Am J Hum Genet 39:562–572, 1986

A Fine Structure Physical Map of the Short Arm of Chromosome 5

JOAN OVERHAUSER,[1] ARTHUR L. BEAUDET,[2] AND JOHN J. WASMUTH[1]

SUMMARY

A series of somatic cell hybrids that retain abnormal chromosomes 5 from 11 different persons with deletions or translocations involving 5p have been isolated. One hundred twenty DNA fragments isolated from a genomic library enriched for sequences from 5p were regionally localized by Southern blot analysis of the hybrid cell deletion mapping panel, including five DNA fragments that reveal restriction fragment length polymorphisms. The fine structure physical map of 5p together with the identification of additional polymorphic loci will facilitate the construction of a complete linkage map of this region. In addition, DNA fragments localized to a region near the 5p15.2-5p15.3 border, which appears to be the segment of 5p that is critical in producing the phenotype associated with the cri du chat syndrome when it is rendered hemizygous by deletion, will be useful in a molecular and DNA level analysis of this deletion syndrome.

INTRODUCTION

Detailed, high-resolution physical and genetic maps of individual human chromosomes are necessary to examine a number of important questions in human genetics at both the basic and applied levels, including the relationship between physical and genetic distances and how this varies from one region to another. Information about the variability in the physical distance-genetic distance relationship is important not only for addressing basic questions about factors that

Received May, 8, 1986.

This work was supported by U.S. Public Health Service grant HD-18642. J. O. was supported in part by U.S. Public Health Service Postdoctoral Training Grant T32-CA-09054.

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

[2] Department of Pediatrics and Howard Hughes Medical Institute, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.

influence the frequency of meiotic crossing over but also has a significant bearing on the strategies being used to isolate genes involved in certain heritable diseases, such as Huntington disease and cystic fibrosis, in which nothing is known about the nature of the relevant genes or gene products.

As more regions of the human genome become saturated with informative genetic markers, the search for linkage relationships between defined markers and other genes of particular interest becomes much more systematic and efficient. The efficiency of saturating a given region with genetic markers, such that any gene in the region will be linked to at least one marker locus, can be significantly enhanced if a library of DNA fragments derived specifically from that region is available and a rapid, accurate means of physically mapping the DNA fragments is possible. Accurate mapping data are particularly important for identifying physical regions that correspond to gaps in a linkage map.

We are particularly interested in developing a fine structure physical map of the short arm of chromosome 5 (5p) in order to study the human 5p deletion syndrome, cri du chat, at the molecular level. The cri du chat syndrome, which is invariably associated with deletions of 5p, is one of the most common human deletion syndromes [1]. The characteristics of this syndrome include severe mental retardation, microcephaly, developmental delay, hypertelorism, and a high-pitched cry similar to the mewing of a cat [1].

Although the size of the segment deleted from 5p varies from patient to patient, there appears to be a critical region near the p15.2-p15.3 border that is common to the deletions in these patients [2]. The notion that this region is critical in terms of the clinical symptoms is supported by reports of at least three persons with deletions of 5p that do not include this segment and who do not have the typical features of cri du chat [3–5]. As a first step in identifying functional genes in the critical region that are involved in the pathology of the disorder when they are rendered hemizygous, we are interested in identifying a large number of DNA fragments located within this segment of 5p [6]. Using a selectable genetic marker in the long arm of chromosome 5, we have isolated somatic cell hybrids that retain abnormal chromosomes 5 from different individuals with rearrangements of 5p [6]. Southern blot analysis of DNA from this panel of cell hybrids using probes from a library enriched for fragments from 5p [6] provides a straightforward means of identifying DNA fragments in the region of interest.

Here, we describe the use of this hybrid cell deletion mapping panel to regionally map 120 DNA fragments to different segments of 5p. The relatively fine structure physical map should facilitate the construction of a complete genetic linkage map of 5p. Toward this goal, we have identified five DNA fragments that reveal restriction fragment length polymorphisms (RFLPs), including one that detects a highly polymorphic locus in p15.2-p15.3.

MATERIALS AND METHODS

Isolation of Cell Hybrids

The Chinese hamster ovary (CHO) cell line UCW56 has a mutation in the LARS gene, rendering leucyl-tRNA synthetase thermolabile and the cell line nonviable at 39°C [7].

TABLE 1

KARYOTYPE AND PHENOTYPE OF INDIVIDUALS WITH REARRANGEMENTS OF 5p

Subject	Cell hybrid	Karyotype alteration	Phenotype
1	HHW339	del(5)(5qter-5p15.1:)	Cri du chat
2	HHW342	del(5)(5qter-5p14:)	Cri du chat
3	HHW686	del(5)(5qter-5p15.1:)	Cri du chat
4	HHW740	del(5)(5qter-5p13::5p15.3-5pter)	Cri du chat
5	HHW661	t(4;5)(p15.1;p15.1)	Normal*
6	HHW659	t(5;18)(p15.3;q12.2)	Normal*
7	HHW720	t(5;18)(p15.3;q12.2)	Normal*
8	HHW764	t(5;13)(p13;q12)	Normal*
9	HHW438	del(5)(5qter-5p14::5p15.1-5pter)	Normal
10	HHW750	del(5)(5qter-5p15.1::5p15.2-5pter)	Mildly retarded
11	HHW711	del(5)(5qter-5p14::5p15.2-5pter)	Mildly retarded

* The phenotypes of offspring from these individuals who inherited translocations in unbalanced form are described in the text.

This cell line was fused to fresh peripheral leukocytes [7] from the 11 different individuals described below with various rearrangements of the short arm of chromosome 5 (5p). Cell hybrids that retained the human LARS gene, which is on 5q and complements the temperature-sensitive phenotype of UCW56, were selected at 39°C. Hybrids isolated as having retained a human chromosome 5 were examined by alkaline-Giemsa staining [8] and trypsin-Giemsa banding [8] to determine if the normal or rearranged chromosome 5 homolog was retained. Hybrids were maintained at 39°C to ensure retention of the selected human chromosome.

Two cell hybrids used in these studies have been characterized previously. HHW105 retains a normal chromosome 5 as its only human chromosome [7, 8], and HHW213 is a derivative of HHW105 in which approximately 95% of the q arm of chromosome 5 has been deleted [6, 8]. Thus, the only detectable human DNA in HHW213 is an intact 5p, the centromere, and part of band 5q11.

Karyotype and Phenotype of Individuals with Rearrangements of Chromosome 5

Karyotype analysis on all of the individuals described below were done by clinical cytogenetic laboratories. Cell hybrids containing abnormal chromosomes 5 were isolated using leukocytes from 11 individuals with different alterations of 5p. An ultimate goal of this project is to define, at the DNA level, a region of 5p that is critical in producing the phenotype of cri du chat when it is deleted. Therefore, it is important to note the phenotype of each of the individuals with a rearrangement of 5p or, in the case of individuals with balanced translocations, the phenotype of offspring who have inherited the translocation in unbalanced form. Table 1 lists, for each person, a description of the abnormal chromosome 5, the number of the corresponding cell hybrid that retains the deleted or derivative chromosome 5, and information about the individual's phenotype.

Individuals 1, 2, and 3 have terminal deletions of 5p, and individual 4 has an interstitial deletion of 5p (case number 34 described by Niebuhr [2]). These four persons all have the typical symptoms of cri du chat including microcephaly, hyperterolism, developmental delay, severe mental retardation, and the cat-like cry.

Individuals 5, 6, 7, and 8 have balanced translocations involving 5p and are clinically

normal. Individual 5 (4;5 translocation) has a child who inherited the derivative chromosome 5 along with the normal 4 and is described clinically as having cri du chat. The chromosome breakpoints in individuals 6 and 7 (5;18 translocation) appear to be identical on both 5p and 18q, even though they are not related. Each of these persons has a child who inherited the derivative 5 as well as the normal 18 and are therefore monosomic for the distal part of 5p15 and trisomic for the distal half of 18q. Both of these children have overriding symptoms of trisomy 18, making it impossible to determine if characteristics of cri du chat were also present. A child of individual 8 (5;13 translocation) who inherited the derivative chromosome 5 and the normal chromosome 13 has cri du chat.

Individuals 9, 10, and 11 have interstitial deletions of different portions of 5p, and none are described clinically as having cri du chat. Individual 9, who has a deletion that removes most of p14, is completely asymptomatic. A molecular characterization of the deletion in this individual has been described [3]. Individual 10, with a deletion that removes most of 5p15.1 and part of 5p15.2, does not have hyperterolism, did not have the cat cry, and is very mildly retarded. The only other noticeable feature is microcephaly. Individual 11 has an interstitial deletion extending from 5p14 to 5p15.1 and has two children who inherited the deleted 5 [4]. The only consistent feature in the parent and two children is moderate mental subnormality. None have or had the cat cry, and only the parent has significant microcephaly [4].

Hybrid HHW693 was derived from HHW661, which retains the derivative chromosome 5 from individual 5. In HHW693, the entire q arm of the derivative 5 has been deleted and the intact p arm has been translocated onto a hamster chromosome. This cell line lacks the small piece of 5q11 that is present in the hybrid HHW213 and was useful for identifying DNA fragments in this region, as discussed in more detail below. A detailed characterization of HHW693 has been presented [9].

Isolation of DNA Fragments from 5p

The preparation of a complete recombinant DNA library from hybrid HHW213 has been described [6]. As discussed above, the only human DNA present in HHW213 is a chromosome 5 derivative with an intact p arm but is missing about 95% of the q arm. The isolation of recombinant phage with human DNA inserts, preparation of phage DNA, and isolation of unique or low-copy DNA fragments from recombinant phage have been described in detail [3].

Genomic Southern Blot Hybridization

High molecular weight DNA extracted from CHO cells, human cells, and the various cell hybrids was digested to completion with *Eco*RI or *Hin*dIII, electrophoresed through 0.8% agarose gels, and transferred to nylon membranes by capillary action as described [3]. Conditions for prehybridization, hybridization, and washing filters were described [3]. Low-copy human DNA fragments from phage from the 5p-enriched library described above were isolated from 0.8% low-melt agarose gels and labeled with ^{32}P [10, 11] and used as hybridization probes for the genomic DNA blots. In some cases, whole recombinant phage DNAs were used as hybridization probes without first purifying low-copy DNA fragments. For these experiments, 50 ng of sonicated phage DNA was labeled with ^{32}P and prehybridized with 500 μg of sonicated human DNA as described by Sealey et al. [12] before adding the labeled DNA to hybridization mixtures.

RFLP Analysis

DNA was extracted from lymphoblastoid cultures established from six unrelated individuals and digested with 30–35 different restriction endonucleases. After size fractionation on agarose gels, the digested DNAs were subjected to Southern blot analysis as described in the preceding section.

RESULTS

Deletion Mapping DNA Fragments on 5p

Trypsin-Giemsa-banded chromosome 5 derivatives from all the cell hybrids that constitute the current 5p deletion mapping panel are shown in figure 1 along with ideograms depicting which region of 5p is deleted from each of these chromosomes 5. One hundred twenty recombinant phage with human DNA inserts were isolated from the 5p-enriched HHW213 library as described in MATERIALS AND METHODS. Low-copy fragments or whole inserts from each of these phage were hybridized to filters containing *Eco*RI- or *Hin*dIII-digested DNA from CHO cells, human cells, and the 14 cell hybrids described in figure 1. DNA fragments from each phage hybridized to at least one human-derived restriction fragment in cell hybrids HHW105 and HHW213, which retain only an intact 5 or 5p, respectively. About 80% of the fragments showed the same hybridization pattern to total human DNA and DNA from hybrids with an intact 5p, indicating that all of the hybridizing genomic fragments are from chromosome 5. The remaining probes detected additional restriction fragments in total human DNA besides the ones present in HHW105 and HHW213. Therefore, these probes detect multiple loci, only some of which are located on chromosome 5.

An examination of the hybridization pattern of each probe to DNA from the various hybrids that are missing different portions of 5p provided a straightforward means of regionally mapping each fragment. Figure 2 shows autoradiograms of representative blot hybridizations using four different fragments to probe the deletion panel of cell hybrids. From these results, probe J018E-C (panel A) can be assigned to region G (see fig. 3), probe J081H-A (panel B) to region D, probe J0183E-A (panel C) to region A, and probe J0187H-A (panel D) to region E. Analogous experiments were performed with an additional 116 probes from the HHW213 library. Figure 3 shows an ideogram of 5p indicating the number of DNA fragments localized to the various regions, as determined by the deletion mapping studies. Several points concerning this physical map of 5p should be noted: (1) DNA fragments have been localized to nine distinct regions delineated by the chromosomal breakpoints. (2) As the number of cell hybrids that comprise the mapping panel has continually been expanded during the last year, not every DNA fragment has been analyzed using the complete panel depicted in figure 1. Thus, some probes will be more precisely mapped in the future as they are reexamined using cell hybrids that were not available when they were first analyzed. This is especially true for fragments below band p14 since the two hybrids with breakpoints in p13 (HHW740, HHW764) were among the last to be isolated. (3) Distribution of the fragments over 5p correlates reasonably well with cytogenetic distances, with the exception that close to 25% of the fragments map between p15.2 and the midregion of p15.3 (regions A and B), which appears to represent no more than 10% of the cytogenetic length of 5p. (4) About 10% of the fragments map to the long-arm side of the centromere, in q11, as determined by their being absent only in HHW693. This result is not surprising since the hybrid from which the library was prepared,

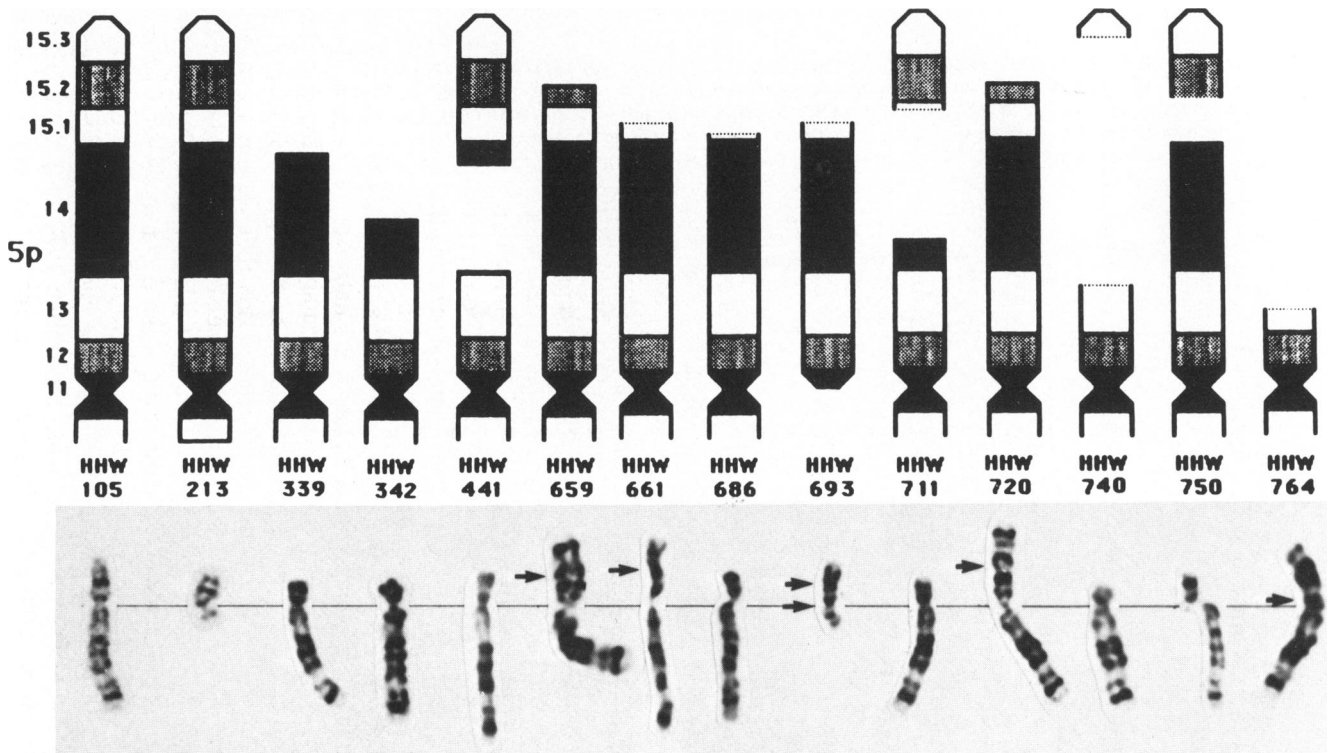

FIG. 1.—Chromosome 5 derivatives retained by somatic cell hybrids. The ideograms indicate which portion of 5p is missing from the chromosome 5 retained by each cell hybrid. For simplicity, the ideograms representing the translocation-derived chromosomes 5 in hybrids HHW659, HHW661, HHW693, HHW720, and HHW764 show only which segments of 5p are deleted and not the translocated fragments from other chromosomes. Translocation breakpoints of these derivative chromosomes 5 are indicated with *arrows* in the examples of G-banded chromosomes 5 shown *below each ideogram*. In all of the hybrids except HHW213 and HHW693, the long arm of chromosome 5 is intact. As described in the text, HHW213 has a terminal deletion of 5q with a breakpoint in q11 while the entire q arm of the derivative chromosome 5 in HHW693 is missing.

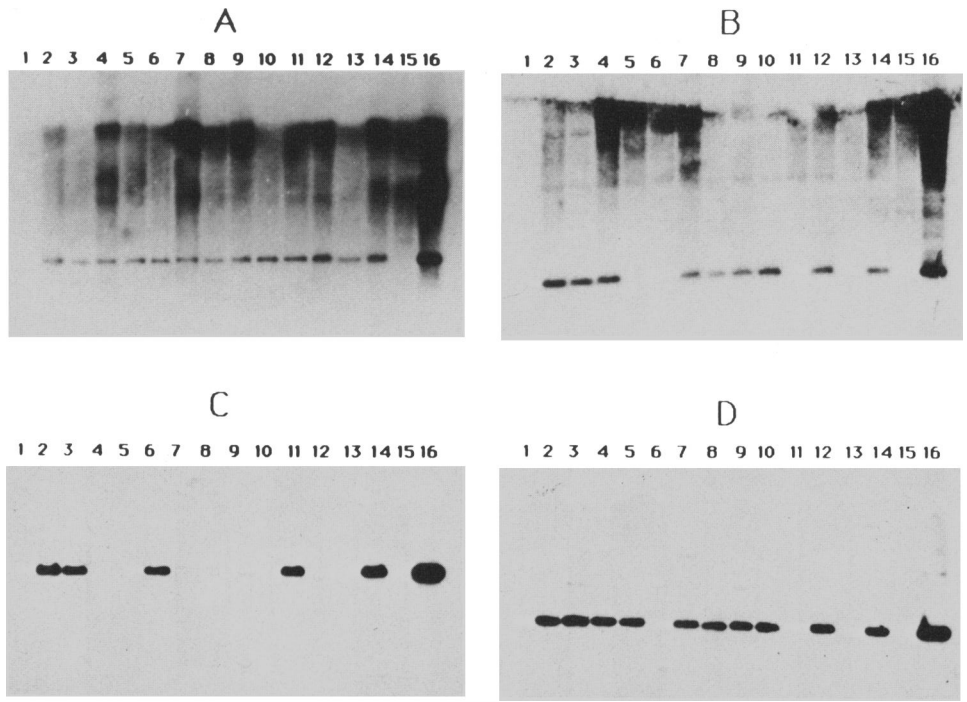

FIG. 2.—Blot hybridization of 5p DNA fragments to restriction endonuclease-digested hybrid cell DNAs. Nylon filters with *Eco*RI- or *Hin*dIII-digested DNA from CHO cells, human cells, and the 14 cell hybrids described in figure 1 were hybridized to the following DNA fragments as described in MATERIALS AND METHODS: probe J018E-C (*panel A*); probe J081H-A (*panel B*); probe J0183E-A (*panel C*); probe J0187H-A (*panel D*). *Lanes 1* contain DNA from CHO cells, and *lanes 16* contain DNA from human cells. The order of the hybrid cell DNAs in *lanes 2–15* is the same as the order of the hybrids shown in figure 1: HHW105 (*2*), HHW213 (*3*), HHW339 (*4*), HHW342 (*5*), HHW441 (*6*), HHW659 (*7*), HHW661 (*8*), HHW686 (*9*), HHW693 (*10*), HHW711 (*11*), HHW720 (*12*), HHW740 (*13*), HHW750 (*14*), HHW764 (*15*).

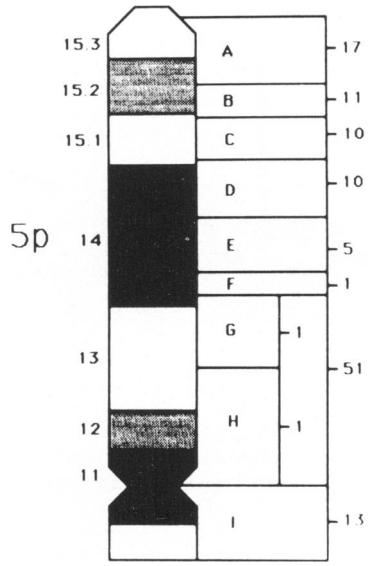

FIG. 3.—Physical location of DNA fragments on chromosome 5. *Letters A–I* indicate nine different regions to which fragments have been assigned based upon hybridization patterns to the hybrid cell panel. *The nos.* indicate how many independent probes have been assigned to each region.

HHW213, has a small fragment of the q arm intact with a breakpoint within q11 [8]. As described above, HHW693 has the short arm of the derivative 5 present in HHW661 translocated onto a hamster chromosome, and all of 5q, including the fragment of q11 present in HHW213, has been deleted [9].

Polymorphic Loci Identified by DNA Fragments from 5p

To begin constructing a genetic map of 5p to complement the physical map, DNA fragments localized to different regions are being tested to identify ones that reveal restriction fragment length polymorphisms (RFLPs). Five of seven fragments examined thus far detect RFLP with at least one restriction endonuclease in a sample of six unrelated people. The autoradiograms shown in figure 4 demonstrate two of the better polymorphisms identified. The five fragments that identify RFLPs are listed in table 2 along with their locations on 5p, which enzymes are informative, and the frequencies of the major alleles. Each of the polymorphisms shows codominant inheritance according to Mendelian expectations. The four different enzymes that show RFLP with probe J0214H-B are either identifying the same DNA sequence variation or are identifying different polymorphisms that are in linkage disequilibrium since the six individuals tested show the same pattern of variation with each enzyme. The same is true for the RFLP detected by *Pst*I, *Sph*I, and *Taq*I with J081H-A and the rare *Sph*I and *Ban*I polymorphisms detected with J0180E-D.

Probe 213-274C identifies a highly polymorphic locus and has been briefly described (J. Overhauser, A. L. Beaudet, J. Wasmuth, submitted for publica-

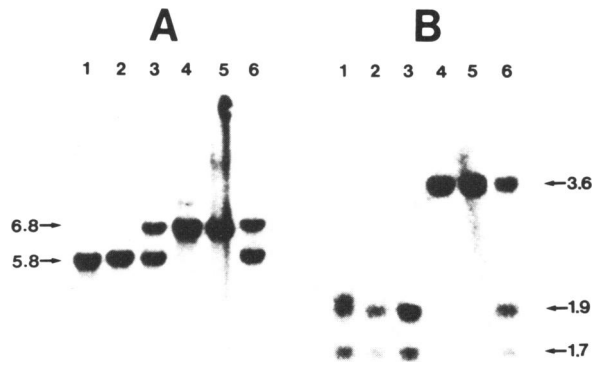

FIG. 4.—Polymorphic loci revealed by DNA fragments from 5p. High molecular weight DNA from lymphoblastoid cultures established from six unrelated individuals was digested with *Xmn*I (*panel A*) or *Msp*I (*panel B*), then subjected to Southern blot hybridization as described in MATERIALS AND METHODS. The filters were hybridized to probe J0214H-B (*panel A*) or probe J0209E-B (*panel B*). The sizes of the various hybridizing DNA fragments are indicated in kilobase pairs.

tion). Using just two enzymes, *Sph*I and *Hin*dII, this probe identified at least seven different alleles in the six people examined, and numerous other enzymes revealed polymorphisms. A more detailed characterization of this very informative genetic marker and the nature of the DNA sequence variation detected by the probe are in progress.

DISCUSSION

The more than 100 DNA fragments regionally mapped on 5p should provide enough fragments that reveal polymorphic loci to construct a complete linkage

TABLE 2

REGIONAL LOCATION OF DNA FRAGMENTS IDENTIFYING POLYMORPHIC LOCI

Probe	Location on 5p	Enzymes revealing RFLP	No. alleles	Frequency of major allele*
J0214H-B	5p15.2-5p15.3	*Hin*dII	2	.50
		*Xmn*I	2	.50
		*Rsa*I	2	.50
		*Fmn*4HI	2	.50
J081H-A	5p14	*Taq*I	2	.83
		*Pst*I	2	.83
		*Sph*I	2	.83
J0209E-B	5p15.1-5p15.2	*Msp*I	2	.58
J0180E-D	5p15.2-5p15.3	*Sph*I	2	.92
		*Ban*I	2	.92
213-274C	5p15.2-5p15.3	Many	>7	N.D.†

* Based on examination of six unrelated individuals.
† Not determined.

map of 5p in the forseeable future. Assuming that 5p represents about 1% of the haploid genome (3×10^7 base pairs [bp] of DNA), the DNA fragments are randomly dispersed throughout the region, and 25% of them reveal polymorphisms, it should be possible to have genetic markers spaced every 1×10^6 bp over the length of 5p. Alignment of a linkage map composed of this many markers with the corresponding physical map should provide an accurate means to identify regions where recombination is greatly increased or decreased relative to the average for the entire genome.

Of the 120 DNA fragments mapped thus far, 28 can be assigned to a segment (regions A and B in fig. 3) that can be considered as the smallest cri du chat critical region defined by the deletion panel. DNA fragments in this region, which represents about 10% of 5p or approximately 3×10^6 bp, are missing in each hybrid with a deletion (or translocation) associated with cri du chat but are present in all hybrids with 5p deletions from persons without cri du chat (HHW438, HHW711, HHW750). To make an analysis of all the DNA in the cri du chat critical region feasible, we would like very much to narrow the definition of its endpoints considerably. In this regard, it is unfortunate that the derivative chromosomes 5 from the two individuals with balanced 5;18 translocations were not informative. The 5p breakpoint on these chromosomes (hybrids HHW659, HHW720) together with the distal breakpoint just above the p15.2-p15.3 border in the cri du chat patient with an interstitial deletion (hybrid HHW740) define a very small region of overlap that represents considerably less than 10% of the cytogenetic length of 5p. Remarkably, 17 of the DNA fragments examined map to this region. As discussed above, however, offspring of the balanced 5;18 translocation carriers who inherited the translocation in unbalanced form are trisomic for much of 18q as well as being monosomic for the relevant segment of 5p. The overriding symptoms of trisomy 18 in these individuals made it impossible to determine whether or not they have the cri du chat syndrome. In the absence of such information, the rearrangement of 5p is not useful in terms of defining the critical region for cri du chat. We are presently trying to obtain samples from individuals with the smallest possible terminal deletions of 5p who clearly do have cri du chat in order to narrow the definition of the critical region at the DNA level.

ACKNOWLEDGMENTS

The excellent technical assistance of Ulla Bengtsson is gratefully acknowledged. We thank Dr. Ladonna Immken, University of Texas Medical School at Houston; Dr. B. A. Smith and Ms. J. L. Walker, Center for Human Genetics and Northern General Hospital, Sheffield, England; Dr. Ellen Magenis, Oregon Health Sciences University; Dr. Eric Niebuhr, University of Copenhagen, Denmark; Dr. David Ledbetter, Baylor College of Medicine; and G. DuMars, University of California, Irvine Medical Center, for their assistance in obtaining and identifying blood samples from individuals with rearrangements of 5p.

REFERENCES

1. NIEBUHR E: The cri du chat syndrome, epidemiology, cytogenetics, and clinical features. *Hum Genet* 44:227–275, 1978

2. NIEBUHR E: Cytologic observations in 35 individuals with a 5p- karyotype. *Hum Genet* 42:142–156, 1978
3. OVERHAUSER J, GOLBUS MS, SCHONBERG SA, WASMUTH JJ: Molecular analysis of an unbalanced deletion of the short arm of chromosome 5 that produces no phenotype. *Am J Hum Genet* 39:1–10, 1986
4. WALKER JL, BLANK CE, SMITH BAM: Interstitial deletion of the short arm of chromosome 5 in a mother and three children. *J Med Genet* 21:465–467, 1984
5. BACCICHETTI C: Del(5p) without "cri du chat" phenotype. *Hum Genet* 60:389, 1982
6. 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
7. DANA S, WASMUTH JJ: Linkage of the *leuS, emtB* and *chr* genes on chromosome 5 in humans and expression of human genes encoding protein synthetic components in human-Chinese hamster hybrids. *Somat Cell Genet* 8:245–264, 1982.
8. DANA S, WASMUTH JJ: Selective linkage disruption in human-Chinese hamster cell hybrids: deletion mapping of the *leuS, hexB, emtB,* and *chr* genes on human chromosome 5. *Mol Cell Biol* 2:1220–1228, 1982
9. WASMUTH JJ, CARLOCK LR, SMITH B, IMMKEN L: A cell hybrid and recombinant DNA library that facilitate identification of polymorphic loci in the vicinity of the Huntington disease gene. *Am J Hum Genet* 39:397–403, 1986
10. FEINBERG AP, VOGELSTEIN B: A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal Biochem* 132:6–13, 1983
11. FEINBERG AP, VOGELSTEIN B. Addendum: A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal Biochem* 137:266–267, 1984
12. SEALEY PG, WHITTAKER PA, SOUTHERN EA: Removal of repeated sequences from hybridization probes. *Nucleic Acid Res* 13:1905–1922, 1985

CALL FOR PROPOSALS FOR SYMPOSIA ON HUMAN GENETICS FOR THE 1988 MEETING OF THE AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE. Symposia at the annual meeting of the American Association for the Advancement of Science are selected primarily from among suggestions submitted by individual scientists. Suggestions made through the various member organizations and sections of the AAAS and supported by them have the best chance of being accepted. Suggested symposia for the 1988 annual meeting, to be held in Boston, Massachusetts, February 11–16, 1988, will be discussed at the 1987 annual meeting to be held on February 14–19, 1987. Anyone interested in organizing a symposium on a topic in the broad area of human genetics should contact: Vincent M. Riccardi, the representative to the AAAS for the American Society of Human Genetics, for additional details. Since the annual AAAS meeting receives very broad national and international press coverage, these symposia represent an excellent opportunity for wide communication of recent scientific developments. For additional information, write to: Vincent M. Riccardi, M.D., Baylor NF Program, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030.