locus, but retained *emtB*, *hexB*, and *leuS*. Class II segregants lost both the human *chr* and *emtB* loci, but have retained *hexB* and *leuS*. Class III segregants retained only the human *leuS* gene. To confirm the gene order proposed above and localize the genes, three independent segregants from each of the three classes were analyzed by cytogenetic means. When metaphase chromosome spreads of rodent-human somatic cell hybrids are stained in Giemsa at alkaline pH, the chromosomes are stained differentially such that human-derived chromosomes appear powder blue whereas the rodent chromosomes are purple (5). Differentially stained metaphase spreads from the nine segregants were examined to ascertain the physical status of human chromosome 5 in each of the segregants. Figure 1 shows representative differentially-stained chromosome spreads from the three parent hybrids (all of which have an intact chromosome 5) and one segregant from each of the three classes. Parent hybrid HHW 105 has no human chromosomes other than number 5, whereas HHW 106 and HHW 108 have two and one additional human

FIG. 1. Differentially stained metaphase chromosome preparations from parent hybrids and class I, II, and III segregants. Metaphase chromosomes were differentially stained by the alkaline Giemsa procedure as described in the text. Human chromosomes stain powder blue and appear lighter in these black-and-white photographs than the Chinese hamster chromosomes, which stain dark purple. Human chromosome 5 and deleted human chromosome 5 derivatives are indicated by solid arrows. The other, nonrelevant, human chromosomes present in hybrids HHW 106, HHW 108, and the segregants derived from them, HHW 217 and HHW 224, are indicated by dashed arrows. HHW 105 and the class III segregant derived from it, HHW 213, have no human chromosomes other than 5. HHW 106 and the class II segregant derived from it, HHW 217, have two human chromosomes in addition to 5. HHW 108 and the class I segregant derived from it, HHW 224, have one human chromosome besides 5.

chromosomes, respectively. For those segregants (class I) which lost the *chr* marker only (HHW 218, 223 and 224), chromosome 5 appears to be the same size in all three and appears to be the same size as an intact chromosome 5 (compare chromosome 5 in HHW 108 and HHW 224 in Fig. 1). In the three Class II segregants, which lost the *chr* and *emtB* markers (HHW 212, 216, and 217), the long arm of chromosome 5 is shortened by approximately one-third in all three (compare chromosome 5 in HHW 106 and HHW 217 in Fig. 1). In Class III segregants, which lost the human *chr*, *emtB*, and *hexB* markers, there is more variability in the size of chromosome 5. In HHW 213 (Fig. 1) virtually all of the long arm is missing, whereas in HHW 208 and HHW 209, chromosome 5 appears metacentric (see below).

Giemsa-banded chromosome spreads of the same nine segregants were then examined to more precisely determine chromosome break

points. Identification of human chromosome 5 in the segregants was simplified by comparing Giemsa-banded spreads to differentially stained spreads and locating chromosomes of the appropriate size. Where considerable portions of chromosome 5 were deleted, identification was sometimes difficult. We found that after Giemsa banding and photographing of chromosome spreads the human and rodent chromosomes could still be differentially stained in alkaline Giemsa. Although banding was usually obscured after differential staining, we could locate spreads that had been photographed after banding and unequivocally identify the human chromosome(s) on prints of the same metaphase spreads. This procedure is described in detail above.

Representative Giemsa-banded chromosome 5 derivatives from each of the nine segregants as well as their phenotypes are presented in Fig. 2. An ideogram of chromosome 5 showing the

CELL LINE	HUMAN GENES EXPRESSED				G-BANDED CHROMOSOME 5		
	leuS	hexB	emtB	chr			
Parent Hybrids	+	+	+	+			
Segregants							
Class I	+	+	+	-			
					HHW 218	HHW 223	HHW 224
Class II	+	+	-	-			
					HHW 212	HHW 216	HHW 217
Class III	+	-	-	-			
					HHW 208	HHW 209	HHW 213

FIG. 2. Human genes expressed and Giemsa-banded (G-banded) chromosome 5 derivatives in parent hybrids and class I, II, and III segregants. The procedures used to determine which human genes were expressed in the various segregants are described in the text. Karyological procedures are also described in the text.

proposed chromosome break point for each segregant is shown in Fig. 3. Based on these results, provisional regional assignments for the four genes can be made (Fig. 4). In all three class I segregants, which lost *chr* only, chromosome 5 appears to be virtually intact, with a possible deletion of the distal tip of 5q in HHW 218 and HHW 224. We believe it unlikely that point mutations in the human *chr* locus account for the *Chr*^r phenotypes of all three of these segregants, although this remains a possibility. Therefore, we can tentatively assign the human *chr* locus to the very terminus of 5q. That this locus is certainly distal to band 5q31 is clear from analyzing the class II segregants. Two class II segregants, HHW 212 and HHW 217, which lost both *chr* and *emtB*, have terminal deletions that extend from 5q23 → 5qter and 5q31 → 5qter, respectively. Thus, the human *emtB* locus, which encodes ribosomal protein S14, can be assigned to this region. We were unable to determine the exact nature of the alteration in chromosome 5 in HHW 216. Only one dark band in the middle region of 5q is apparent, and this is followed by a long lightly stained region and a

FIG. 3. Ideogram of human chromosome 5 indicating the proposed location of break points in chromosome 5 derivatives from segregants.

FIG. 4. Ideogram of human chromosome 5 showing provisional regional assignments for the *leuS*, *hexB*, *emtB*, and *chr* genes.

tight dark band at the terminus. As discussed before, George and Francke localized the human *hexB* gene to segment 5cen $\rightarrow$ 5q13 (7). Two class III segregants, HHW 208 and HHW 209, which do not express human Hex B, have deletions that extend from 5q13 $\rightarrow$ 5qter (Fig. 2 and 3). These segregants have thus retained the 5cen $\rightarrow$ 5q12 region, which allows us to assign the *hexB* locus relatively precisely to the middle region of band 5q13. The *leuS* gene must be located very close to the centromere on the long arm of chromosome 5 or on the short arm, since the segregant HHW 213 expresses the human leucyl-tRNA synthetase yet it has lost virtually the entire long arm of chromosome 5. As discussed below, we have preliminary evidence to indicate that *leuS* is, in fact, located very near the centromere on the long arm of chromosome 5.

Differential staining and Giemsa-banding analyses were then carried out on the segregant, HHW 207, that lost the two internal markers, *emtB* and *hexB*, but retained the two outside markers, *chr* and *leuS*. As mentioned above, we presumed this segregant to have an interstitial deletion of chromosome 5. Differential staining revealed that about two-thirds of the long arm of human chromosome 5 is deleted in HHW 207, such that the chromosome appears to be almost metacentric (Fig. 5). However, the banding pattern on the long arm of the partially deleted chromosome 5 in this cell line is such that we

were unable to determine the location of the break points. Thus, the loss of human gene function in HHW 207 can also be explained by partial chromosome deletion rather than some other mechanism.

DISCUSSION

By using spontaneous segregants from Chinese hamster-human somatic cell hybrids selected for the expression of one human gene, *leuS*, and loss of a second linked marker, *emtB* or *chr*, regional assignments for four genes on human chromosome 5 have been made. Terminal deletion of segments of the long arm of chromosome 5 appears to be the prevalent mechanism for disruption of linkage, leading to three major classes of temperature-resistant segregants and making regional mapping by cytogenetic means relatively easy. Interstitial deletions may be less frequent events which give rise to segregants, HHW 207 for example. This same battery of segregants should prove useful for regional mapping of any other marker located on human chromosome 5 provided there is a means of detecting the presence of that gene or its gene product.

In two of the class I segregants, which lost the *chr* locus only, it appeared that the very distal tip of 5q was missing. However, since the chromosomal alterations are not terribly obvious, we cannot rule out the possibility that other mechanisms, such as point mutations or gene inactivation, are involved in loss of the human *chr* gene function in these segregants. In this laboratory, we have found the frequency of spontaneous *Chr^r* mutants in CHO cells to be $\sim 10^{-6}$, an order of magnitude below the frequency with which *Chr^r* segregants arise at 39°C from the Chinese

FIG. 5. Differentially stained metaphase chromosome preparation from segregant HHW 207. The partially deleted human chromosome 5 is indicated by the arrow.

hamster-human hybrids. Thus, it seems unlikely that this mechanism is responsible for generating the *Chr*^r phenotype in the segregants. The possibility of gene inactivation of the human *chr* locus in the segregants cannot be ruled out at this time. However, the *chr* locus is almost certainly deleted in two segregants, HHW 212 and HHW 217, that have also lost *emtB* and have deletions extending from 5q23 → 5qter and 5q31 → 5qter, respectively. The *chr* locus is therefore located distal to band 5q31, most likely at the distal tip of 5q. The same two segregants, HHW 212 and HHW 217, also enable us to assign the gene for ribosomal protein S14, *emtB*, to the 5q23 → 5qter segment. As discussed previously (4), this is the first ribosomal protein gene to be given a chromosomal assignment in humans.

Our results, together with those of George and Franke (7), allow us to assign the *hexB* gene to a fairly precise region at band 5q13. In addition, segregant HHW 213 defines the location of the gene encoding leucyl-tRNA synthetase as being very near the centromere on 5q or on 5p. In selecting *Emt*^r and *Chr*^r segregants at 39°C, we assumed *leuS* was proximal to the centromere in relation to either of these markers. Nothing in our results contradicts this idea. More recently, we constructed secondary hybrids derived from HHW 105, which were isolated as follows. HHW 105 cells were irradiated with high doses of γ radiation (2 to 10 Krads) to fragment the chromosomes and were immediately fused to the original UCW 56 CHO mutant; secondary hybrids were isolated at 39°C to select for retention of the human *leuS* gene. One such cell line also expresses human Hex B. This cell line has a single, small fragment of human chromosome 5 translocated onto Chinese hamster chromosome 8, which can be visualized after alkaline Giemsa staining. This fragment does not contain the centromere and is about 20% of the length of human chromosome 5. Based upon this preliminary evidence, therefore, we would predict that *leuS* is located very near the centromere on the long arm of chromosome 5.

In view of the fact that the vast majority of *Emt*^r and *Chr*^r segregants isolated at 39°C from our hybrids result from breaks in human chromosome 5, these cells provide a very simple and quantitative test to determine the potency of chromosome breaking agents, or clathrogens. Thus, one could simply compare the frequency of *Emt*^r or *Chr*^r segregants that arise at 39°C in clathrogen-treated and untreated cultures. The difference between these values would represent the relative clathrogenic potency of the agent being examined.

Another point that deserves mention is the extensive homology between human chromosome 5 and Chinese hamster chromosome 2.

Five genes linked on chromosome 2 in Chinese hamster cells are linked on chromosome 5 in humans, including the four genes examined in this study as well as the gene encoding the diphtheria toxin receptor (17). In addition, the relative order of the *leuS*, *emtB*, and *chr* genes is the same on the long arm of Chinese hamster chromosome 2 and human chromosome 5. Perhaps the most striking thing about the retention of the linkage relationships between the two species, however, is that the genes are so far apart. Thus *chr* and *leuS* are separated by essentially the entire long arm of chromosome 5 in humans, yet the linkage relationship between these genes, and *emtB*, as well as their relative order, has been retained in species as divergent as Chinese hamsters and humans. Although it is not unusual for the linkage of closely linked autosomal loci to be conserved among different mammalian species, the *leuS*, *emtB*, and *chr* loci represent an extreme example of conservation of gene organization.

Finally, we used cell lines derived from the hybrid containing only human chromosome 5, HHW 105, as a starting point in attempts to clone the gene encoding leucyl-tRNA synthetase. As mentioned above, after γ -irradiation of this hybrid and refusion to the UCW 56 parent, we isolated a secondary hybrid that retained the human *leuS* gene, but only a small fragment of chromosome 5. We have since subjected this cell line to further rounds of irradiation and refusion, again selecting for retention of the human *leuS* gene. We have quantitated the amount of human DNA in cell lines isolated in these experiments, by using a technique that measures the extent of hybridization of DNA from these cell lines to radioactive human middle repetitive sequence probes (R. Cirullo, S. Dana, and J. Wasmuth, manuscript in preparation). In one such cell line which still expresses the human *leuS* gene, less than 0.1% of the DNA is human. We are now preparing a λ phage genomic DNA library from this cell line and will identify those recombinants containing human DNA specifically as described by Gusella et al. (12). A complete library of all of the human DNA for such a cell line (which must include *leuS* sequences), assuming 0.1% of the DNA is human, would be contained in about 600 recombinants with average size inserts of 20 kilobases. Thus, the sequences of interest will be purified at least 1,000-fold in this one step alone. Further screening of these clones, using more standard techniques, should enable us to identify those containing the genomic human sequences encoding leucyl-tRNA synthetase. These will then be used to isolate the corresponding sequences in a Chinese hamster genomic DNA library. In view of the large number of somatic cell mutants

and revertants that have been, and can be, isolated with alterations in this gene, DNA probes specific for *leuS* would be valuable tools to analyze the relationship between gene structure, mutation, and gene expression.

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