Am J Hum Genet 37:839–852, 1985

Deletion Mapping of Human Chromosome 5 Using Chromosome-Specific DNA Probes

LEON R. CARLOCK,[1] DOUGLAS SKARECKY, SHARON L. DANA, AND JOHN J. WASMUTH

SUMMARY

A complete genomic DNA library was prepared from a Chinese hamster-human cell hybrid that contains human chromosome 5 as its only human DNA. Unique or low-copy DNA fragments, isolated form recombinant bacteriophage that contained human DNA inserts, were regionally mapped on chromosome 5 using Southern blot analysis of genomic DNA from a series of hybrid cell lines that were selected as having deletions of various portions of 5q. The chromosome 5-specific DNA library, together with a genetic selective procedure allowing the isolation of hybrid cell lines with deletions of virtually any portion of 5q, will provide a means to construct very accurate physical and recombinational maps of this human chromosome. This system represents an excellent opportunity to examine very precisely the relationship between physical and genetic distances for many loci along the length of this autosome.

INTRODUCTION

The development of a detailed map of the human genome will provide an invaluable aid for examining many important problems in human genetics, including the establishment of close linkage between identifiable genetic markers and loci involved in heritable disorders. Such linkage relationships can potentially provide a means to distinguish between carriers and noncarriers of mutant alleles for various genetic disorders, which is most critical for diseases

Received September 11, 1984; revised February 25, 1985.

[1] All authors: Department of Biological Chemistry, California College of Medicine, University of California, Irvine, CA 92717.

This work was supported by U.S. Public Health Service grant GM-25339 from the National Institute of General Medical Sciences. L. R. C. was supported by U.S. Public Health Service Postdoctoral Training Grant T32-CA-09054.

 0002-9297/85/3705-0001$02.00

in which the basic biochemical defect is unknown. The study of interspecific, human-rodent somatic cell hybrids, which preferentially segregate human chromosomes, has provided a means to assign hundreds of genes to specific chromosomes or regions of chromosomes in humans [1]. Until a few years ago, gene assignments made using somatic cell hybrids required that one be able to detect a product of the human gene in question and be able to distinguish it from the homologous rodent gene product. However, with the advent of recombinant DNA technology, it has become possible to assign loci defined by virtually any nucleic acid probe, either a cloned gene of known function or random fragments of DNA, to specific chromosomes by Southern blot analysis of DNA from interspecific cell hybrids containing different human chromosomes [2]. This technology has already had a dramatic impact on the development of a comprehensive human gene map [3]. Furthermore, any nucleic acid probe that detects common DNA sequence or restriction endonuclease fragment length polymorphism (RFLP) provides a useful genetic marker for linkage analysis of the type described above [4]. The usefulness of this approach to human gene mapping and linkage studies was dramatically demonstrated by Gusella et al. [5] in studies with Huntington disease. The use of molecular probes that detect RFLP has also provided important insights into genetic alterations in somatic tissues that may be involved in the expression of mutant alleles in certain types of human cancer, incluing retinoblastoma [6] and Wilms tumor [7–10]. In addition, an extensive human gene map will also aid in the understanding of aneuploidy and deletion syndromes by helping to identify genes or loci located in regions either duplicated or deleted in these classes of genetic disorders. At a more basic level, the development of detailed maps of individual human chromosomes is an essential requirement for studying and defining the relationship between physical and genetic distance or recombination frequency. Studies of this type, which have recently been reported for X-linked loci [11], can help to determine the uniformity of recombination frequencies over the entire genome and possibly identify regions where recombination is either impeded or enhanced.

For many of the studies described above, it is critical to determine, with a reasonable degree of precision, the intrachromosomal location of different loci. Intrachromosomal or subchromosomal localization of genes can be accomplished using any one of several approaches, including: (1) in situ hybridization of radiolabeled nucleic acid probes to metaphase chromosomes [12], (2) the use of DNA probes that detect RFLP in conjunction with established human cell lines containing deletions of defined chromosomal regions to determine if heterozygosity for the marker loci can be detected [13], (3) analysis of interspecies cell hybrids derived from cells from persons with defined chromosome rearrangements [14], and (4) analysis of interspecific cell hybrids with spontaneous or induced rearrangements of retained human chromosomes, which have occurred after the isolation of the hybrid cell lines [15, 16]. In a previous report from this laboratory, we described the use of a combination of selective conditions that, when applied to certain Chinese hamster-human cell hybrids that contain human chromosome 5, require the hybrids to retain one human

gene (LARS) on chromosome 5 but lose another gene or genes (RPS14 or CHR) on the same chromosome [16]. The use of these combined selective pressures resulted in the isolation of a series of cell lines with well-defined deletions of different portions of the long (q) arm of human chromosome 5 [16]. These segregants with deletions of various parts of 5q, and others that have since been isolated in a similar manner, have been used in conjunction with a genomic DNA library specific for human chromosome 5 in order to develop an extensive DNA probe map of the long arm of human chromosome 5. This genetic system for fine structure mapping of chromosome 5 provides an excellent opportunity to examine recombination frequencies for various loci on an autosome whose physical locations on the chromosome are well defined. In addition, the ability to localize DNA fragments to relatively precise positions on this chromosome may prove helpful in defining the region of 5q, which, when it becomes monosomic in certain somatic cells, predisposes individuals to certain malignant hematologic disorders [17–20].

MATERIALS AND METHODS

Hybrid Cell Lines and Segregants with Deletions of 5q

The isolation and characterization of interspecific cell hybrids between the Chinese hamster ovary triple mutant UCW 56 and normal human leukocytes was described [21]. UCW 56 has mutations in three genes: LARS (previously referred to as leuS), RPS14 (previously referred to as emtB), and CHR [22]. The mutation in the LARS gene renders leucyl-tRNA synthetase thermolabile and the cell line nonviable at 39°C [23]. The mutations in the RPS14 and CHR genes render the cell line resistant to normally cytotoxic concentrations of emetine and sodium chromate, respectively [22]. Hybrids between UCW 56 and human leukocytes were selected at 39°C to ensure that viable hybrids retained human chromosome 5 containing the human LARS gene, which complements the temperature-sensitive phenotype of UCW 56. Maintaining the hybrids at 39°C ensures all viable cells retain human chromosome 5. All such hybrids are also sensitive to both emetine and sodium chromate because the human RPS14 and CHR genes are also on chromosome 5 and their expression results in sensitivity to these compounds [22]. Three such hybrids were examined in the most detail: HHW 105, HHW 106, and HHW 108 [16, 22]. Using a combination of alkaline Giemsa (G-11)-staining and trypsin-Giemsa-banding, it was determined that HHW 105 retains human chromosome 5 exclusively [16, 22], HHW 106 contains human chromosomes 3 and Y in addition to 5, and HHW 108 retains only the Y chromosome in addition to 5. The presence of the Y chromosome in HHW 106 and HHW 108 was also confirmed by the presence in these cell lines of a Y-chromosome-specific 4.3-kilobase (kb) *Eco*RI restriction fragment that is detected by a cDNA probe for the enzyme argininosuccinic acid synthetase ([24], see RESULTS).

Spontaneous segregants were isolated at 39°C from these three hybrids to ensure retention of the human LARS gene, in medium containing either 0.3 μM emetine or 20 μM sodium chromate, to select for loss of the human RPS14 or CHR gene functions, respectively [16]. Segregants isolated in this manner were subjected to cytogenetic analysis and were found to have terminal or interstitial deletions of various portions of 5q [16]. Figure 1 shows photographs of the trypsin-Giemsa-banded chromosome 5 derivatives present in the various segregant cell lines used in the present study, whose deletion breakpoints have been described [16, 25, 26]. Ideograms of these chromosome 5 derivatives are included in figure 4. Segregants HHW 207, HHW 208, HHW 213, and HHW 406 were derived from HHW 105 and have no human chromosomes besides a deleted 5. Segregants HHW 209 and HHW 217 were derived from HHW 106 and have

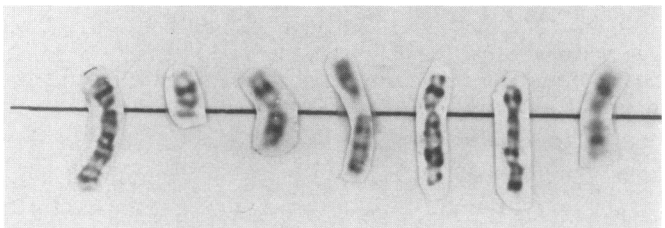


Fig. 1.—Trypsin-Giemsa-banded chromosome 5 derivatives present in hybrid cell lines. Metaphase chromosome preparations were trypsin-Giemsa-banded as described [16, 26]. The chromosomes 5 pictured are from (*left to right*): HHW 105 (normal 5); HHW 213 (del 5pter → 5q11:); HHW 208 (del 5pter → 5q13:); HHW 406 (del 5pter → 5q23:); HHW 217 (del 5pter → 5q23:); HHW 212 (del 5pter → 5q23:); HHW 207 (del 5pter → 5q12::5q34 → 5qter). The G-banding patterns of the chromosome 5 derivatives in HHW 207 and HHW 208 appear similar. However, HHW 207 but not HHW 208 retains two genes, FMS and CHR, which are localized to 5q34 and 5qter, respectively, confirming the cytogenetic interpretation of an interstitial deletion in the former cell line [16, 25].

chromosomes 3 and Y in addition to a deleted 5. HHW 212 was derived from HHW 108 and has only the Y chromosome in addition to a deleted 5.

Preparation of a Human Chromosome 5-Specific Genomic DNA Library

DNA from HHW 105, which contains an intact human chromosome 5 as its only human DNA, was used to prepare a complete recombinant bacteriophage library with human DNA fragments derived specifically from chromosome 5. High molecular weight DNA from HHW 105 was partially digested with *Mbo*I using conditions that resulted in an optimal yield of DNA fragments from 18 to 25 kb. Size-fractionated DNA fragments between 18 and 25 kb were cloned into the λ vector EMBL-4 [27] as described for the preparation of a DNA library specific for the short arm of chromosome 5 [28]. Sufficient in vitro packaging reactions [29] were carried out to yield approximately 1.5×10^6 recombinant phage, which should represent, with greater than 99% certainty, all the DNA sequences in HHW 105 and all the DNA sequences present on human chromosome 5 [30]. The complete library was amplified as plate lysates by plating 10^5 in vitro packaged phage particles on *E. coli* strain LE392 on 15 replicate 30 × 45 baking dishes.

Identification of Recombinant Phage with Human DNA Inserts and Isolation of Low-Copy DNA Fragments

Phage with human DNA were identified by in situ plaque hybridization [31] using ^{32}P-labeled total human DNA as a probe [32]. Phage identified as having human DNA in this manner were plaque purified and rescreened a second time to verify the presence of human DNA and the absence of Chinese hamster DNA by hybridizing duplicate nitrocellulose filters with phage DNA with either ^{32}P-labeled total human or total Chinese hamster DNA.

Chromosome 5-derived low-copy or unique-sequence DNA fragments devoid of repetitive DNA sequences were identified and isolated from various recombinant phage as described [13, 28]. Following extraction of the appropriate DNA fragments from agarose gels, they were either subcloned into a suitable plasmid vector or were used directly as probes for blot hybridization experiments.

Blot Hybridization Analysis

High molecular weight DNA was extracted from UCW 56, human fibroblasts, HHW 105, and various of the segregants described above with deletions of 5q. The hybrid and segregant cell lines were grown at 39°C prior to harvesting for DNA extraction to ensure

retention of the human chromosome 5 derivative. Ten to 20 μg of DNA was digested to completion with various restriction endonucleases, electrophoresed through 0.8% agarose gels, and DNA fragments transferred onto nitrocellulose filters [33]. Conditions for prehybridization, hybridization, and washing of filters were exactly as described [28]. The DNA probes for these experiments were low-copy or unique-sequence fragments derived from the HHW 105 library as described above, which had been labeled with ^{32}P via nick-translation to a specific activity of at least 1×10^8 cpm/μg of DNA. Following hybridization and washing of filters, they were exposed to X-ray film at −70°C with an intensifying screen for 16–48 hrs.

RESULTS

A complete genomic DNA library was prepared from the hybrid cell line HHW 105, which contains human chromosome 5 exclusively, as described in MATERIALS AND METHODS. Prior to preparation of the library, a segregant derived from HHW 105 that has lost all of human chromosome 5, as judged by cytogenetic analysis [21], was screened using a sensitive nucleic acid dot blot hybridization assay [34] to determine if any residual human DNA could be detected following segregation of human chromosome 5. By this assay, no human DNA (less than 0.05% of the total DNA of the segregant) was detected. Approximately 4.5% of the phage in the HHW 105 library contained human DNA inserts as judged by in situ plaque hybridization to ^{32}P-labeled total human DNA. The size of the human DNA inserts in various recombinants ranged from 14.5 kb to 21 kb. Human DNA fragments containing unique or low-copy sequences devoid of repetitive elements were isolated from phage from this genomic DNA library as described in MATERIALS AND METHODS. These DNA fragments were used as probes for blot hybridization analyses of restriction endonuclease-digested DNA from the hybrid cell lines that contain either an intact or partially deleted human chromosome 5. Each of the probes tested hybridized to one or more human-specific restriction fragments present in HHW 105 that were not present in UCW 56, the CHO parent of all the hybrid cell lines. Some of the probes also gave weak hybridization signals to restriction fragments derived from the CHO genome, which were present in UCW 56 and all the hybrid cell lines. However, cross-hybridizing hamster fragments were very seldom the same size as their homologs on human chromosome 5. For each of the human specific restriction fragments detected in HHW 105, the corresponding fragment was present in DNA from human cells. Not surprisingly, some of the probes tested hybridized to additional restriction fragments in human DNA that were not present in HHW 105, indicating that these DNA sequences, or sequences closely related to them, are also present on human chromosomes other than number 5. However, when cross-hybridization to human restriction fragments not localized to chromosome 5 was observed, the hybridization signals were invariably much less intense than the signals produced by fragments from chromosome 5. In addition, a few probes that were examined gave high background hybridization to total human DNA. It is likely that probes of this type are not completely devoid of repetitive element sequences. However, simply subcloning these fragments further to remove all traces of a repetitive element will correct this problem.

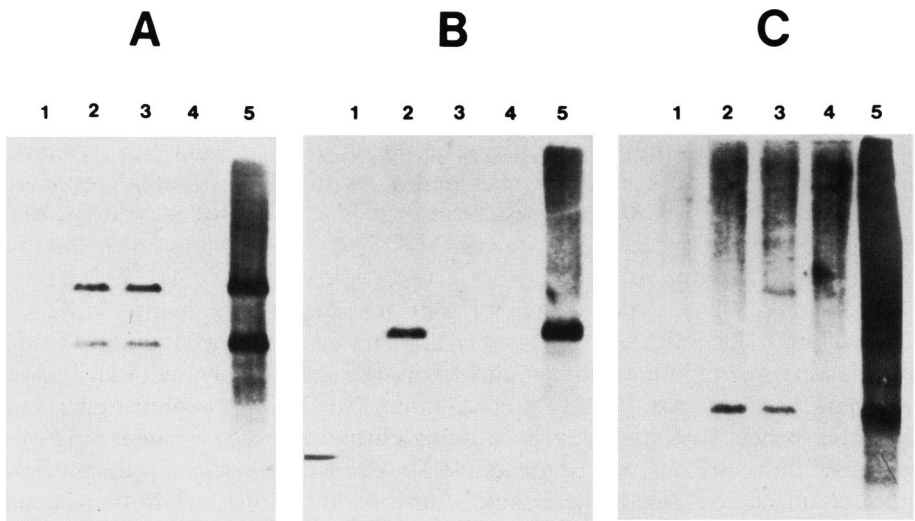


Fig. 2.—Blot hybridization analysis of chromosome 5 probes. High molecular weight DNA from UCW 56 (*lanes 1*), HHW 105 (*lanes 2*), HHW 212 (*lanes 3*), HHW 213 (*lanes 4*), or human fibroblasts (*lanes 5*) was digested to completion with *Eco*RI, electrophoresed through 0.8% agarose gels, and transferred to nitrocellulose filters. The blots were prehybridized, and washed as described in MATERIALS AND METHODS. The probes used for the different blots are: *A*, D5UCI 12; *B*, D5UCI 14; *C*, D5UCI 11.

The subchromosomal localization of restriction endonuclease fragments from chromosome 5 that hybridize to various probes is accomplished by determining whether or not the restriction fragments in question are detected in segregants with deletions of various portions of 5q. These analyses are initially carried out on a small panel of hybrids: HHW 105 with an intact chromosome 5, HHW 213 with a deletion extending from 5q11 → 5qter, and HHW 212 with a deletion extending from 5q23 → 5qter (see fig. 4) in order to get a rough idea of the intrachromosomal localizations of the probes. Figure 2, panels *A*, *B*, and *C*, show the results of Southern blot analyses with this panel of segregants using three different chromosome 5-derived sequences as probes. Probe D5UCI 12 hyridized to two *Eco*RI fragments (4.3 kb and 2.3 kb) in total human DNA, both of which are on chromosome 5 as evidenced by their presence in HHW 105. Both of these fragments are also present in HHW 212 but absent in HHW 213, indicating that they are localized to the region of 5q11 → 5q23. Probe D5UCI 14 hybridizes to a single 2.3-kb *Eco*RI fragment in human DNA, which is present in HHW 105 but missing in HHW 212 and HHW 213. This fragment is therefore located centromere distal to band 5q23. Probe D5UCI 11 detects a single 3.8-kb *Eco*RI fragment which is present in total human DNA, HHW 105, and HHW 212 but which is absent in HHW 213, indicating that it is in the region from 5q11 → 5q23. A second 6.5-kb *Eco*RI fragment is also present only in HHW 212, which contains the human Y chromosome in addition to the deleted chromosome 5. This cross-hybridizing fragment, which is not present in the human DNA sample (prepared from female cells), can therefore be assigned to the Y

chromosome. This assignment was confirmed by the observation that this 6.5-kb *Eco*RI fragment was also present in hybrid HHW 106, which contains chromosomes 3, 5, and Y but was not present in a hybrid containing just chromosomes 3 and 5 (data not shown).

Depending upon the outcome of this type of initial study, some probes are subsequently screened against an expanded panel of hybrids, containing cell lines with other types of deletion, to define their location more accurately. As shown in figure 3*A*, probe D5UCI 1 has been localized to band 5q23, based upon the single 1.0-kb *Eco*RI fragment it detects being present in HHW 212 but absent in HHW 406, both of which have terminal deletion endpoints in band 5q23 (see fig. 4). This result reiterates the fact that molecular analysis of chromosomal alterations can help define them beyond the level obtainable by cytogenetic analyses using the light microscope. The results shown in figure 3*B* for another probe, D5UCI 7, demonstrate its localization to the region 5q13 → 5q23 based upon the absence of the single 3.8-kb *Eco*RI fragment it detects in all the cell lines tested, except HHW 212. Subsequent analysis of additional cell lines using this probe have not provided a more accurate localization at this point.

In addition to random probes derived from chromosome 5, we have also determined the regional location of argininosuccinic acid synthetase (ASAS) pseudogenes. ASAS has approximately 14 different pseudogenes, distributed on 11 different chromosomes, which can be detected using pAS1, an ASAS cDNA probe [35]. Two *Eco*RI fragments detected by this probe, 11.0 and 9.5 kb, respectively, have been assigned to chromosome 5 [35]. As shown in figure 3*C*, both of these fragments are present in HHW 105, confirming their assignment to chromosome 5. In addition, both fragments are missing in HHW 213, only the 9.5-kb fragment is present in HHW 207, and both fragments are present in HHW 209 and HHW 212. Based upon the breakpoints of the deletions of 5q in these cell lines, the 9.5-kb ASAS pseudogene fragment can be assigned to the region 5q11 → 5q12 and the 11.0-kb fragment can be assigned to the region 5q12 → 5q13. Thus, it appears that the different *Eco*RI fragments that hybridize to the pAS1 probe are derived from two distinct pseudogenes on chromosome 5. Additional bands detected by this probe in cell lines HHW 209 (7.7 kb and 4.3 kb) and HHW 212 (4.3 kb) are derived from human chromosomes 3 (7.7 kb fragment) and Y (4.3 kb fragment) [24, 35].

In addition to the probes described thus far, seven additional chromosome 5-derived low-copy DNA fragments were analyzed in a similar fashion. The results of all these analyses are summarized in figure 4. For each probe examined, the restriction fragments (produced by a given enzyme) it detects in the human genome are listed along with an indication of which fragments are on chromosome 5 and their regional localization on this chromosome. Restriction fragments present in total human DNA that gave very weak hybridization signals, relative to the fragments derived from chromosome 5, are not listed. Only probes D5UCI 8 and D5UCI 11 produced reasonably strong hybridization signals to fragments not on chromosome 5. Both of these probes detected

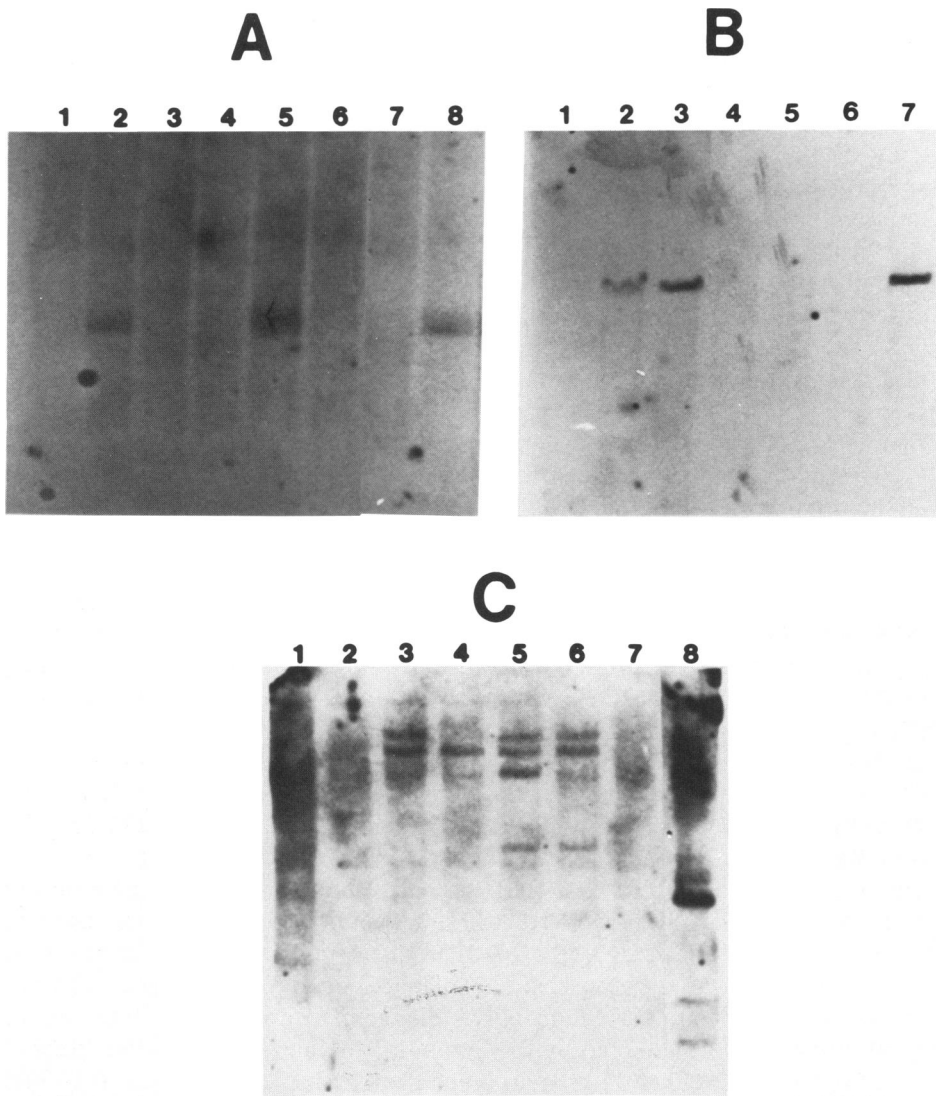


Fig. 3.—Blot hybridization analysis of probes using expanded hybrid panels. The blot hybridizations were carried out as described in the legend to figure 1 and in MATERIALS AND METHODS. *A*, Probe D5UCI 1 was hybridized to a blot containing DNA from UCW 56 (*lane 1*), HHW 105 (*lane 2*), HHW 207 (*lane 3*), HHW 208 (*lane 4*), HHW 212 (*lane 5*), HHW 213 (*lane 6*), HHW 406 (*lane 7*), and human fibroblasts (*lane 8*). *B*, Probe D5UCI 7 was hybridized to a blot containing DNA from UCW 56 (*lane 1*), HHW 105 (*lane 2*), HHW 217 (*lane 3*), HHW 209 (*lane 4*), HHW 207 (*lane 5*), HHW 213 (*lane 6*), and human fibroblasts (*lane 7*). *C*, Probe pAS1 was hybridized to a blot containing DNA from HHW 188, a segregant from HHW 105 that has lost all of chromosome 5 (*lane 1*), UCW 56 (*lane 2*), HHW 105 (*lane 3*), HHW 207 (*lane 4*), HHW 209 (*lane 5*), HHW 212 (*lane 6*), HHW 213 (*lane 7*), and human fibroblasts (*lane 8*).

*Eco*RI fragments derived from the Y chromosome. Any of the probes listed in figure 4 that detects a common RFLP will provide a useful genetic marker on chromosome 5.

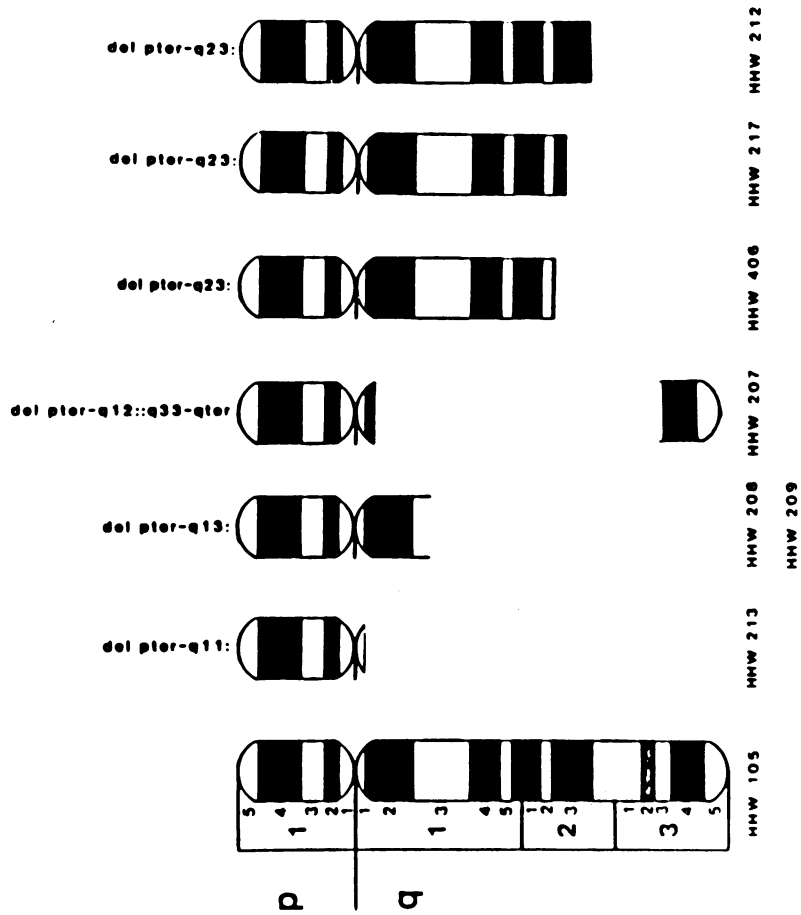
DISCUSSION

One goal of the work outlined in this report is to construct a fine structure physical map of human chromosome 5 as a prerequisite to constructing a recombinational map of this chromosome. As mentioned earlier, the two maps together, one providing accurate physical locations of many markers and the other providing recombination frequencies for the same markers, will hopefully help to increase our knowledge of the relationship of physical and genetic distances in various regions of the genome and determine how uniform this relationship is from region-to-region. Toward these goals, we are currently screening a large number of DNA probes derives from chromosome 5, including those described in this report, to identify those that detect common RFLP, which provides a means to distinguish various alleles at loci defined by the probes. In addition, we are currently analyzing another large set of segregants with deletions of 5q to identify cell lines with deletion endpoints in the regions from 5q13 → 5q23 and 5q31 → 5q34 in order to be able to define the location of probes in these regions more precisely. By testing a large number of probes against an expanded panel of cell lines with deletions, we should be able to map each probe to a specific major G band on 5q, providing a very detailed physical map.

Molecular probes in the distal one-third of 5q may be of particular interest in analyzing deletions present in malignant cells of some persons with de novo or secondary acute nonlymphocytic leukemia. One of the most common chromosomal alterations in persons with these disorders is a deletion of a portion of 5q [17–20, 36, 37]. The region from 5q23 → 5q33, in particular, appears to be common to the deletions in most individuals with acute nonlymphocytic leukemia who have detectable deletions of 5q [20]. DNA probes in this area may help to define, at the molecular level, a "critical" region associated with this disorder.

In a related series of studies, we are utilizing DNA probes derived specifically from the short arm of chromosome 5 to analyze the relatively common human deletion syndrome, cri du chat, or $5p^-$ syndrome, at the DNA level [28]. These experiments have identified DNA fragments that are missing from the deleted chromosome 5 homolog in some individuals with this disorder and will hopefully provide a means to define a critical region of 5p that is involved in producing the phenotype characteristic of the cri du chat syndrome when it is hemizygous [28]. In addition, these analyses and others using interspecific hybrids with a chromosome 5 from individuals with different alterations of 5p should provide a means to construct an accurate physical map of this arm of human chromosome 5 as well.

The analysis of hybrid cell lines that contain one or two human chromosomes in addition to 5, using probes derived from chromosome 5, can also facilitate the localization of DNA sequences that are not on chromosome 5 but which


del pter-q23:
del pter-q23:
del pter-q23:
del pter-q12::q33-qter
del pter-q13:
del pter-q11:
HHW 212
HHW 217
HHW 406
HHW 207
HHW 208
HHW 209
HHW 213
HHW 105
p
q

Probe	Fragments Detected							
D5UCI 1	(E) 1.0-5	+	-	-	-	-		+
D5UCI 2	(E)13.0-5	+	-					+
	(E) 6.5-5	+	-					+
D5UCI 3	(E) 4.2-5	+	+					+
D5UCI 5	(H) 6.6-5	+	-					+
D5UCI.6	(E) 6.5-5	+	-					+
D5UCI 7	(E) 3.8-5	+	-	-	-	+	+	
D5UCI 8	(E) 7.0-5	+	-					-
	(E) 6.3-Y*							
	(E) 2.0-5	+	-					-
D5UCI 9	(E) 2.2-5	+	-	-	-			-
D5UCI 11	(E) 6.5-Y*							
	(E) 3.8-5	+	-					+
D5UCI 12	(E) 4.3-5	+	-					+
	(E) 2.3-5	+	-					+
D5UCI 14	(E) 2.3-5	+	-					-
D5UCI 15	(E)25.0-5	+	-					+
pAS 1	(E)11.0-5	+	-	+	-			+
	9.5-5	+	-	+	+			+

Fig. 4—Regional location of restriction fragments detected by chromosome 5 probes. The ideograms indicate the region of chromosome 5 that is deleted in the different cell lines, with the breakpoints of the deletions listed above. For each probe, the sizes of the restriction fragments it detects (in kb pairs) in total human DNA are listed, as in the chromosome the various fragments are derived from. Fragments produced by digestion with *Eco*RI are prefaced with (*E*) while fragments produced by digestion with *Hin*dIII are prefaced with (*H*). For example, (*E*) 1.0-5 is a 1.0-kb *Eco*RI fragment located on chromosome 5 that is detected by probe D5UCI 1. Fragments detected by a probe that are not on chromosome 5 are further identified with *an asterisk*. For each of the restriction fragments located on chromosome 5, a (+) under an ideogram indicates the presence of that fragment in the corresponding cell line, a (−) indicates that the fragment is absent, and a *blank space* indicates that the probe was not tested with the cell line. For the ASAS cDNA probe, pAS1, only the two *Eco*RI fragments on chromosome 5 are listed.

cross-hybridize to a probe under study. An example of this is the identification of a Y-chromosome-specific restriction fragment detected by the chromosome 5-derived probe D5UCI 11 as described in RESULTS. The use of such probes, which detect a small number of restriction fragments on different human chromosomes, can facilitate the analysis of interspecific somatic cell hybrids as well as the search for RFLP at different loci, as evidenced by the results of Su et al. with the ASAS cDNA probe [35].

Finally, it should be mentioned that in addition to the mapping information presented in this report, several other genes or DNA probes of known origin have been regionally mapped on human chromosome 5 using hybrid cell lines with deletions of this chromosome. These include the LARS, RPS14, and CHR genes mentioned above [16]; the human c-fms oncogene (band q34) [25]; the functional dihydrofolate reductase gene (band q23) [26]; the HEXB gene (band q13) [16, 38]; and the gene (DTS) that confers sensitivity to diphtheria toxin [39]. In addition, we have preliminary evidence that indicates that besides the gene encoding leucyl-tRNA synthetase (LARS), the genes encoding at least two other aminoacyl-tRNA synthetases, histidyl-tRNA synthetase (HARS) and arginyl-tRNA synthetase (RARS), are on chromosome 5 as well (L. Carlock and J. Wasmuth, unpublished results, 1985).

ACKNOWLEDGMENTS

We thank Dr. A. L. Beaudet (Baylor College of Medicine, Houston, Texas) for kindly providing the pAS1 cDNA probe, Darlene Wise for her help in preparation of the manuscript, and Ulla Bengston for expert technical assistance in performing cytogenetic analysis of hybrids.

REFERENCES

1. McKusick VA, Ruddle F: The status of the gene map of the human chromosomes. *Science* 196:390–405, 1977
2. Skolnick MH, Willard HF, Menlove LA: Report of the Committee on Human Gene Mapping by Recombinant DNA Techniques, *in Human Gene Mapping 7 (1984): Seventh International Workshop on Human Gene Mapping. Cytogenet Cell Genet* 37:210–273, 1984
3. Seventh International Workshop on Human Gene Mapping: *Human Gene Mapping 7 (1984): Seventh International Workshop on Human Gene Mapping. Cytogenet Cell Genet* 37:Nos. 1–4, 1984
4. Botstein D, White RL, Skolnick M, Davis RW: Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am J Hum Genet* 32:314–331, 1980
5. Gusella JF, Wexler NS, Conneally PM, et al.: A polymorphic DNA marker genetically linked to Huntington's disease. *Nature* 306:234–238, 1983
6. Cavanee WK, Dryja TP, Phillips RA, et al.: Expression of recessive alleles by chromosomal mechanisms in retinoblastoma. *Nature* 305:779–784, 1983
7. Koufos A, Hansen MF, Lampkin BC, et al.: Loss of alleles at loci on human chromosome 11 during genesis of Wilms' tumour. *Nature* 309:170–172, 1984
8. Orkin SH, Goldman DS, Sallan SE: Development of homozygosity for chromosome 11p markers in Wilms' tumour. *Nature* 309:172–174, 1984
9. Reeve AE, Housiaux PJ, Gardner RJM, Chewings WE, Grindley RM, Millow LJ: Loss of a Harvey ras allele in sporadic Wilms' tumour. *Nature* 309:174–176, 1984

10. FEARON ER, VOGELSTEIN B, FEINBERG AP: Somatic deletion and duplication of genes on chromosome 11 in Wilms' tumour. *Nature* 309:176–178, 1984
11. DRAYNA D, DAVIES K, HARTLEY D, ET AL.: Genetic mapping of the human X chromosome by using restriction fragment length polymorphisms. *Proc Natl Acad Sci USA* 81:2836–2839, 1984
12. HARPER ME, SAUNDERS GF: Localization of single copy DNA sequences on G-banded human chromosomes by in situ hybridization. *Chromosoma* 83:431–439, 1981
13. CAVENEE W, LEACH R, MOHANDAS T, PEARSON P, WHITE R: Isolation and regional localization of DNA segments revealing polymorphic loci from human chromosome 13. *Am J Hum Genet* 36:10–24, 1984
14. FRANCKE U, DE MARTINVILLE B: Mapping of DNA sequences to chromosome regions in somatic cell hybrids, in *Banbury Report 14: Recombinant DNA Applications to Human Disease*, edited by CASKEY CT, WHITE RL, Cold Spring Harbor, N.Y., Cold Spring Harbor Laboratory, 1983, pp 175–187
15. GUSELLA J, VARSANYI-BREINER A, KAO T.-T., ET AL.: Precise localization of human β-globin gene complex on chromosome 11. *Proc Natl Acad Sci USA* 76:5239–5243, 1979
16. DANA S, WASMUTH JJ: Selective linkage disruption in human-Chinese hamster cell hybrids. Deletion mapping of the *leu*S, *hex*B, *emt*B, and *chr* genes on human chromosome 5. *Mol Cell Biol* 2:1220–1228, 1982
17. ROWLEY, JD, GOLUMB HM, VARDIMAN JW: Nonrandom chromosome abnormalities in secondary acute luekemia: relevance of ANLL de novo. *Blood* 50:759–770, 1977
18. TEERENHOVI L, BORGSTROM GH, LINTUAL R, ET AL.: The 5q$^-$ chromosome in preleukaemia. *Scand J Haematol* 27:119–129, 1981
19. ROWLEY JD, TESTA JR: Chromosome abnormalities in malignant hematologic disease. *Adv Cancer Res* 36:103–148, 1982
20. FOURTH INTERNATIONAL WORKSHOP ON CHROMOSOMES IN LEUKEMIA, 1982: Deletions of 5q. *Cancer Genet Cytogenet* 11:296–299, 1984
21. DANA S, WASMUTH JJ: Linkage of the *leu*S, *emt*B and *chr* genes on human chromosome 5 and expression of human genes encoding protein synthetic components in human-Chinese hamster cell hybrids. *Somat Cell Genet* 8:245–264, 1982
22. WASMUTH JJ, CHU L-Y: Linkage in cultured Chinese hamster cells of two genes, leuS and emtB, involved in protein synthesis and isolation of cell lines with mutations in three linked genes. *J Cell Biol* 87:697–702, 1980
23. THOMPSON LH, HARKINS JL, STANNERS CP: A mammalian cell mutant with a temperature-sensitive leucyl-transfer RNA synthetase. *Proc Natl Acad Sci USA* 70:3094–3098, 1973
24. DAIGER SP, WILDIN RS, SU T-S: Sequences on the human Y chromosome homologous to the autosomal gene for argininosuccinate synthetase. *Nature* 298:682–684, 1982
25. GROFFEN J, HEISTERKAMP N, SPURR N, DANA S, WASMUTH JJ, STEPHENSON JR: Chromosomal localization of the human c-fms oncogene. *Nucleic Acids Res* 11:6331–6339, 1983
26. MAURER BJ, CARLOCK L, WASMUTH J, ATTARDI G: Assignment of the human dihydrofolate reductase gene to band q23 of chromosome 5 and of the related pseudogene HD1 to chromosome 3. *Somat Cell Mol Genet* 11:79–85, 1985
27. FRISCHAUF A-M, LEHRACH H, POUTSKA A, MURRAY N: Lambda replacement vectors carrying polylinker sequences. *J Mol Biol* 170:827–842, 1983
28. CARLOCK LR, WASMUTH JJ: A molecular approach to analyzing the human 5p deletion syndrome, cri du chat. *Somat Cell Mol Genet* 11:267–276, 1985
29. HOHN B: *In vitro* packaging of lambda and cosmid DNA. *Methods Enzymol* 68:299–309, 1979
30. CLARKE L, CARBON J: A colony bank containing synthetic Col E1 hybrid plasmids representative of the entire *E. coli* genome. *Cell* 9:91–99, 1976

31. BENTON W, DAVIS R: Screening λgt recombinant clones by hybridization to single placques in situ. *Science* 196:180–182, 1977
32. GUSELLA JF, KEYS C, VARSANYI-BREINER A, ET AL.: Isolation and localization of DNA segments from specific human chromosomes. *Proc Natl Acad Sci USA* 77:2829–2833, 1980
33. SOUTHERN E: Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* 98:503–517, 1975
34. CIRULLO RE, DANA S, WASMUTH JJ: Efficient procedure for transferring specific human genes into Chinese hamster cell mutants: interspecific transfer of the human genes encoding leucyl- and asparaginyl-tRNA synthetases. *Mol Cell Biol* 3:892–902, 1983
35. SU T-S, NUSSBAUM RL, AIRHART S, ET AL.: Human chromosomal assignments for 14 argininosuccinate synthetase pseudogenes: cloned DNAs as reagents for cytogenetic analysis. *Am J Hum Genet* 36:954–964, 1984
36. FOURTH INTERNATIONAL WORKSHOP ON CHROMOSOMES IN LEUKEMIA, 1982: Secondary leukemias associated with neoplasia: treated and untreated. *Cancer Genet Cytogenet* 11:319–321, 1984
37. ARTHUR DC, BLOOMFIELD CD: Banded chromosome analysis in patients with treatment-associated acute nonlymphocytic leukemia. *Cancer Genet Cytogenet* 12:189–199, 1984
38. GEOERGE DL, FRANCKE U: Evidence for localization of the gene for hexosamindase B to the cen → q13 region of human chromosome 5 using mouse × human hybrid cells. *Cytogenet Cell Genet* 22:408–411, 1978
39. GEORGE DL, FRANCKE U: Regional mapping of the human genes for hexosamindase B and diphtheria toxin sensitivity on chromosome 5 using mouse × human hybrid cells. *Somat Cell Genet* 3:629–638, 1977

MEETING: *International Congress of Human Genetics*, September 22–26, 1986, West Berlin.