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Genetics

Human J chain gene: Chromosomal localization and associated restriction fragment length polymorphisms

(immunoglobulin/*in situ* hybridization/somatic cell hybrids/tandem repeats/chromosome 4)

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ABSTRACT **Gene clones encoding the human J chain, the protein that links immunoglobulin monomers, were recently described. Using probes from J chain clones we have now investigated the chromosomal location of this gene by analysis of somatic cell hybrids and by *in situ* chromosome hybridization. The gene is located on the long arm of chromosome 4 in band q21, the chromosomal band in which a consistent translocation with chromosome 11 has been observed in some acute leukemias. An additional human sequence that cross-hybridizes with some J chain gene probes is located on a different chromosome. Restriction fragment length polymorphisms deriving from length variations in a tandemly repeated region 5′ to the J chain gene were detected; these should facilitate the analysis of genetic linkage between this gene and other markers on chromosome 4.**

J chain is a 137-amino acid protein that is synthesized in B lymphocytes and serves two known functions: linking immunoglobulin monomers (IgM to pentamers, IgA to dimers) and binding these immunoglobulins to secretory component (1). J chain shows no evidence of primary sequence homology to the immunoglobulin superfamily, though it may resemble immunoglobulins in its three-dimensional structure (2) and in the regulation of its expression (3). Southern blot analysis previously demonstrated that the human genome contains two J chain-related sequences: the functional gene and another related sequence that cross-hybridizes to some, but not all, J chain probes (4). We have now analyzed the chromosomal locus of the J chain gene, in part to learn the relationship between these two cross-hybridizing sequences. The location was determined by Southern blot analysis of somatic cell hybrids between human and rodent cells and by *in situ* chromosomal hybridization.

Restriction fragment length polymorphisms (RFLPs) associated with the J chain gene have potential utility for studies correlating the physical position of this gene on its chromosome with data on genetic linkage to other markers on the same chromosome. We investigated the possibility of polymorphisms due to variation in the number of copies of a tandemly repeated DNA segment located 5′ of the J chain gene (unpublished data); such polymorphisms were found and were shown to be inherited in a Mendelian fashion.

MATERIALS AND METHODS

Chromosomal Assignment by Somatic Cell Hybrids. The human–rodent somatic hybrids used to obtain a chromosomal assignment for the J chain gene were from a set of previously described fusion lines (5, 6). The human chromosomes retained in these lines were characterized primarily by electrophoretic analysis of isozyme markers derived from genes of known chromosomal origin as well as by Southern blot hybridization with probes from previously localized genes. For the specific case of chromosome 4, hybrids were scored positive if they demonstrated either human phosphoglucomutase 2 activity or the human α-fetoprotein gene, both markers for chromosome 4. DNA samples isolated from the hybrid lines by the method of Polsky (7) were digested with *Eco*RI and analyzed by Southern blotting (8) using a 0.6-kilobase (kb) *Pvu* II to *Xba* I hybridization probe corresponding to exon 4 of the human J chain gene (4).

Gene Mapping by *in Situ* Chromosome Hybridization. Human metaphase chromosome spreads were prepared and hybridized with a J chain gene probe using previously described techniques (9). The probe was a plasmid containing a 1.6-kb *Xba* I fragment of the human J chain gene including most of exons 3 and 4 (4). The plasmid was radiolabeled with ^3H to a specific activity of 1.5×10^7 cpm/μg by nick-translation as described (10). After autoradiography, development, and fixation, the slides were stained in a 0.005% quinacrine mustard dihydrochloride solution in McIlvaine's buffer (pH 5.4) (11). The chromosome spreads were observed using a combination of incident ultraviolet and transmitted visible light that permitted data collection to be performed at the microscope. A standard ideogram of human chromosomes at the 400-band stage (12) was used to record the position of silver grains.

RFLPs. DNA for analysis of RFLPs was obtained from lymphoblastoid lines derived from eight families that have been used in our laboratory for molecular genotyping analyses of other genes (13–15). Restriction endonuclease digestions were performed using precautions to prevent incomplete digestion (15). Routine screening of single enzyme digests was done by the standard Southern technique of capillary blotting from agarose gels onto nitrocellulose (8). For analysis of small DNA fragments generated by double enzyme digestions with *Eco*RI plus *Ava* II, electrophoresis was performed in 5% acrylamide gels and the DNA was electroblotted onto Genescreen*Plus* (New England Nuclear) using protocols supplied with that product, with minor modifications. These electroblots were probed with a 334-base-pair (bp) *Alu* I to *Ava* II fragment 5′ of the human J chain gene radiolabeled by nick-translation; conditions of prehybridization, hybridization, and washing were as described in the directions for Genescreen*Plus*.

RESULTS

The chromosomal location of the human J chain gene was investigated by a combination of somatic hybrid analysis and *in situ* hybridization to chromosome spreads. For the somatic cell hybrid analysis a total of 32 human–mouse and 31 hu-

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Abbreviations: kb, kilobase(s); RFLP, restriction fragment length polymorphism; bp, base pair(s).

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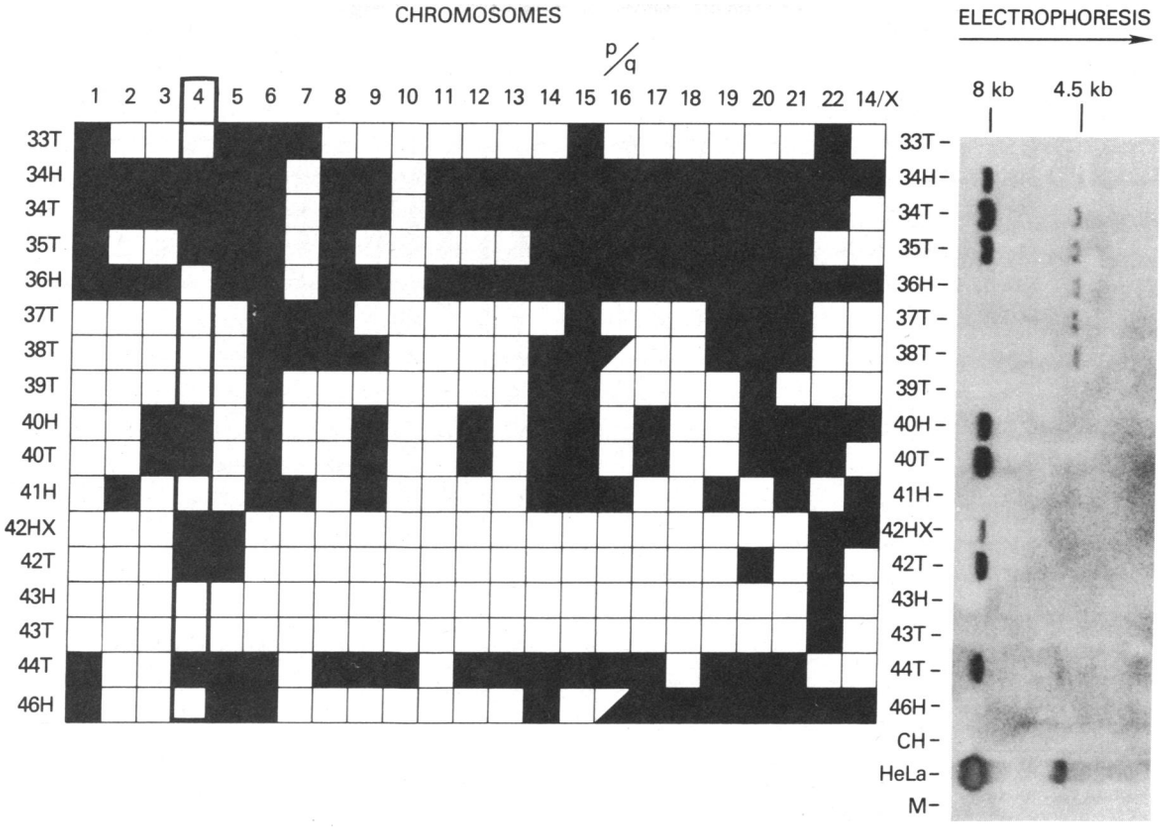


FIG. 1. Assignment of human J chain gene to chromosome 4. Data on 17 of the 63 human–rodent somatic hybrid lines examined in the present study are shown. The chromosomal content of the hybrids is indicated by the checkerboard figure at the left (black indicates presence of the chromosome; the p and q arms of chromosome 16 are indicated separately for two hybrids with fragments of this chromosome). A Southern blot of *Eco*RI-digested DNA from the same somatic hybrids—as well as from Chinese hamster (CH) and HeLa cells—is shown at the right. Fragment sizes were estimated from markers in lane M. The cells demonstrating the 8-kb band are congruent with those containing chromosome 4.

man–hamster hybrid lines, all previously characterized as to their content of human chromosomes, were tested for the presence of the human J chain gene by Southern blot analysis of extracted DNA. The analysis of a typical hybrid set is shown in Fig. 1. These hybrids were constructed from a fusion of Chinese hamster fibroblasts with a human cell line containing a 14/X translocated chromosome (6). The Southern blot on the right of Fig. 1 shows that under the conditions of this experiment the human probe did not hybridize with Chinese hamster DNA (lane CH at bottom of Fig. 1). The lane with human DNA (derived from HeLa cells) demonstrates the two J chain-related sequences known to exist in the human genome on the basis of earlier studies from our laboratory (4); the 8-kb *Eco*RI fragment contains the functional J chain gene and the 4.5-kb fragment contains a related sequence that cross-hybridizes with certain J chain gene probes (4). In the 17 hybrids examined in Fig. 1, the presence of the 8-kb *Eco*RI band correlates uniquely with the retention of chromosome 4. Additional hybrids were also examined (data not shown), yielding concordance data shown in Table 1. These data firmly establish the chromosomal assignment of the functional human J chain gene to chromosome 4. Although the data of Fig. 1 suggest the assignment of the 4.5-kb *Eco*RI fragment to chromosome 8, weakness of hybridization of this band with the J chain probe prevented unambiguous chromosomal assignment of this sequence. However, it is clear that the 4.5-kb *Eco*RI fragment segregates independently from the 8-kb fragment in the hybrids and is therefore not derived from chromosome 4.

To localize the J chain gene to a particular band on chromosome 4, we used the technique of *in situ* hybridization, probing human metaphase chromosomes with a ^{3}H-labeled plasmid containing a fragment of the cloned J chain gene. Fig. 2 *Left* illustrates an example of such a spread demonstrating a silver grain over the q21 band of chromosome 4. In analyses of 106 metaphase preparations, approximately 17% were found to have a silver grain either on or directly beside the q21 band of at least one chromosome 4. Of the 484 grains observed on or beside chromosomes, 22 (4.5%) were localized at band q21. These data are summarized in Fig. 2 *Right* and establish the chromosomal location of the J chain gene to chromosome 4, band q21. A secondary peak of silver grains at band 13q14 is currently unexplained; the somatic hybrid data argue against its identification with either the functional gene (discordancy = 46%, Table 1) or the sequence represented by the 4.5-kb *Eco*RI fragment (discordancy = 40%). Other examples of secondary peaks of unknown origin have been reported in *in situ* chromosome hybridization analyses (9); the existence of such peaks reinforces the usefulness of combining data from somatic hybrids and *in situ* chromosome studies to obtain the best genetic mapping assignments.

To facilitate the analysis of genetic linkage of the human J chain gene to other markers on chromosome 4, RFLPs were sought. An initial screen of DNAs from several unrelated individuals using several single enzyme digests revealed only a single polymorphism in one individual for the enzyme *Msp* I. This polymorphism was shown to be inherited in the children of this individual as expected for a Mendelian trait (data not shown); but because of its rarity, this polymorphism may be of limited utility in linkage studies. Previous sequence analysis of the J chain gene had revealed the existence of a segment of tandem repeats of a 27-bp sequence 5′ to the gene (unpublished data). Since similar tandem repeats in other

Table 1. Segregation of J protein gene in rodent–human hybrids

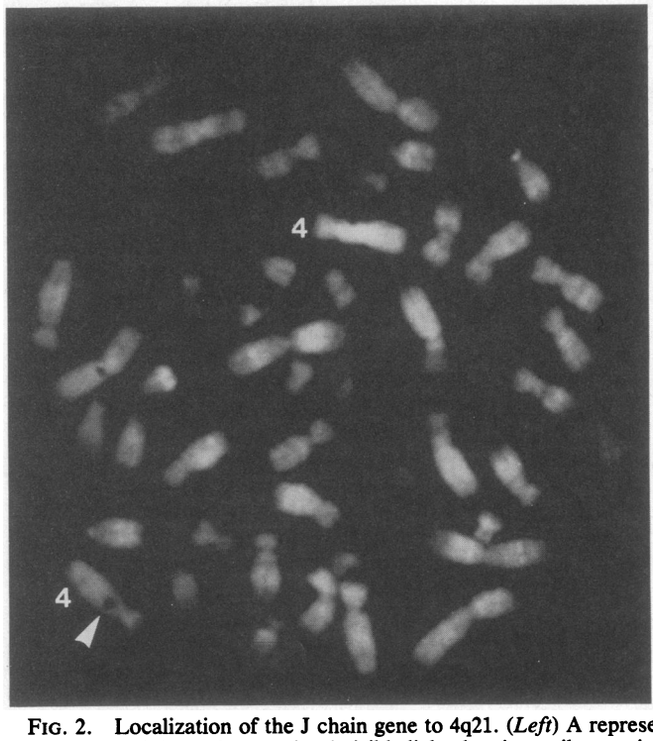
Human chromosome	% discordancy		
	Hamster–human hybrids	Mouse–human hybrids	Total hybrids
1	35	45, 47	40, 41
2	48, 42	28, 22	38, 32
3	23	25	24
4	0	3	<2
5	39	53	46
6	39, 35	53, 69	46, 52
7	58	59	59
8	35	57	46
9	42	45	43
10	26	45	35
11	26, 32	38	32, 35
12	23	47, 56	35, 40
13	35	56	46
14	26	66	46
15	61	56	59
16	26, 32	69, 63	48, 48
17	35	41	38
18	45	38	41
19	45	41	43
20	35	31	33
21	35	47	41
22	32	45	38
X	42	45	43

In this analysis a hybrid line is considered discordant for the indicated human chromosome if it contains the chromosome but not the human J chain gene (represented by the 8-kb *Eco*RI band) or vice versa. When two numbers are given, they refer to discordance with short arm and long arm markers, respectively. Discordancy of a single cell line for chromosome 4 may be due to chromosome breakage, with retention of only a fragment in the cell line. The human–hamster hybrids consisted of 24 primary clones and 7 subclones; 15 of these 31 lines were positive for the J chain gene. The human–mouse hybrids consisted of 15 primary clones and 17 subclones; 20 of these 32 lines were positive for the gene.

genes have been found to produce polymorphisms as a result of expansion or contraction in the number of repeats (16–18), we screened for 27-bp length variations in the tandem repeat region. Genomic DNAs were digested with *Eco*RI plus *Ava* II, two enzymes with recognition sites that closely flank the tandem repeats and that together yield a fragment of 451 bp in the cloned J chain gene (see map at bottom of Fig. 3). As size markers, plasmid DNA containing this region was cut with the same two enzymes and was mixed with DNA cut with *Eco*RI plus *Dra* I; the latter digest yields a 394-bp fragment, close to the 397-bp size predicted for an *Eco*RI–*Ava* II fragment lacking two of the 27-bp repeats. The top panel of Fig. 3 shows a Southern blot of DNAs from 15 unrelated individuals (F = father, M = mother) from eight families previously studied in our laboratory. (Individual S5 represents one of the siblings in family 5, for which the father is unavailable.) Of these 15 unrelated individuals, 4 demonstrate variant restriction fragments, which apparently derive from at least three different alleles. Compared to the size of the most frequent allele, individual 2F shows a band of the size expected for "+1" extra repeats, whereas 5M and 1AF show a "−1" band and 8M shows a "−3" band. To investigate the heritability of these polymorphisms, all of the members of family 1A and family 8 were studied; the lower panel of Fig. 3 clearly demonstrates that the polymorphic bands were propagated to the next generation.

DISCUSSION

The present investigations have localized the human J chain gene to chromosome 4, band q21. This position is of particular interest because it is a consistent site of translocations with chromosome 11 in some acute lymphocytic leukemias (ALL) (19–21). In one series (21) t(4;11) translocations occurred in 18 of 218 ALL patients with karyotypic abnormalities and always involved the 4q21 band. Translocations in a variety of malignancies have been associated with chromosomal breakage near genes that are highly and spe-



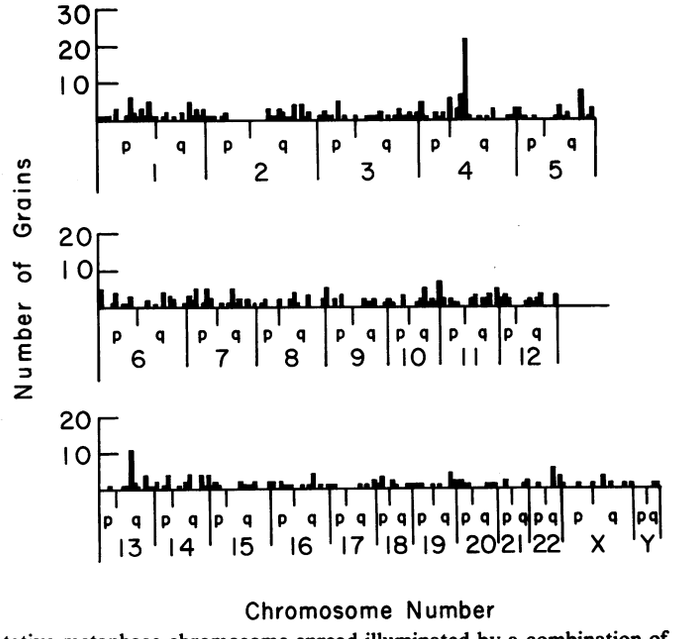


FIG. 2. Localization of the J chain gene to 4q21. (*Left*) A representative metaphase chromosome spread illuminated by a combination of incident ultraviolet and transmitted visible light showing a silver grain at 4q21. (*Right*) Histogram showing the distribution of silver grains from 106 metaphase spreads hybridized with the J chain probe. A peak of silver grains was observed at 4q21. (The smaller secondary peak seen at 13q14 is unexplained; see text.)

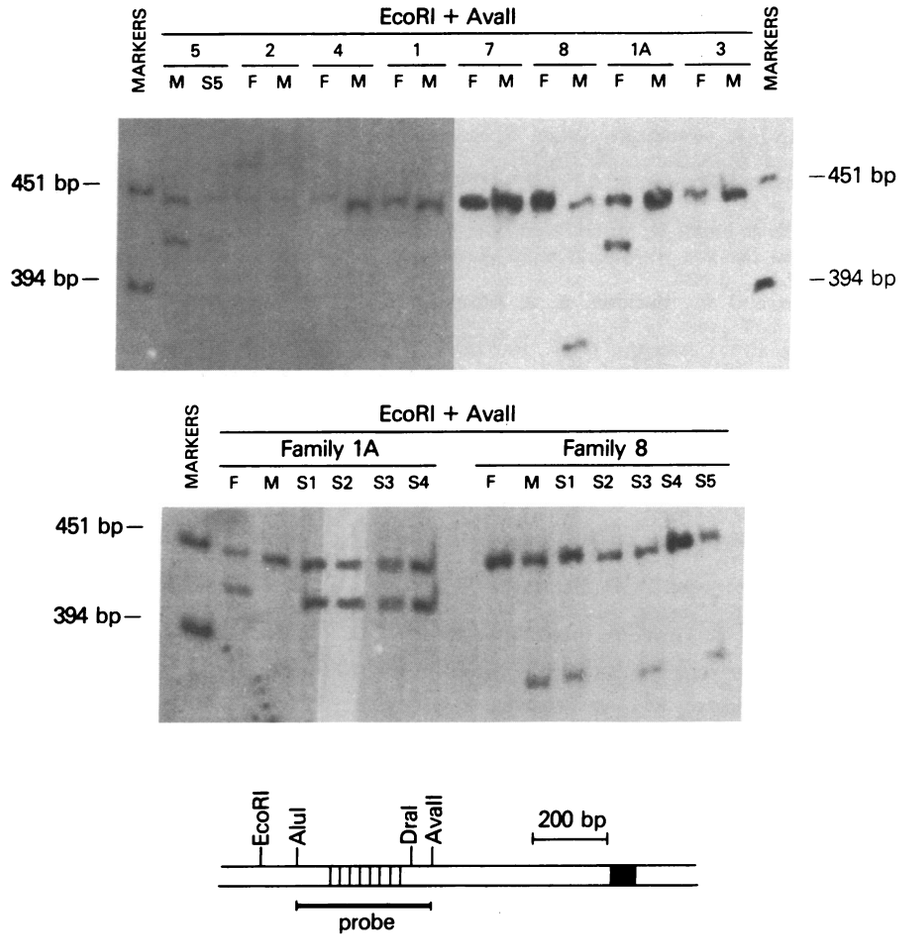


FIG. 3. J chain gene RFLPs. The top Southern blot panel represents DNA from 16 individuals, 2 from each of eight families, digested with *Eco*RI plus *Ava* II and hybridized with a probe for the tandem repeats 5′ of the human J chain gene, as shown in the map below. In the map the black box represents the first exon of the gene; the seven white rectangles represent the seven exact repeats. The markers are *Eco*RI–*Dra* I and *Eco*RI–*Ava* II fragments of the cloned gene (see map). The lower Southern blot represents a similar analysis of all of the members of two families selected because one of the parents demonstrated a variant band.

cifically expressed in the cell type undergoing transformation (22)—e.g., immunoglobulin genes in malignancies of B-cell lineage (23–25) or T-cell receptor genes in malignancies of T-cell lineage (26, 27). It is tempting to speculate that the J chain gene, which is highly expressed in most B cells (4), may be near the site of chromosomal breakage in leukemias associated with the 4;11 translocations at q21.

The murine J chain gene has been assigned to mouse chromosome 5. This chromosome also includes four other genes whose human homologs have been mapped near the centromere of human chromosome 4 (28, 29), although exact subband localizations are not available. The human genes are phosphoglucomutase 2 (4p14-4q12), peptidase S (4p11-4q12), albumin (4q11-4q13), and α-fetoprotein (4q11-4q13). These four genes, plus the newly mapped J chain gene, represent a conserved linkage group between human chromosome 4 and mouse chromosome 5.

The RFLPs that were discovered near the J chain gene are consistent with (though not definitive proof for) genetic variation in the number of repeats of a 27-bp sequence that is tandemly reduplicated 5′ of the human J chain gene. In our cloned gene, which apparently represents the most frequent allele, we find seven exact repeats of this sequence, flanked on each side by one or two less exact repeats (unpublished data). The function, if any, of these repeats is unknown. The experiments presented here (and other data not shown) indicate that this tandem repeat sequence is not found elsewhere in the human genome. Other tandemly repeated sequences have been found frequently in the genome (16–18); some appear to play a role in the regulation of expression of associated genes (30–34). The variation often seen in the number of repeats is thought to arise from "slipped mispairing" or other mechanisms based on homologous recombination between repeated elements. Regardless of the function of this region or the mechanism of its variation, the polymorphisms we have discovered should prove useful in genetic linkage studies in a region of chromosome 4 heretofore relatively barren of markers definitively localized to a particular subband (29).

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