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CHROMOSOMAL ASSIGNMENT OF HUMAN GENES FOR GASTRIN, THYROTROPIN (TSH)- β SUBUNIT AND C-erbB-2 BY CHROMOSOME SORTING COMBINED WITH VELOCITY SEDIMENTATION AND SOUTHERN HYBRIDIZATION

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Human genes for gastrin, thyrotropin (TSH)- β subunit and c-erbB-2 were assigned to specific chromosomes using a single-laser cell sorter. For this purpose, condensed human chromosomes prepared from a karyotypically normal lymphoblastoid cell line were preliminarily fractionated by velocity sedimentation, and then sorted using a fluorescence-activated cell sorter. DNA was then extracted from the chromosomes, cleaved by restriction enzymes, and subjected to Southern hybridization using gene-specific radioactive probes. When the assignment of specific chromosomes was not possible due to chromosomal size overlapping, sorted chromosomes from cell lines carrying chromosomal translocation or from hybrid cells carrying known human chromosomes were used in addition. The results indicate that human genes for gastrin, TSH- β , and c-erbB-2 are located on chromosomes 17, 1 and 17, respectively. © 1986 Academic Press, Inc.

Chromosomal gene mapping is becoming increasingly important in basic, as well as applied research in biology and medicine. It is usually done either by in situ hybridization (1) or by discordance analysis using somatic hybrid cell panels (2).

Both techniques present some drawbacks, since in situ hybridization requires time to obtain a decisive conclusion, and the hybrid cell panel method is subject to inconsistent results mostly due to the unstability of human chromosomes. In contrast to these two techniques, analysis using a cell sorter to fractionate chromosomes (3 - 7) seems to be the most powerful method for rapid and correct gene assignments.

However, with a single-laser cell sorter, chromosomes can not be sorted out into individual components. We have overcome this problem by sorting chromosomes from cell lines that carry translocated chromosomes or

Abbreviations: TSH, thyrotropin; NaDodSO₄, sodium dodecylsulfate; bp, base pair.

from human-rodent hybrid cells that carry known human chromosomes. Southern blot analysis using the sorted chromosomes (3, 4), rather than blotting directly the chromosomes (5 - 7) greatly improved the accuracy of the assignments.

MATERIALS AND METHODS

1. Cell lines and chromosome preparation

Two human lymphoblastoid cell lines GM0131: 46, XX and GM3197: 46, XX, t(17;22) (17qter-17p13::22q11-22qter;22pter-22q11::17p13-17pter) (8) were obtained from "The Human Genetic Mutant Cell Repository (Camden, NJ)". These cell lines were grown in RPMI 1640 medium supplemented with 10% fetal calf serum under 5% CO₂/95% air at 37°C.

Human-mouse hybrid cells TA1A and TA3A (9) were kindly provided by Dr. N. Schimizu at Keio University. TA1A carries human chromosomes 1, 4, 7, 13, 15, 18, 19 and TA3A carries human chromosomes 3, 13, 14. These hybrids were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. The cells were then incubated for 16 hrs in the presence of colcemid (0.2µg/ml). Chromosome suspensions were prepared according to the polyamine method of Sillar and Young (10) with some modifications: The cells containing condensed chromosomes were harvested by centrifugation, resuspended in 75mM KCl, and kept on ice for 30 min. They were then collected by centrifugation for 10 min, washed with polyamine buffer (10), resuspended in the same buffer containing 0.1% digitonin, followed by vigorous agitation for 1 min using a Vortex mixer to release chromosomes.

2. Velocity sedimentation and sorting

The chromosome suspensions (50µl) from 5×10^5 mitotic cells were layered over a 16 ml linear sucrose gradient (5 - 30%, w/v) in polyamine buffer, centrifuged using a swing bucket at 900 rpm for 30 min at 4°C, and then fractionated into sixteen 1 ml aliquots.

To each fraction, ethidium bromide (50µg/ml) was added and the stained chromosomes were sorted using a fluorescence-activated cell sorter CHROSS 1 (Japan Spectroscopic Co., LTD.) monitored by the MULTI 16 computer (Mitsubishi Electric, Tokyo). For excitation, a Spectra-Physics model 2025-05 laser at 488 nm/300mW was used. The flow rate of sorting was 1000 - 2000 chromosomes/sec. The preliminary fractionation by sucrose gradient sedimentation facilitated rapid sorting. To obtain meaningful signals in Southern blot analysis of chromosomal DNA, about 4×10^5 sorted chromosomes were used.

3. DNA extraction

The sorted chromosomes were collected by centrifugation at 14 krpm for 5 min. DNA was prepared by incubating the samples for 5 hrs at 37°C in extraction buffer (75mM NaCl, 24mM EDTA, 10mM Tris-HCl, pH 7.5) containing proteinase K (100µg/ml), yeast carrier tRNA (10µg/ml) and 1% NaDodSO₄, followed by phenol extraction and ethanol precipitation (11).

4. DNA probes

The following probes were used: human gastrin, a 260 bp HindIII-HindIII fragment from cDNA (12); human TSH-β, a 210 bp EcoRV-RsaI fragment from genomic DNA (13); human c-erbB-2, a 440 bp KpnI-XbaI fragment from genomic DNA (14).

5. Southern hybridization

The DNA from sorted chromosome was digested with EcoRI (gastrin, TSH-β) or PvuII (c-erbB-2), placed in 2mm slots in a 0.7% agarose gel and

electrophoresed. Blotting onto nitrocellulose filters and hybridization with ^{32}P -labeled DNA probe (specific activity $2 - 5 \times 10^8$ cpm/ μg) were done as described by Southern (15).

RESULTS AND DISCUSSION

To sort chromosomes, metaphase chromosomes in a cell lysate of GM0131 which has a normal karyotype (46, XX) were fractionated by velocity sedimentation through sucrose (7, 16), and each fraction was then subjected to flow sorting. Fig. 1 shows relations between sucrose fractions and histograms. By combining sedimentation with flow sorting, the sorting rate increased by a factor of 5 - 10. Eight chromosome fractions (A to H: see

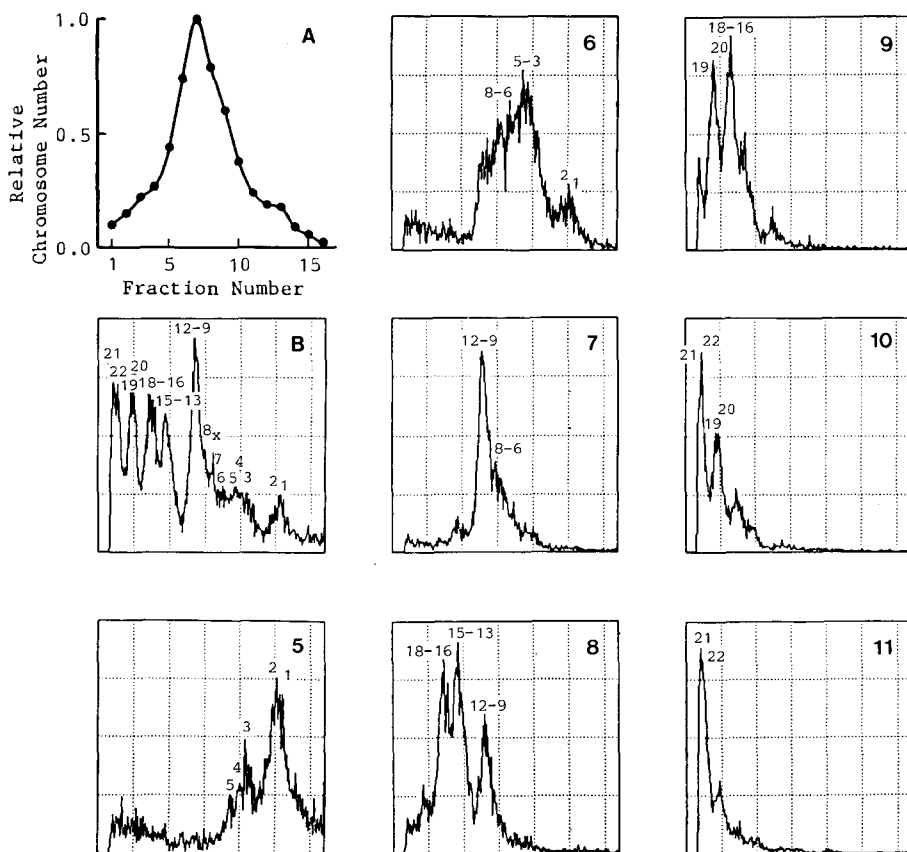


Figure 1. Sedimentation profile (Panel A) and flow histograms of human chromosomes prepared from the cell line GM0131. Chromosomes were preliminarily separated into 7 fractions (5 to 11) by velocity sedimentation through sucrose. Each fraction was mixed with ethidium bromide and analysed by a fluorescence-activated cell sorter. ordinate: relative number of chromosomes; abscissa: relative fluorescence intensity. Panel B is the histogram from an unfractionated sample. Panels 5 - 11 show histograms of the corresponding sucrose fractions. Numbers in the histograms represent the positions of human chromosomes. For example, sucrose fraction 5 is enriched with chromosomes 1, 2 and 3 to 5.

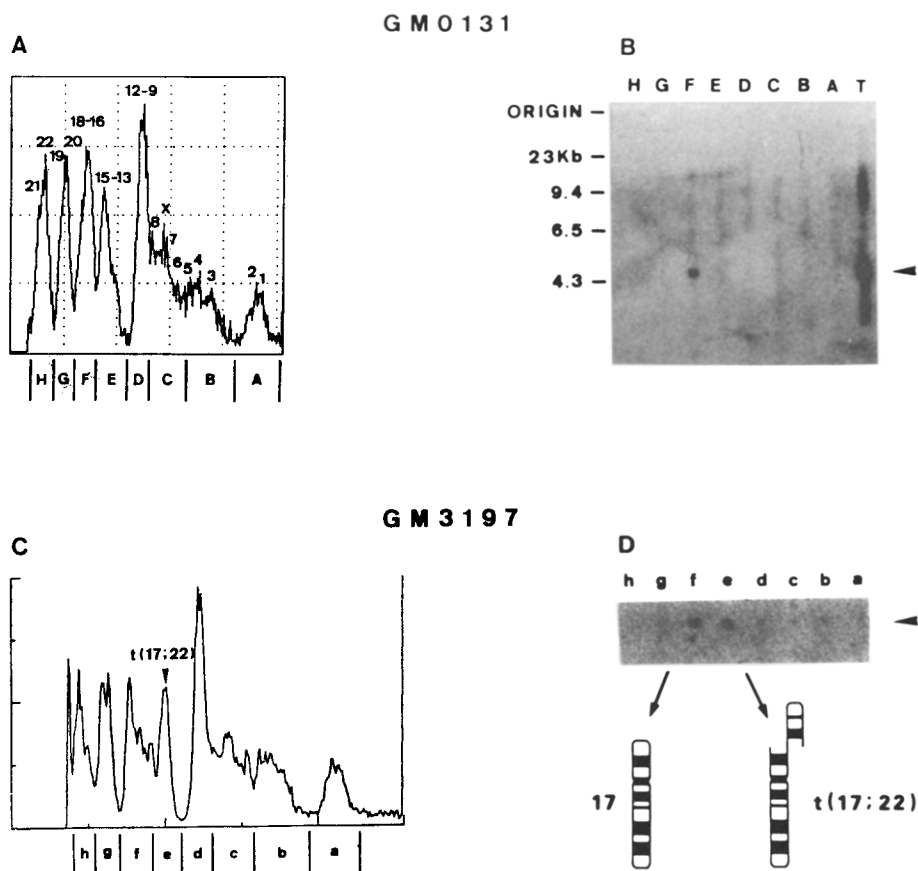


Figure 2. Assignment of human gastrin gene to chromosome 17. (A) Flow histogram of normal human chromosomes from the cell line GM0131. Numbers in the figure represent the positions of human chromosomes. Fractions A to H were separately obtained as described in the text. (B) DNA was prepared from the sorted chromosomes, digested by EcoRI, and subjected to Southern blot analysis using 32 P-labeled human gastrin gene (12) as a probe. Lane T: total human lymphocyte DNA. Lanes A to H; DNA from each sorted chromosome hybridizing to the probe. The arrow indicates the position of the DNA fragment hybridizing to the probe. Positions of marker DNAs using HindIII-digested λ phage DNA are shown on the left. The gastrin gene is in fraction F that contains chromosomes 16, 17, 18. (C) Flow histogram of chromosomes from a human cell line GM3197 that carries a reciprocal translocation t(17;22). Chromosomes were sorted into fractions a to h as shown at the bottom of the histogram. The arrow head indicates the position of the t(17;22)(17qter-17p13::22q11-22qter) chromosome. (D) DNA was prepared, cleaved with EcoRI, and subjected to Southern blot analysis as in (B). The gastrin gene is in fraction e that contains chromosomes 13, 14, 15, t(17;22) as well as in fraction f that contains chromosomes 16, 17, 18. Therefore, chromosome 17 should carry the gastrin gene.

Fig. 2A) were obtained in a two-day operation and were used in one filter assay. DNA was extracted from each chromosome fraction, digested with appropriate restriction enzymes, and then analysed by Southern hybridization which gives unambiguous signals, as compared to spot blot

analysis (5 - 7). The filter was hybridized to nick-translated human genes including gastrin, TSH- β or c-erbB-2.

The gastrin probe showed a positive signal in fraction F that corresponds to the pool of chromosomes 16, 17 and 18 (Fig. 2B). In the next step, chromosomes from another cell line, GM3197 were sorted similarly. This cell carries a reciprocal translocation t(17;22) and the translocated chromosome, (17qter-17p13::22q11-22qter) is collected in fraction e that contains chromosomes 13, 14 and 15 (Fig. 2C). Analysis of DNA showed signals in fractions e and f (Fig. 2D). These results clearly demonstrate that the human gastrin gene is located on chromosome 17. In the same way, a cellular oncogene, c-erbB-2 was mapped on chromosome 17 (data not shown).

The human TSH- β gene probe hybridized to fraction A containing sorted chromosomes from GM0131 cells. This fraction contains chromosomes 1 and 2. In the next step, we used a human-rodent hybrid cell line TA1A that contains human chromosome 1, but not 2. The fraction that corresponds to human chromosomes 1 and 2 (see Fig. 3A) showed a positive signal by Southern blot analysis (Fig. 3B). In contrast, no positive signal was detected in the corresponding chromosome fraction obtained from another hybrid cell TA3A that does not contain human chromosomes 1 or 2. Independent studies on chromosomes from the cell line GM3876 that carries reciprocally translocated chromosomes t(1;20) supported this conclusion. In this case, positive signals were detected in two fractions, one that contains normal human chromosomes 1 and 2, and another that contains the translocated chromosomes (data not shown). These results indicate that human TSH- β gene is located on chromosome 1.

At present, most of the gene mapping studies are performed using hybrid cells. In principle, chromosome sorting is unnecessary when working with a panel of hybrid cells. However, we found that the chromosome sorting technique is more powerful since in many hybrid cell lines, human chromosomes are unstable, resulting in frequent loss of the once-identified

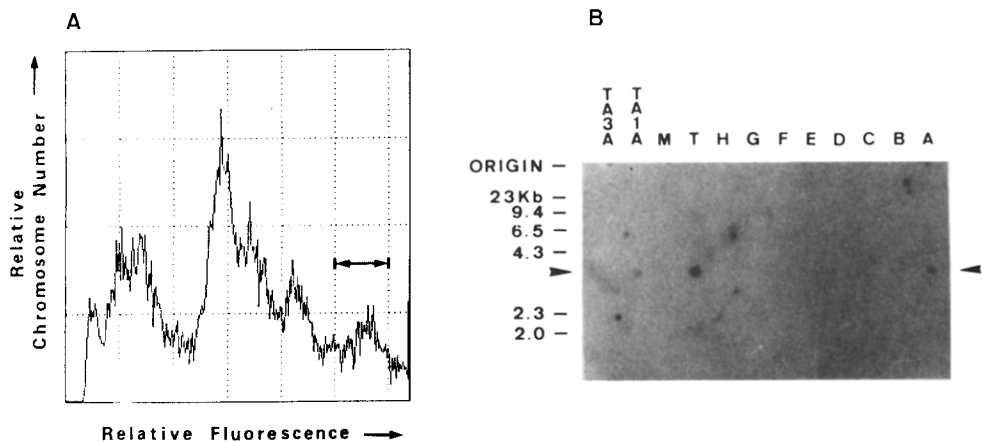


Figure 3. Assignment of human TSH- β gene to chromosome 1. (A) Flow histogram of chromosomes from a human-mouse hybrid cell TA1A that carries human chromosome 1, but not 2. TA1A carries, in addition, human chromosomes 4, 7, 13, 15, 18, 19. The chromosome fractions corresponding to the position for human chromosomes 1 and 2 (shown by a horizontal arrow) were pooled. (B) EcoRI analysis of the DNA from sorted chromosome samples using 32 P-labeled human TSH- β gene (13) as a probe. Lanes A to H contain DNA samples prepared in the same way as those shown in Fig. 2. Human chromosomes 1 and 2 (if they are present) in TA1A and TA3A, as recovered from the fraction shown by an arrow in (A), were analysed similarly. Lanes T and M: total human DNA and total mouse DNA, respectively. An arrow indicates the position of the fragment which hybridizes to the human TSH- β gene probe. Thus, the TSH- β gene must be located on chromosome 1.

chromosomes or in frequent translocations to rodent chromosomes (6). In fact, we tested 7 hybrid cell lines, and obtained inconsistent results. Therefore, when hybrid cells are used, the purification of the corresponding chromosomal fraction using a cell sorter greatly helps to reduce the complexity of these problems.

Recently, further development of sorting techniques using a dual-laser cell sorter has made it possible to sort chromosomes into 21 unique chromosomal types (6). Our technique, viz. combination of preliminary assignment with chromosome sorting from translocated or hybrid cell lines, is another practical way to assign genes to chromosomes.

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