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Localization of human immunoglobulin κ light chain variable region genes to the short arm of chromosome 2 by *in situ* hybridization

(human κ genes/gene mapping/chromosome translocation/Burkitt lymphoma)

S. MALCOLM*, P. BARTON*, C. MURPHY†, M. A. FERGUSON-SMITH†, D. L. BENTLEY‡, AND T. H. RABBITTS‡

*Department of Biochemistry, Queen Elizabeth College, Campden Hill Road, London W8, England; †Institute of Medical Genetics, Royal Hospital for Sick Children, Yorkhill, Glasgow, Scotland; and ‡Laboratory of Molecular Biology, The MRC Centre, Hills Road, Cambridge CB2 2QH, England

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ABSTRACT **The genes for human immunoglobulin κ light chains have been localized in normal lymphocyte and fibroblast chromosomes by *in situ* hybridization of probes from cloned DNA fragments of the κ variable region locus. The localization was achieved by counting grains (after autoradiography) over chromosomes in a number of karyotypes. The variable region gene probes hybridized in a cluster on a region of the chromosome 2 short arm close to the centromere (2cen→p12). This location was confirmed in lymphocytes from a balanced translocation carrier 46XXt (2; 16) (q13; q22). Our results show that human κ light chain genes are located in the region of the break point observed in specific chromosomal translocations associated with Burkitt lymphoma.**

The three sets of unlinked genes code for antibody proteins: the heavy chain, the κ light chain, and the λ light chain genes. Attempts to map these genes in man have been hindered by the lack of variants suitable for family studies and the loss of immunoglobulin expression in somatic cell hybrids. Despite these problems, chromosome assignments for some human immunoglobulin genes have been made by using panels of mouse–human hybrid cell lines with different human chromosome complements. Correlation between immunoglobulin expression and the presence of specific human chromosomes in hybrids of mouse myeloma cells and human B cells has led to assignment of λ light chains to chromosome 22 (1) and of heavy chains to chromosome 14 (2). Human heavy chain genes have also been assigned to chromosome 14 on the basis of filter hybridizations between cloned heavy chain genes and DNA from a panel of mouse–human hybrid cell lines with different human chromosome complements (3).

A completely different technique—with the advantage of using normal cells rather than a panel of hybrid cell lines—is that of *in situ* hybridization to fixed metaphase chromosomes. This technique previously has been used to confirm the location of human α- and β-globin genes to their respective chromosomes (4, 5). In this paper we describe the localization of human κ light chain genes by *in situ* hybridization. Two cloned genomic DNA fragments, each containing a single κ variable region (Vκ) gene, were used as hybridization probes. The results of the *in situ* hybridization with these Vκ probes localize the κ chain genes to chromosome 2 in man.

MATERIALS AND METHODS

Chromosome Preparation. Translocation carrier and normal human metaphase chromosome spreads prepared from phytohemagglutinin-stimulated peripheral blood cultures (6) were banded by mild treatment with 1% lipsol detergent (LIP Equipment and Services, Shipley, Yorkshire, United Kingdom), stained with Giemsa, and photographed. Chromosomes were prepared from fibroblast cultures as described by de Grouchy *et al.* (7) except that the culturing medium used contained Harris F-10 medium with 10% newborn calf serum (GIBCO, Paisley, Scotland), 10% fetal calf serum (GIBCO), penicillin (Glaxo, Middlesex, United Kingdom; 1,000 units/ml), and streptomycin (Glaxo; 25 mg/ml).

***In Situ* Hybridization.** Prior to hybridization, chromosomes were denatured in 60% (vol/vol) formamide/0.1 mM EDTA/5 mM Hepes, pH 7, at 55°C for 15 min, washed in 0.30 M NaCl/0.03 M sodium citrate, and dehydrated through a series of alcohols. The [^{3}H]cRNA used as a probe was prepared as described (5) under conditions that gave random initiation and produced equal transcription of vector and inserted DNA. [^{3}H]cRNA (specific activity, 1.7×10^8 dpm/μg) in 50% formamide/0.6 M NaCl/5 mM Hepes, pH 7.6/0.1 mM EDTA was hybridized to denatured chromosomes at 43°C for 20 hr (approximately 20 ng of cDNA in 5 μl per slide). Unhybridized cRNA was removed by mild RNase treatment and extensive washes in 0.30 M NaCl/0.03 M sodium citrate; the slides were then dipped in Ilford (Essex, United Kingdom) K2 dipping emulsion and exposed for 23–26 days. Previously photographed spreads were relocated and scored for silver grains occurring over chromosomes. The method of prehybridization banding allows unequivocal identification of chromosomes, which is otherwise hampered by changes in morphology during denaturation, and also prevents observer bias in choice of spreads.

Alternatively, HK101 was labeled by nick-translation with ^{125}I-labeled dCTP to a specific activity of about 5×10^8 cpm/μg. This material was hybridized to metaphase chromosomes by using 50 ng of heat-denatured ^{125}I-labeled HK101 per slide in 20 μl as above except for the addition of 10% dextran sulfate. Exposure time was 6 days.

RESULTS

Characteristics of Cloned Hybridization Probes. Two fragments of human fetal liver DNA, cloned in λ phage Charon 4A (8), were used for our experiments. Clone HK101 contains a single Vκ gene of subgroup 1 in 18.6 kilobases of chromosomal DNA and has been fully described elsewhere (9, 10). Clone HK122 was isolated from the same library of fetal liver DNA as HK101 and contains a 10.5-kilobase-long insert including a single Vκ gene also of subgroup 1. These two Vκ genes are about 87% homologous in their coding sequences (unpublished data). No highly repeated sequences were detected in either HK101

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Abbreviation: Vκ, κ variable region.

or HK122 by hybridization with nick-translated total human DNA (11). Cloned segments of flanking region sequences from HK101 exhibit a low copy number in human DNA when hybridized under conditions of high stringency (10). Clones HK101 and HK122 do not cross-hybridize to any significant degree except within the Vκ coding segments themselves.

Hybridization of HK101 Probe to Normal Stimulated Lymphocyte Preparations. [^{3}H]cRNA made from clone HK101 was hybridized to metaphase chromosome spreads from peripheral blood lymphocytes. A total of 106 prephotographed spreads from seven slides were analyzed for the presence of silver grains in the region of the individual chromosomes. Data from each slide were treated separately and from all slides as a whole. The grain distribution over the chromosomes was analyzed in two ways. First, the number of grains per unit length for each chromosome was calculated by dividing the total number of grains found on each chromosome by the percentage of the genome contained in that chromosome. This procedure was adopted to allow for the difference in background found on different length chromosomes. Chromosome 2 was more heavily labeled than any other chromosome, with a grains/unit length value of 93.5 compared to a median of 68.4 and falling 2.75 SD away from the median. Because this method of calculation will produce a bias against the longer chromosomes (because the site-specific grains will be averaged over the whole chromosome), a second method of analysis was used.

In the second method the number of grains expected on each chromosome, if all grains were randomly distributed along equal lengths of the genome, was calculated and the deviation from this random value was calculated (Fig. 1*A*). The excess

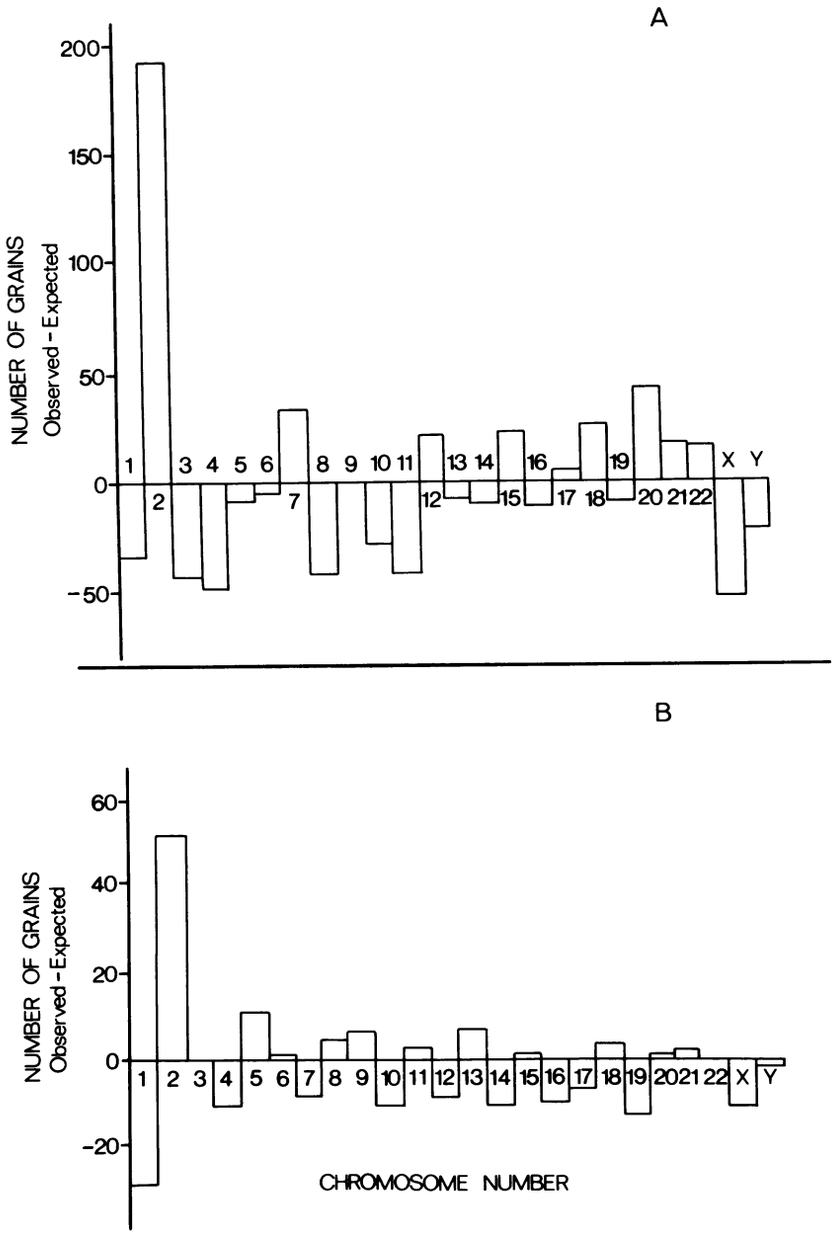

FIG. 1. *In situ* hybridization of HK101 [^{3}H]cRNA to human chromosomes: distribution of grains over the genome. (*A*) Hybridization to lymphocyte chromosomes from 106 karyotyped cells hybridized with HK101 [^{3}H]cRNA (18 ng/5 μl; 1.67×10^8 dpm/μg). Autoradiographs were exposed for 23 days. Expected values are derived from a proportional distribution of the 6,999 grains counted according to chromosome lengths. (*B*) Hybridization to fibroblast chromosomes from nine karyotyped cells hybridized with HK101 [^{3}H]cRNA (21 ng/5 μl; 1.6×10^8 dpm/μg). Autoradiograph was exposed for 26 days. Expected values are calculated from a proportional distribution of the 1,525 grains counted.

grains on chromosome 2 are extremely striking (+194) and fall outside (3.94 SD from the mean) the range of deviation, positive and negative, found on the other chromosomes. *In situ* hybridization of human peripheral blood lymphocytes was also carried out with iodinated HK101 labeled by nick-translation using ^{125}I-labeled dCTP (12). It is clear from the karyotype analysis (Fig. 2) that both chromosomes 2 show a localization of silver grains whereas no other pairs of chromosomes display this consistent labeling. In four other pairs of chromosome 2 (Fig. 2 *Lower*), all members show labeling at the same approximate position. Therefore, the results obtained with [^{3}H]cRNA and ^{125}I-labeled DNA were identical in demonstrating hybridization of the human κ light chain probes to chromosome 2 in lymphocytes.

Hybridization of HK101 to Metaphase Chromosomes Prepared from Fibroblast Cell Cultures and a Cell Line with a Balanced Translocation of Chromosome 2. Although we used phytohemagglutinin-stimulated lymphocytes [consisting mainly of T cells which generally show no rearrangement of their κ genes (13)], we controlled against any possible rearrangement of the immunoglobulin genes in the stimulated lymphocytes by *in situ* hybridization of [^{3}H]cRNA probes to cultured fibroblasts. Nine spreads from two slides were analyzed. The chromosomal distribution of grains, shown in Fig. 1*B*, indicates that chromosome 2 was significantly labeled, on the basis of grains per unit length, compared with the other chromosomes. Therefore, these data show that the κ light chain probe hybridizes to chromosome 2 in fibroblasts as it did in peripheral blood lymphocytes.

As a further check on the specificity of hybridization and to define the position of the gene with respect to a cytogenetically defined break point, we carried out *in situ* hybridization of HK101 [^{3}H]cRNA to chromosomes from a carrier of a balanced translocation between chromosomes 2 and 16 [46XXt (2; 16) (q13; q22)]. The break point in chromosome 2 is at q13—i.e., in the long arm just below the centromere. Cells from this individual contain one copy each of the normal chromosomes 2 and 16, an abnormal 2/16 (2pter→2q13; 16q22→16qter), and an abnormal 16/2 chromosome (16pter→16q22; q13→2qter). Results of an analysis of grain distribution obtained from 22 karyotypes are shown in Fig. 3. Both the normal 2 and the abnormal 2/16 chromosomes show significant hybridization with the κ probe, thus confirming the location of the Vκ gene to chromosome 2 and locating the position above the break point q13.

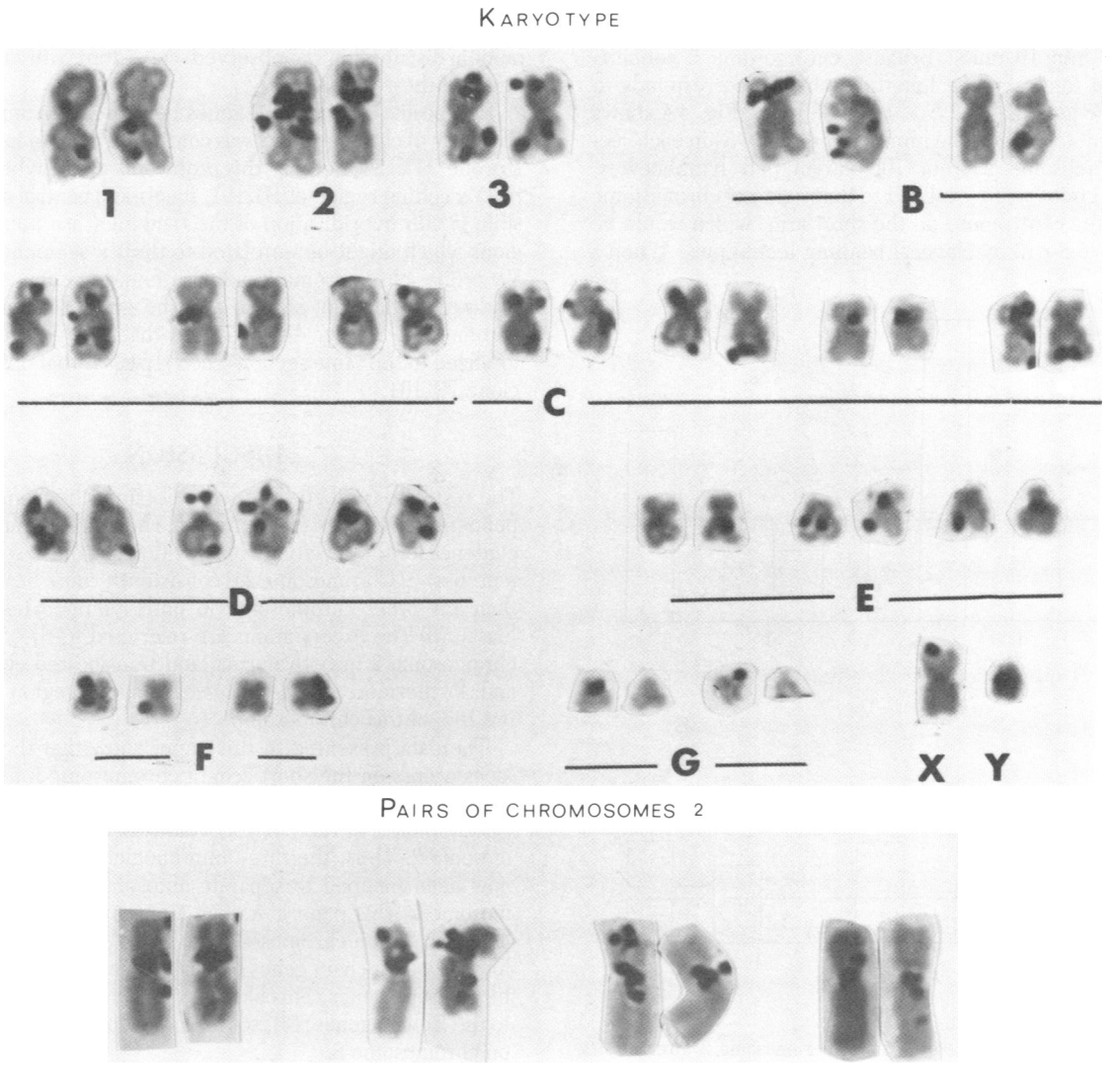

FIG. 2. *In situ* hybridization of ^{125}I-labeled HK101 to human chromosomes. Chromosome spreads from blood lymphocytes were hybridized with HK101 ^{125}I-labeled by nick-translation (12): 50 ng of heat-denatured ^{125}I-labeled HK101 DNA hybridized in 20 μl for 20 hr. Autoradiography was for 6 days. (*Upper*) Karyotype of lymphocyte chromosomes with associated grains after hybridization. (*Lower*) Pairs of chromosomes 2 taken from four other hybridized chromosome spreads.

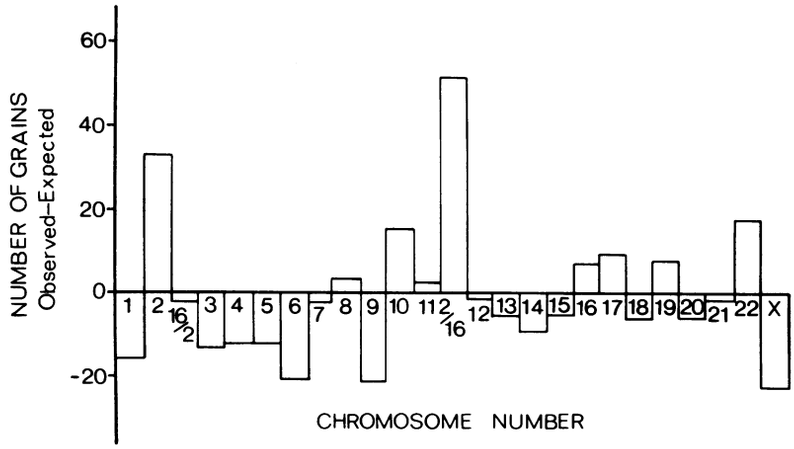

FIG. 3. *In situ* hybridization of HK101 [^{3}H]cRNA (18 ng/5 μl; 1.67 $\times$ 10^8 dpm/μg) to translocated (2; 16) chromosomes: distribution of grains over each chromosome from 22 karyotyped cells. Autoradiographs were exposed for 26 days. Expected grain values are derived from a proportional distribution of the total grain count according to chromosome length and number of chromosomes counted.

Location of κ Light Chain Genes Within Chromosome 2. In order to locate the κ gene locus more precisely, each photograph (data from Fig. 1*A*) of chromosome 2 (arm ratio q:p, 1.57) was divided into 10 units. Because chromosome 2 contains 7.76% of the total genome length, each unit corresponds to 0.776% of the genome or 2.3 $\times$ 10^7 base pairs. Fig. 4*A* shows the result of determining the number of grains within each section of chromosome 2 from 106 karyotyped lymphocytes. Clearly, the grains were localized to the region of chromosome 2 closest to the centromere on the short arm, which would be defined as 2cen→p12 by classical banding techniques. When a similar analysis of the grains over the same number of chromosomes 7 or chromosomes 20 was carried out [both of which showed small positive values in grain distribution (Fig. 1)], a random distribution was observed, consistent with a nonspecific origin of these grains.

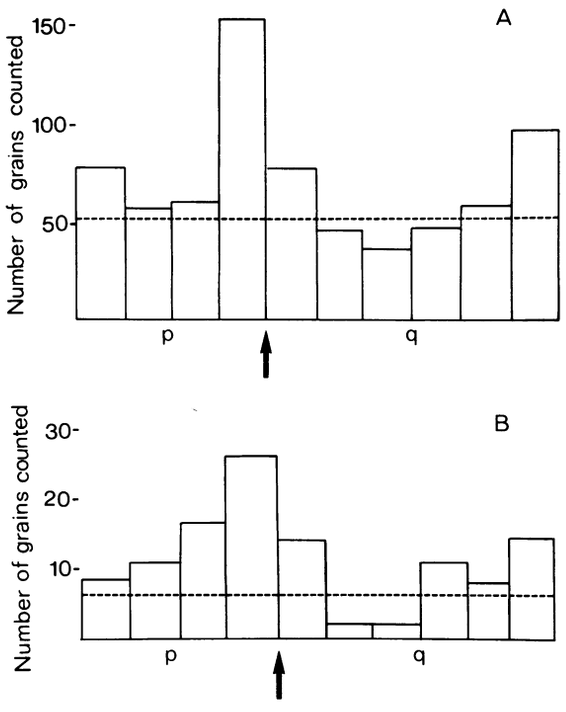

FIG. 4. Distribution of grains along chromosome 2 after *in situ* hybridization of [^{3}H]cRNA transcribed from Vκ probes. p and q, short and long arms; vertical arrow, position of centromere; broken line, number of grains expected from a proportional distribution of the total number of grains on all chromosomes. (*A*) Distribution after hybridization to HK101 (data from Fig. 1*A*). (*B*) Distribution after hybridization to HK122 (data from 18 karyotypes).

The localization of the κ genes near the centromere on the short arm of chromosome 2 was confirmed with an independent κ probe, HK122. Because this probe only cross-hybridizes with the Vκ coding region of HK101, it acts as a control against possible *in situ* hybridization of the flanking (i.e., noncoding) regions which might be unrelated to the Vκ segment. With this Vκ probe, chromosome 2 was also found to show specific hybridization (data not shown) and the grain distribution along chromosome 2 (Fig. 4*B*) indicated that the hybridization was localized to the same section (2cen→1p12) as that observed with clone HK101.

DISCUSSION

The results described here were obtained by using two independently derived κ chain probes. There are two main lines of evidence for the specificity of hybridization of these two cloned κ probes. (*i*) Chromosome 2 is consistently more heavily labeled than any other chromosome in both lymphocytes and fibroblasts. (*ii*) The excess grains are restricted to the short arm of chromosome 2 in both normal and translocated chromosomes and, furthermore, they are clustered in one region encompassing 10% of this chromosome.

The data presented in this paper show that the human Vκ locus occurs on the short arm of chromosome 2 near the centromere (2p12). We assume that the κ constant region gene is closely linked to the Vκ genes and therefore also is on chromosome 2. Thus, the three immunoglobulin loci in man have now been mapped to separate autosomes, κ chains on chromosome 2 (this paper), λ chains on chromosome 22 (1), and heavy chains on chromosome 14 (2, 3). A corollary of mapping human κ light chain genes to chromosome 2 concerns the Kidd blood group genes. This blood group locus appears to be linked to the κ chain genes (14), so this locus would also seem to reside on chromosome 2.

The position of the κ chain genes within chromosome 2 is of interest because it is in the same area (2p12) as the specific break point in 8;2 translocations, which have been described in some Burkitt lymphomas (15, 16). Any possible relationship between this specific translocation and the transformation process is ob-

scure at present, but it has also been observed that other Burkitt lymphomas show a translocation of a similar piece of chromosome 8 to either chromosome 14 (which carries the immunoglobulin heavy chain genes) or chromosome 22 (which carries the immunoglobulin λ light chain genes). In connection with the translocations, it is interesting that the immunoglobulin genes undergo extensive DNA rearrangement during the activation of human lymphocytes: this can involve variable region rearrangement to joining segments near the constant regions or switching between heavy chain constant region genes. It is possible, therefore, that these rearrangements facilitate the specific translocations that occur between immunoglobulin-encoding chromosomes and chromosome 8 in the Burkitt lymphomas and that these specific chromosome translocations represent the neoplastic transformation event.

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