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Chromosome Measurement and Sorting by Flow Systems

(isolated chromosomes/purified chromosomes/flow microfluorometer/DNA cytophotometry/karyotype)

J. W. GRAY, A. V. CARRANO, L. L. STEINMETZ, M. A. VAN DILLA, D. H. MOORE II, B. H. MAYALL, AND M. L. MENDELSOHN

Biomedical Division, Lawrence Livermore Laboratory, University of California, Livermore, Calif. 94550

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ABSTRACT **A flow microfluorometer was used to measure metaphase chromosomes in suspension at rates up to 100,000 per min. Chromosomes from cells of the Chinese hamster M3-1 cell line were isolated, stained for DNA with the fluorescent dye ethidium bromide, and analyzed for DNA content. Nine distinct peaks were resolved that correspond well with independent chromosomal DNA measurements made with a high-resolution scanning cytophotometer. Chromosomes were sorted from each peak by an electronic cell sorter. Visual examination of each fraction indicated the purity of the sorted chromosomes. This novel technology allows separation of purified populations of individual chromosomes suitable for biochemical and biological characterizations.**

Metaphase chromosomes can be described by several properties, including length, area, DNA content, and sedimentation velocity (1, 2). Of these, DNA content is the most fundamental chromosomal descriptor and the only one insensitive to shape and degree of compaction. Heretofore, chromosomal DNA content has been measured cytophotometrically in conventional slide preparations (2–4); the method is complex and time-consuming, and cannot be used to prepare fractions of purified chromosomes. Bulk techniques such as centrifugation can sort the chromosomes, but only into gross fractions of low purity (5). The advent of flow microfluorometry and flow sorters allows application of precise DNA measurement, rapid processing, and individual sorting to metaphase chromosomes. We report here the first such application. Individual chromosomes of a clonal derivative (650A) from the M3-1 Chinese hamster cell line were isolated, stained for DNA with ethidium bromide, and analyzed by the flow system. A distribution of DNA for the chromosomes in suspension was obtained that showed nine distinct peaks. The chromosomes corresponding to each peak were separated for visual scoring by an electronic cell sorter. The relative DNA content (peak mean), chromosomal frequency (peak area), and chromosome type (peak content as determined visually) were compared with measurements of similar parameters made with a high-resolution scanning cytophotometer on chromosomes from metaphase spreads of cells from the same culture. The agreement was excellent.

MATERIALS AND METHODS

Chromosome Preparation. Chromosomes for flow systems analysis were isolated from mitotic cells of a clonal derivative (650A) of M3-1 male Chinese hamster cells (kindly supplied by Dr. H. J. Burki, University of California, Berkeley) using Pipes [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)] buffer at pH 6.5 according to the procedure of Wray and Stubblefield (6). The procedure was modified by placing the cells in hypotonic KCl (75 mM) while cooling at 4° to permit more gentle shearing. One milliliter of cells (about 4 to 8 × 10^6 cells) was syringed five times through a 22-gauge needle to break the cell membranes and yield the isolated chromosomes. The final suspension contained approximately 10^8 chromosomes in 1 ml of Pipes buffer.

Chromosome spreads of cells from the same culture were also prepared for karyotyping and analysis by CYDAC (computer-oriented image analysis microscope system) according to procedures described elsewhere (2).

Chromosome Staining. For flow system analysis, the chromosomes, still suspended in the Pipes solution, were stained for DNA by adding an equal volume of 0.004% ethidium bromide in Pipes buffer. The chromosomes were uniformly stained within 10 min; they remained in the stain solution throughout flow system analysis. Chromosomal fluorescence was stable from 0.1 hr to 2 days after staining.

Other DNA dyes were used also. Propidium iodide, substituted for ethidium bromide, gave almost identical results. Chromosomes prepared according to the procedure of Burki *et al.* (5) and stained with the dye 33258 Hoechst (7) or acriflavine (8) gave similar but less precise results. Chromosome spreads for analysis by CYDAC first were stained with quinacrine hydrochloride for identification of chromosomes and then restained specifically for DNA with gallocyanin-chrome alum (2). Chromosomes were banded for karyotyping both by trypsin-Giemsa (9) and quinacrine procedures (10).

Flow Systems. The flow microfluorometer and electronic cell sorter used in this work are described elsewhere (8, 11). Briefly, stained chromosomes in the flow microfluorometer flow one at a time through an intense beam of laser light at rates of about 100,000 per min. As each chromosome passes through the beam, the resulting fluorescent light pulse is detected photoelectrically and processed to yield a value proportional to the total fluorescence intensity. This value is stored in the memory of a multichannel pulse-height analyzer, which thereby accumulates the fluorescence distribution histogram of the population. The sorter has an additional feature: if the measured value for a chromosome falls within a preset range, a group of three droplets, one of which contains the desired chromosome, is electrically charged and subsequently deflected into a special container. Droplets with the undesired chromosomes are not charged and are collected in a different container.

Abbreviation: Pipes, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid).

TABLE 1. *Flow system and CYDAC data on chromosomes of the M3-1 line of Chinese hamster cells (male)*

Flow system				CYDAC			
Peak designation	Predominant morphology of sorted chromosomes*	Relative chromosome frequency	Relative DNA content	Relative DNA content	Chromosome frequency per cell	Morphology	Chromosome designation
A	LM (0.84)	1.97	1.00	1.03	2	LM	1
B	LM (0.94)	2.03	0.86	0.87	2	LM	2
C	MM (0.84)	3.26	0.55	0.54	3	MM	4, t(X;5)
D	MM (0.80)	1.03	0.47	0.45	1	MM	5
E F	A (0.90)	4.88	0.36	0.36	5	A	6, 7, Y
G	A (0.99)	1.99	0.30	0.29	2	A	8
H	SM (0.76)	2.87	0.23	0.22	3	SM, A	9, M1
I	SM (0.90)	4.71	0.14	0.14	5	SM	10, 11, M2

* LM, large metacentric; MM, medium metacentric; SM, small metacentric; A, acrocentric. Numbers in parentheses are the fractions of the predominant morphology.

Typically, 10^5 to 10^6 chromosomes were measured to give the distribution of DNA content (i.e., fluorescence) per chromosome. The peaks in this distribution were analyzed by computer to determine the area and mean for each peak. In analysis, Gaussian functions plus an exponential function were fitted by least squares to the experimental data points. The Gaussian functions yield estimates of peak areas and means; the exponential function yields an estimate of the magnitude of any underlying continuum. The peak areas indicate relative chromosomal frequency, and the peak means indicate relative chromosomal DNA content.

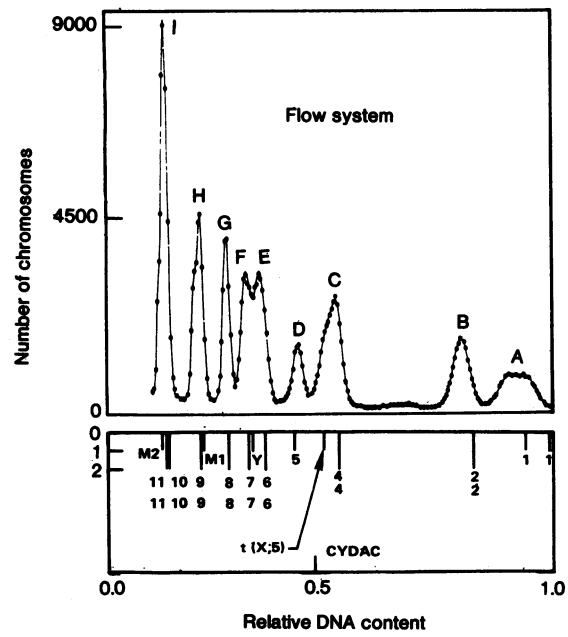

FIG. 1. Flow system and CYDAC data on chromosomes from Chinese hamster M3-1 line (clone 650A) cells. The points in the flow system histogram are experimental data; the solid line represents a computer fit to these data. The length of the bars in the CYDAC plot indicates the number of chromosomes found per metaphase cell. The CYDAC data indicate a difference of 6% in the DNA content between the two homologues of the number 1 chromosome. The data have been plotted to indicate this. Normalization of flow systems and CYDAC results is based on the total DNA content of the karyotype; small differences between flow microfluorometer peak means and CYDAC means are probably due to staining and instrumental effects.

CYDAC. The CYDAC system is described elsewhere in detail (2, 3). Briefly, the stained chromosomes are scanned on a flying-spot microscope, and the scans are digitized and processed by computer; the end results are measurements of the integrated absorbance (i.e., DNA content) for each chromosome.

RESULTS

Flow System Analysis. The distribution of fluorescence intensity for chromosomes of the M3-1 line (650A) is given by the points in Fig. 1. Nine distinct peaks are seen superimposed on a low continuum. Since each peak is produced by chromosomes of similar DNA content, it can be associated with a single or limited number of chromosome types. The computer fit to these data points is given by the solid line. The relative peak means and areas as determined by the computer analysis are shown in Table 1. The peak means were normalized to 1.00 for the largest mean. The peak areas were normalized so that the combined area of peaks A and B is equal to 4 (the expected number per metaphase cell of chromosomes 1 and 2).

The chromosomes within each peak of the histogram of Fig. 1 were identified visually after they were sorted onto microscope slides. The sorting window was centered at the modal value of each peak and covered about half the peak-width. Conventional morphological criteria were used for visual classification. The chromosomes were assigned to four classes: large, medium, and small metacentrics, and acrocentrics. Table 1 shows the frequency of the predominant class of sorted chromosomes found for each peak.

The large metacentric chromosomes are found in peaks A and B, the medium metacentrics in peaks C and D, the acrocentrics in peaks E, F, and G, and the small metacentrics in peaks H and I; peak H also contains small acrocentrics. Contamination of any sorted fraction of chromosomes by chromosomes of different morphology was less than 20%, except for peak H, which contained the expected mixture of acrocentric and metacentric chromosomes.

Cytogenetic Analysis. Chromosome spreads from cells of the same culture used in the flow system work were analyzed by

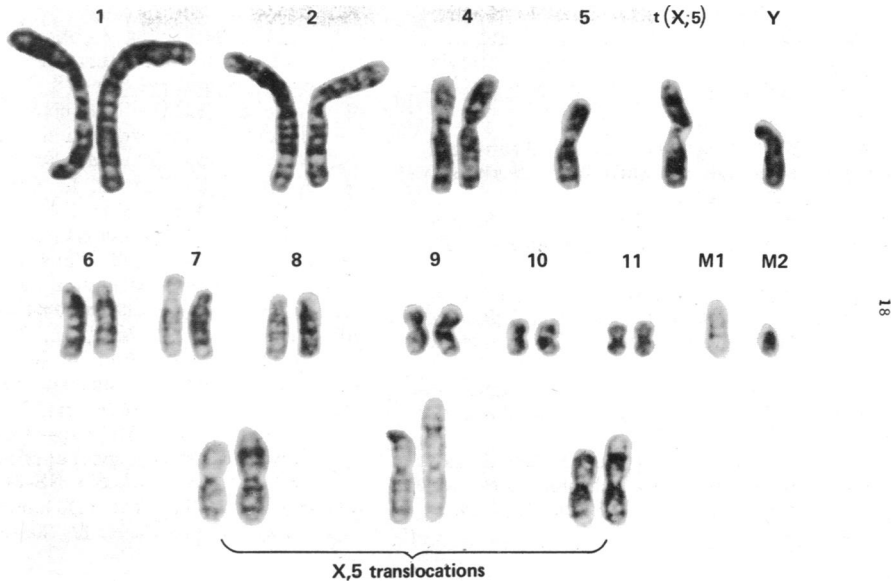

FIG. 2. Trypsin-Giemsa banded karyotype of clone 650A of the M3-1 Chinese hamster cell line. Over forty banded metaphase cells were examined to identify the rearrangements. The bottom line shows the normal number 5 (left) and t(X;5) (right) chromosomes from three additional cells. The translocated chromosome has been inverted in these pairs to demonstrate the similarity in the bands to chromosome 5. A difference in the banding patterns between the homologues of chromosome 1 is not evident even though the cytophotometry indicated that the two homologues differed in DNA content by 6%.

conventional cytogenetic methods. The karyotype was established both by trypsin-Geimsa and quinacrine banding procedures (Fig. 2). The 650A clone has 23 chromosomes. Comparison of its banding patterns with those of the normal male Chinese hamster suggests that the following rearrangements occurred. Both the X chromosome and one of the number 5 chromosomes broke in the region of the centromere. The long arm (and possibly the proximal region of the short arm) of one number 5 chromosome was translocated to the long arm of the X chromosome to produce a new translocated chromosome, t(X;5). The short arm of chromosome 5 could not be found in the cell and is presumed lost. The short arm of the X chromosome apparently retained the centromere and is present as a small acrocentric chromosome, marker no. 1 (M1). There appears to be a partial deletion of the short arm of the Y chromosome. A small metacentric chromosome, marker no. 2 (M2) is present; it cannot be identified with certainty because its banding is too diffuse.

CYDAC Analysis. The DNA stain content of each chromosome from eight metaphase spreads of these M3-1 cells was measured by CYDAC. These results are presented in Table 1 for comparison with the flow system results. The CYDAC measurements were normalized so that the total DNA content for all the chromosomes is the same as in the normalized flow system results. The normalized values for several of the chromosomes were averaged so that a direct comparison can be made between the CYDAC and the flow system measurements where peak overlap occurs. In addition, the normalized CYDAC values for each of the 14 different chromosomes are plotted on the lower portion of Fig. 1.

The resolution of the flow microfluorometric histogram is affected by chromosomal preparation and staining methods, by instrumental broadening, and by variability of chromosomal DNA content. The coefficient of variation of measurements of chromosomes of a single type varied from 2.2% for chromosome number 2 (peak B) to 3.3% for chromosome number 8 (peak G). The situation for chromosome number 1 is discussed below.

DISCUSSION

The agreement seen in Table 1 between the flow system and CYDAC measurements of the M3-1 line chromosomes is striking, particularly since the chromosomes were prepared, stained, and measured by entirely different methods. This agreement allows the definitive assignment of chromosomes to each of the peaks seen in Fig. 1. For DNA content, the largest discrepancy between the flow systems and CYDAC measurements is less than 5%. The agreement between the relative chromosomal frequencies obtained from the two systems indicates that the isolated chromosome suspensions were representative; i.e., there was little preferential loss of chromosomes of a specific type. The morphology of the chromosomes sorted from each peak is consistent with the assignment for each peak made from the CYDAC analysis. Another indication of the agreement between the flow system and CYDAC data is seen from the data on the number 1 chromosome (peak A in the flow system data). Peak A is non-Gaussian in shape; computer analysis reveals the existence of two populations of chromosomes differing in mean DNA content by approximately 5%. Similarly, CYDAC measurements of the number 1 chromosomes show a highly significant ($P < 0.005$) difference in the homologues of approximately 6% (12).

The measurement of this small difference between the number 1 chromosomes shows that flow systems techniques can resolve objects whose DNA contents differ by about 6×10^{-14} g.

These results demonstrate that we have measured and sorted chromosomes from a cloned line of M3-1 Chinese hamster cells with a rapidity, resolution, and purity that should permit flow-oriented karyotype analysis and offer a new preparative method for biochemical and biological studies of large numbers of individual chromosomes.

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