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Regional Gene Mapping of Human Chromosomes Purified by Flow Sorting

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A. Introduction

Analysis and sorting of chromosomes using fluorescence-based high speed flow systems was reported in 1975 (Gray et al. 1975a). In the ensuing 5 years these principles have been applied to karyotype analysis (Gray et al. 1975b; Stubblefield et al. 1975; Carrano et al. 1976, 1978, 1979a,b; Otto and Oldiges 1978; Gray et al., 1979) and to chromosome biochemistry and cytochemistry (Sawin et al. 1979; Lebo et al. 1979; Langlois et al. 1980). The results of these investigations indicate that the use of these systems for chromosome analysis and sorting is an important adjunct to existing cytogenetic techniques and a primary resource for molecular cytogenetic studies of individual chromosomes. This brief report describes one application of this approach, namely, regional gene mapping of human chromosomes.

B. Methodology for Flow Systems Analysis and Sorting of Chromosomes

For human studies metaphase chromosomes are routinely isolated from diploid fibroblast cultures. Cells are grown in roller bottles, metaphase cells accumulated by the addition of Colcemid, and the cultures rotated to dislodge the mitotic cells. These cells are then collected by centrifugation, distributed into aliquots of 8×10^6 cells, and resuspended in hypotonic KCl (0.075 *M*) at 4° C for 30 min. After centrifugation each cell aliquot is resuspended in 0.5 ml chromosome isolation buffer (25 m*M* Tris-HCl, pH 7.5/0.75 *M* hexylene glycol/0.5 m*M* $CaCl_2$/1.0 m*M* $MgCl_2$) and sheared with a Virtis homogenizer. The isolated chromosomes are then stained with an appropriate fluorochrome. For high resolution human chromosome analysis 33258 Hoechst (2 μg/ml, final concentration) has been excellent. The final suspension containing approximately $2–4 \times 10^8$ stained chromosomes in about 1 ml buffer is used directly for analysis and sorting.

The principles and operational aspects of flow cytometry and sorting have been previously described (Van Dilla et al. 1974; Horan and Wheeless 1977). Basically, the stained chromosomes flow in a narrow stream past a laser beam of high intensity UV light (360 nm at 0.7 – 1.0 W). The size of the chromosome stream and width of the laser beam are such that the emitted fluorescence can be measured from single chromosomes at rates of several thousand per second. Separation of chromosomes with a preselected fluorescence intensity is accomplished by electronically charging and deflecting liquid droplets containing the chromosome of interest. Two preselected "windows" of fluorescence intensity are sorted simultaneously. Sort rates for human chromosomes generally range from 100 to 200 chromosomes per second. Thus to collect 5×10^6 human chromosomes (about 1 μg DNA for an average size human metaphase chromosome) requires about 7–14 h. The sorted chromosomes can be fixed onto microscope slides for cytologic analysis or collected in tubes for subsequent DNA extraction.

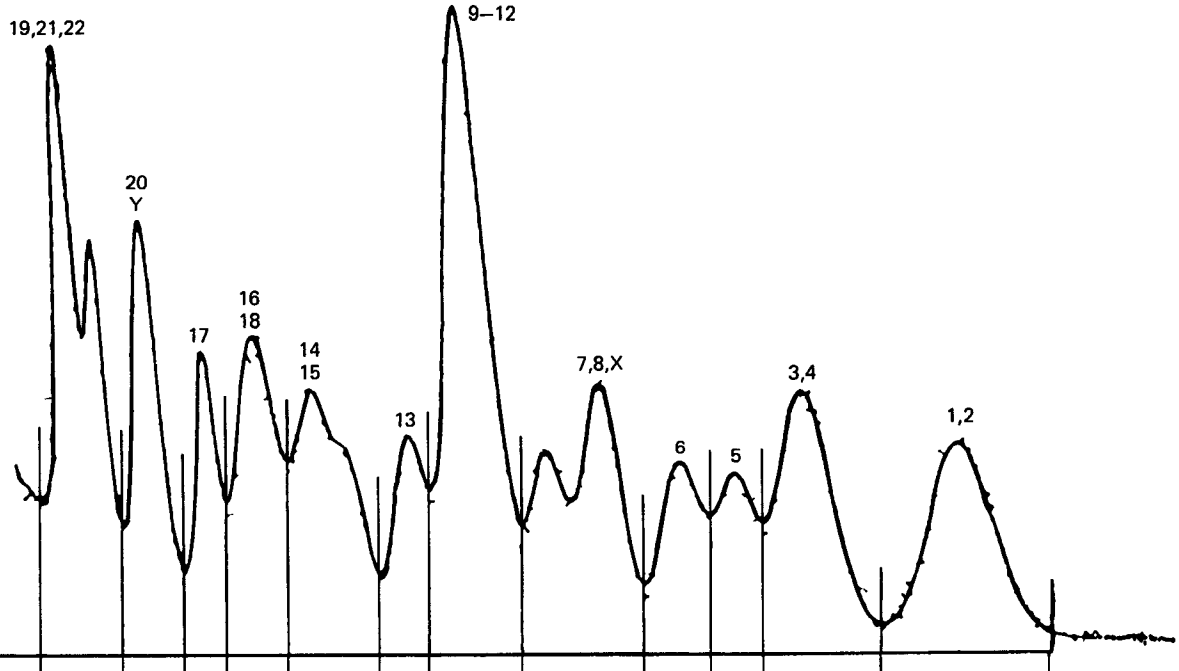
C. Application to Regional Gene Mapping

There are three requirements for regional gene mapping: (1) the ability to resolve the chromosome of interest in the flow distribution; (2)

a DNA probe specific for the gene to be mapped; and (3) cell strains possessing rearrangements of the chromosome to be mapped. The regional mapping of the human β-, γ-, and δ-globin genes illustrates the principles involved.

The 33258 Hoechst distribution of a normal human diploid fibroblast strain is shown in Fig. 1. The ordinate represents the relative number of chromosomes measured and the abscissa, the relative fluorescence intensity. Fifteen peaks are distinguishable and each can be characterized by a mode and area determined by computer fit. The peak mode is a measure of the relative Hoechst fluorescence and the area a measure of the relative number of chromosomes in the peak. These values are given in the figure and the chromosomes associated with each peak (identified by quinacrine banding analysis of the sorted chromosomes) are shown on the top of the distribution. There is reasonably good agreement between the relative Hoechst fluorescence and DNA stain content of the chromosomes, although there are exceptions, e.g., chromosomes 18 and 19. The variations between the observed and expected number of chromosomes for some peaks are attributed primarily to inaccuracies in the fit code. The purity of the chromosomes obtained by sorting is also shown. This ranges from 63% to 98%. Since only four chromosome types (No. 5, 6, 13, and 18) are compeletely resolved, identifying a gene on any other chromosome requires segregation of at least one homolog of the chromosome from the peak – hence the utility of chromosomal rearrangements.

In order to map the β-, γ-, and δ-globin genes we purified chromosomes from several peaks in the distribution. The DNA from each was extracted, digested with EcoRl, and applied to a single well on an agarose gel. After electrophoresis the DNA was transferred to nitrocellulose filters and the filters hybridized with ^{32}P-labeled cDNA prepared by reverse transcription of human globin mRNA. The filters were then washed, dried, and exposed for autoradiography. Positive hybridization occurred between the cDNA for the β-, γ-, and δ-globin genes and DNA extracted from the peak containing chromosomes 9 through 12.



Purity of fraction (%)	98		92	71	89	75		84	63	81		65	56	80	78
Relative Hoechst	0.19	0.22	0.26	0.31	0.36	0.41	0.44	0.50	0.56	0.62	0.68	0.75	0.80	0.86	1.0
Area observed	4.7		2.9	1.6	3.7	4.1		2.1	8.5	5.7		2.5	1.9	4.4	4.1

Fig. 1. Flow distribution of metaphase chromosomes isolated from diploid human fibroblasts and stained with 33258 Hoechst. The *ordinate* represents the number of chromosomes analyzed and the *abscissa*, the fluorescence intensity

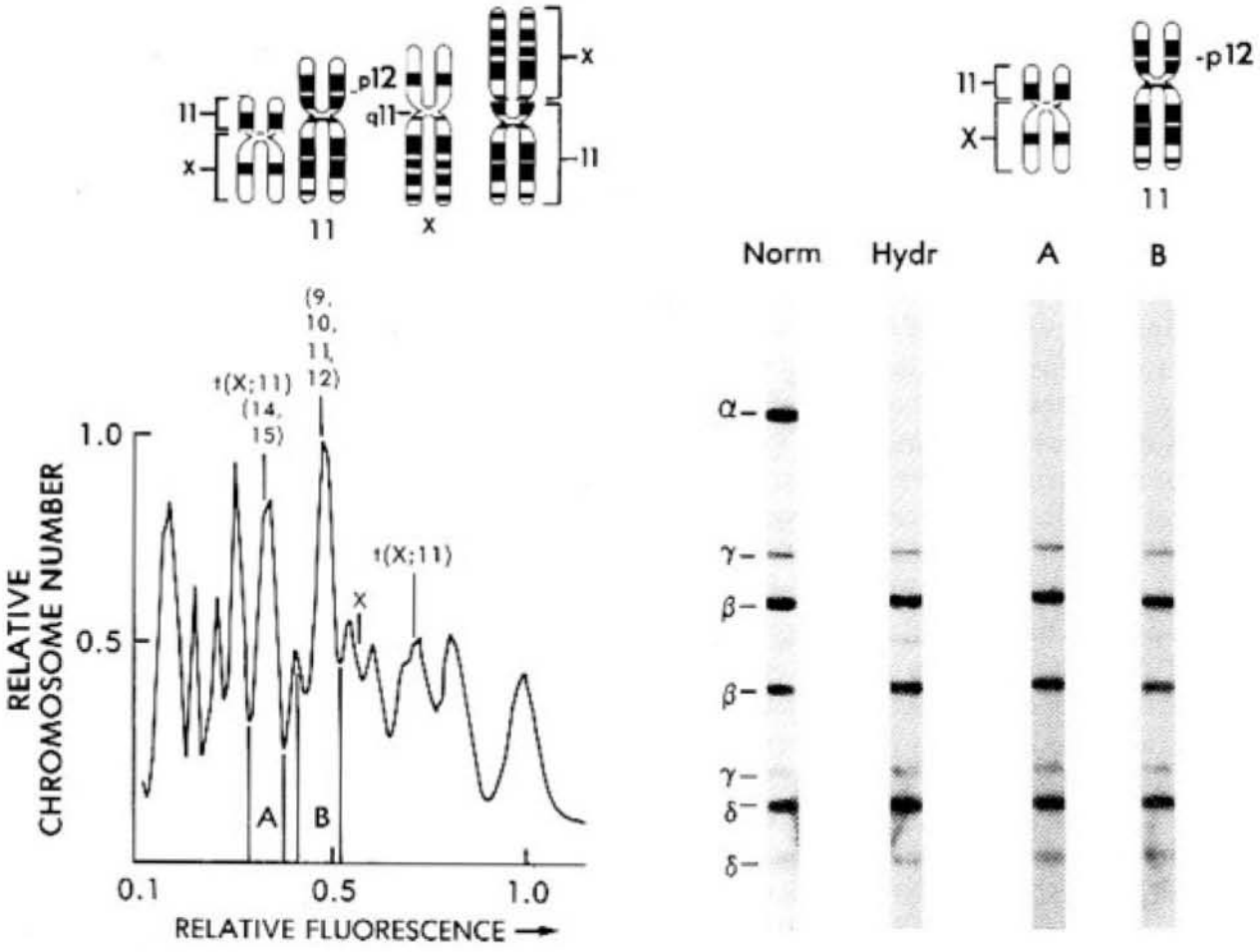


Fig. 2. *Left:* The 33258 Hoechst flow distribution of a human fibroblast strain carrying a translocation, t(X;11) (q11;p12). The normal and derivative chromosomes are shown above the distribution. *Right:* Autoradiographs of ^{32}P-labeled cDNA from human globin RNA hybridized to restricted total cell DNA from a normal *Norm* and hydrops *(Hydr)* individual or to restricted DNA from chromosomes sorted from peaks A and B. The location of the α-, β-, γ-, and δ-globin EcoRI restriction fragments are indicated

Since these genes had been provisionally mapped to chromosome 11 by somatic cell hybridization (Deisseroth et al. 1978), we used three translocations of chromosome 11 to further localize the genes. The 33258 Hoechst flow distribution, the nature of the third translocation, and the autoradiographs from the restricted DNA are shown in Fig. 2. As illustrated by the chromosome diagram above the flow distribution, peak B contained one normal chromosome 11, while peak A contained chromosomes 14, 15, and the derivative chromosome consisting essentially of the short arm of the X and the short arm of chromosome 11 (bands p12 → pter). The other derivative chromosome containing the remainder of chromosomes 11 and X shifted to a higher fluorescence intensity. The restriction enzyme patterns of total cell DNA from a normal individual are shown in the gel at the left. A single band is present for α-globin, while two bands each are present for β-, γ-, and δ-globin. The total hydrops DNA lacking the α-globin is shown in the next gel. Gels A and B were prepared from DNA extracted from chromosome peaks A and B in the flow distribution. Positive hybridization in both gels identified the location of the β-, γ-, and δ-globin genes to chromosome 11, bands p12 → pter. This is supported by independent somatic cell hybridization studies (Gusella et al. 1979).

D. Conclusions

In addition to gene mapping studies, chromosomes purified by flow sorting have potential application to gene transfer and to the molecular biology of chromosome structure and function. It should now be possible to examine DNA or protein unique to a single chromosome and, for example, to examine the DNA associated with specific chromosomal alterations in hematological disorders such as the

translocation between chromosomes 9 and 22 in chronic myelogenous leukemia (Rowley 1973; Mayall et al. 1977). The full potential of this flow systems approach to chromosome analysis has not yet been realized.

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