Assessment of Persisting Chromosome Aberrations by Flow Karyotyping of Cloned Chinese Hamster Cells

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A preparation, staining and measuring protocol for high resolution flow cytometry of chromosomes was developed. This method allows us to identify all chromosome types and is suited for characterization of permanent cell lines and cell clones by establishing their flow karyotypes. In cell clones this procedure can be used for the detection of chromosomal aberrations which appear spontaneously or are induced by mutagen treatment and persist in the cell population.

Introduction

In 1980 we studied the flow karyotypes of uncloned and cloned Chinese hamster cell cultures [1]. Though the resolution of the chromosome histograms was unsatisfactory at that time, it became evident, that the quality of the histograms obtained for cloned cell lines surpassed that for uncloned cells. Thus, we recommended the use of cell clones for flow cytometric investigations in experimental genetics and mutagenicity testing. Using advanced preparation and measuring techniques, these conclusions were now confirmed. Suitable clones yield an excellent resolution which allows the identification of all chromosome types and opens a way for the detection of persisting aberrations. In earlier approaches to utilize flow cytometric chromosome histograms for mutagenicity studies, the authors analyzed the first mitoses after treatment and used the broadening of the peaks and/or the increasing background for the quantitation of the mutagenic effect [2–7]. These approaches remained unsatisfactory, because the sensitivity was low and the specificity was impaired by cytotoxic effects.

Materials and Methods

The Chinese hamster cell line M3-1 and clones derived from this line were used for the methodological development and mutagenicity experiments. The cells were grown as monolayer cultures in Eagle's minimum essential medium supplemented with 5% fetal calf serum and gentamycin.

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For the preparation of chromosomes, exponentially growing cultures were treated with 0.7 μmol of colcemid/l for 5 h. Metaphase cells were harvested by shaking, kept on ice for 45 min, pelleted by centrifugation for 10 min at $100 \times g$, and resuspended in a hypotonic solution consisting of tris-HCl buffer (10 mmol/l, pH 7.4), KCl (30 mmol/l), MgCl (5 mmol/l), and dithioerythritol (DTE; 3 mmol/l). The cells were incubated for 10 min at room temperature. Triton X-100 was then added to a final concentration of 0.5%. The cell membranes were disrupted and the chromosomes dispersed by syringing through a 27 gauge cannula. The chromosomes were stained by adding the same volume of hypertonic solution consisting of NaCl (350 mmol/l), Na citrate (40 mmol/l) and containing 4′,6-diamidino-2-phenylindole (DAPI; 5 μmol/l) which was introduced to flow cytometry by Göhde *et al.* [8]. Measurements were performed using a PAS II flow cytometer (Partec AG, Arlesheim, Switzerland) equipped with a UG 1 excitation filter, a TK 420 dichroic mirror and a GG 435 barrier filter.

For quantitative evaluation of the histograms, a computer program (Partec AG) was used to calculate the median of each peak (= mean relative DNA content of the chromosome), the integral (relative frequency of the chromosome) and the coefficient of variation (CV) of each peak.

Results and Discussion

1. Flow karyotyping of Chinese hamster cell lines and cell clones

The flow karyotypes of established Chinese hamster cell lines exhibit considerable differences [1].

The 3 examples shown in Fig. 1 demonstrate the variability in the region of the small chromosomes and the tendency towards polymorphism of chromosome 1. The two homologues of chromosome 1 differ in DNA content by more than 5% in line M3-1 and by 2% in line V79. In B14F28 cells there is also a small difference as can be seen from the width of the peak. Chromosome 2 proved to be very stable. The established cell lines contain mixtures of different karyotypes and therefore produce complex chromosomal histograms, particularly in the region of the small chromosomes.

Clones originate from single karyotypes and therefore exhibit relatively simple chromosome histograms. Fig. 2 shows the histograms of the parental cell line M3-1 and of 3 clones selected from a series of clones obtained by a subcloning experiment.

The histograms of the clones allow us to identify all chromosome types. Particularly, clone H exhibits a karyotype favorable for flow cytometry with 22 chromosomes represented in the form of 15 well separated peaks. The chromosome number can be determined easily from the histogram on the basis of clear-cut groups of frequencies: There is one peak representing 3 chromosomes, five peaks containing pairs of chromosomes with homologues exhibiting the same DNA content, and nine peaks containing single chromosomes depicting homologues with different DNA content.

This homogeneity of the karyotype remains for some time before new chromosomal changes develop in the cell population. During this period the cell clone can be used to study spontaneous and induced chromosome mutations.

2. *Detection of persisting chromosome aberrations*

In order to check the stability of the flow karyotype and prove the feasibility of a new mutagenicity assay based on the detection of persisting chromosomal aberrations, the following experiment was performed:

One flask of the cell clone M3-1-H was taken as the control. From this flask a series of subcultures were grown, each starting with a very low number of cells (4 cells on average). The subcultures were propagated for at least 22 cell generations. Then chromosomes were prepared for flow cytometry. Evaluation of the histograms (4 examples are given in Fig. 3) showed that 11 out of 12 subcultures exhibited an unchanged flow karyotype, one subculture exhibited a deviation in the region of the smallest chromosomes. The peak of the smallest chromosome was reduced and a new peak had appeared at a position of higher DNA content. It is assumed that these two changes are produced by one chromosomal aberration, probably a translocation. Taking into account that the originating cell number in the 12 subcultures was 48, the spontaneous aberration rate observed in this experiment is about 2%.

Another flask of the same cell clone was treated with the mutagen N-methyl-N′-nitro-N-nitrosoguadinine (MNNG; 10 μmol/l for 24 h). Thereafter subcultures were established, propagated for 22 cell generations, and the chromosomes were prepared for flow cytometry. Evaluation of the histograms (4 examples are given in Fig. 4) showed that 6 out of 9 subcultures exhibited paired or multiple alterations in the flow cytometric pattern. These alterations are considered as induced aberrations. Considering the initial cell numbers of the 9 subcultures in this experiment, the number of events underlying the observed deviations can be estimated to be at least 8 alterations induced in 36 cells, thus corresponding to an aberration rate of about 22%.

From theoretical considerations it becomes obvious that in this type of experiment each chromosome mutation event (*e.g.* deletion or translocation) produces a pair of changes in the histogram, namely a reduction or disappearance of a peak plus an appearance of a new peak (or less frequently an increase of an existing peak). From the position of a new peak the type of aberration can be concluded. The reduction or disappearance of a peak in connection with a new peak at a position of higher DNA content must be assigned to a translocation, in connection with a new peak at a position of lower DNA content to a chromosomal deletion. The results of the present experiments have to be confirmed by microscopical analyses and the morphological characterization of the aberrations remains to be done. However, the first observations already demonstrate that persisting chromosome aberrations may be analyzed by this type of assay.

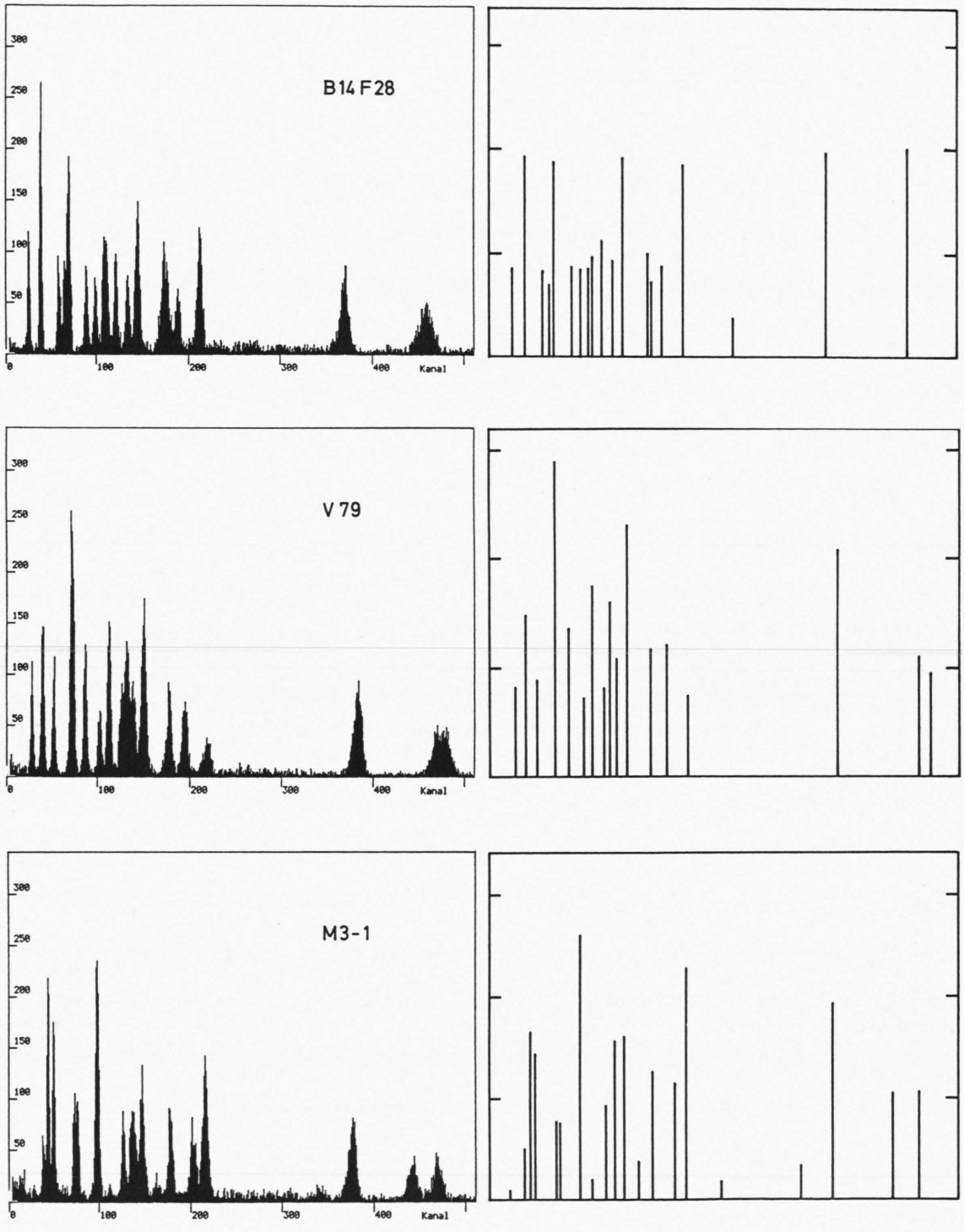

Fig. 1. Flow cytometric chromosome histograms of 3 permanent Chinese hamster cell lines. In the histogram evaluations (right) the position of each line depicts the mean relative DNA content and the height of each line represents the relative frequency of the chromosome type.

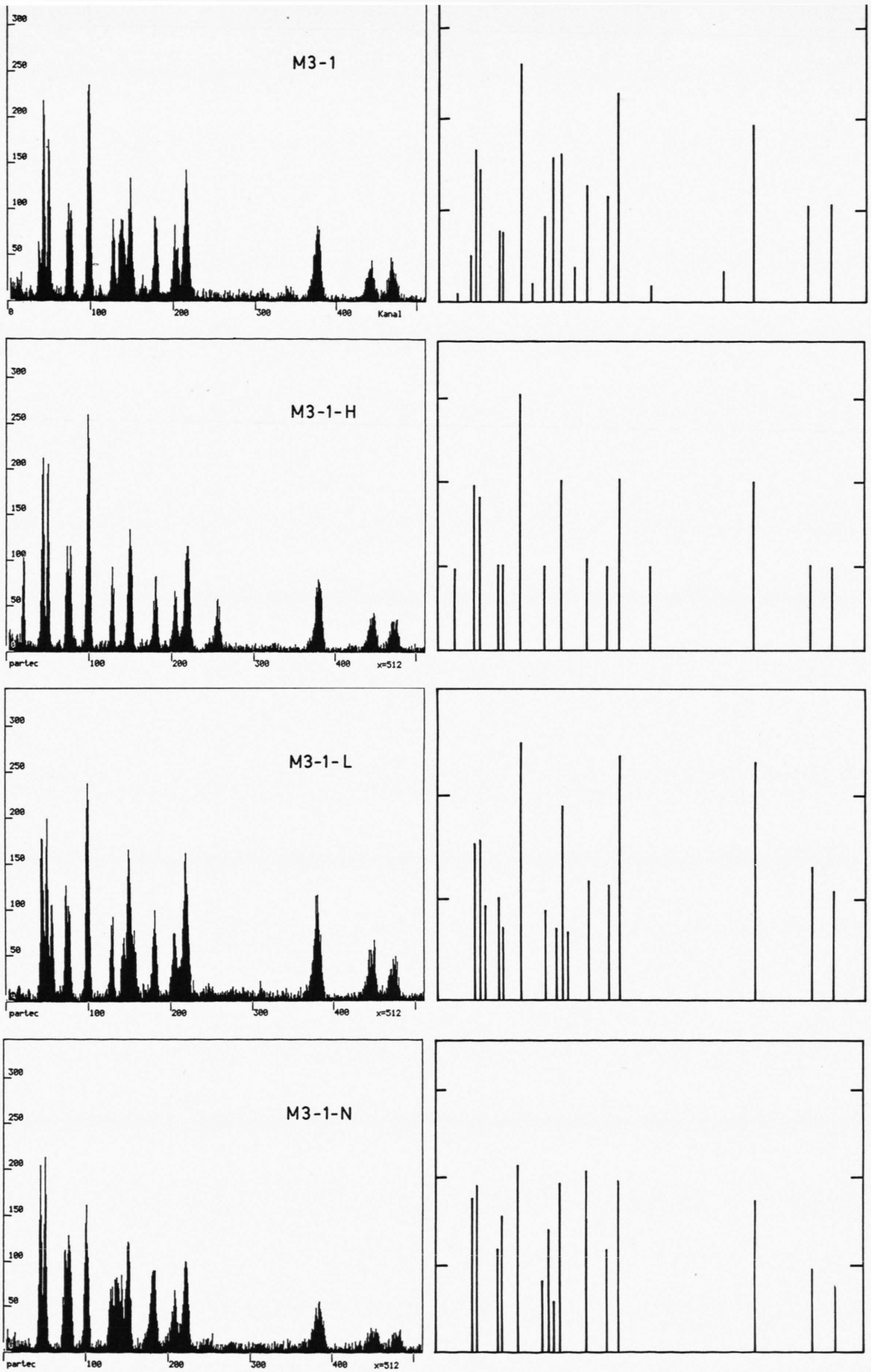

Fig. 2. Flow cytometric chromosome histograms of the parental cell line M3-1 and 3 clones derived from this line.

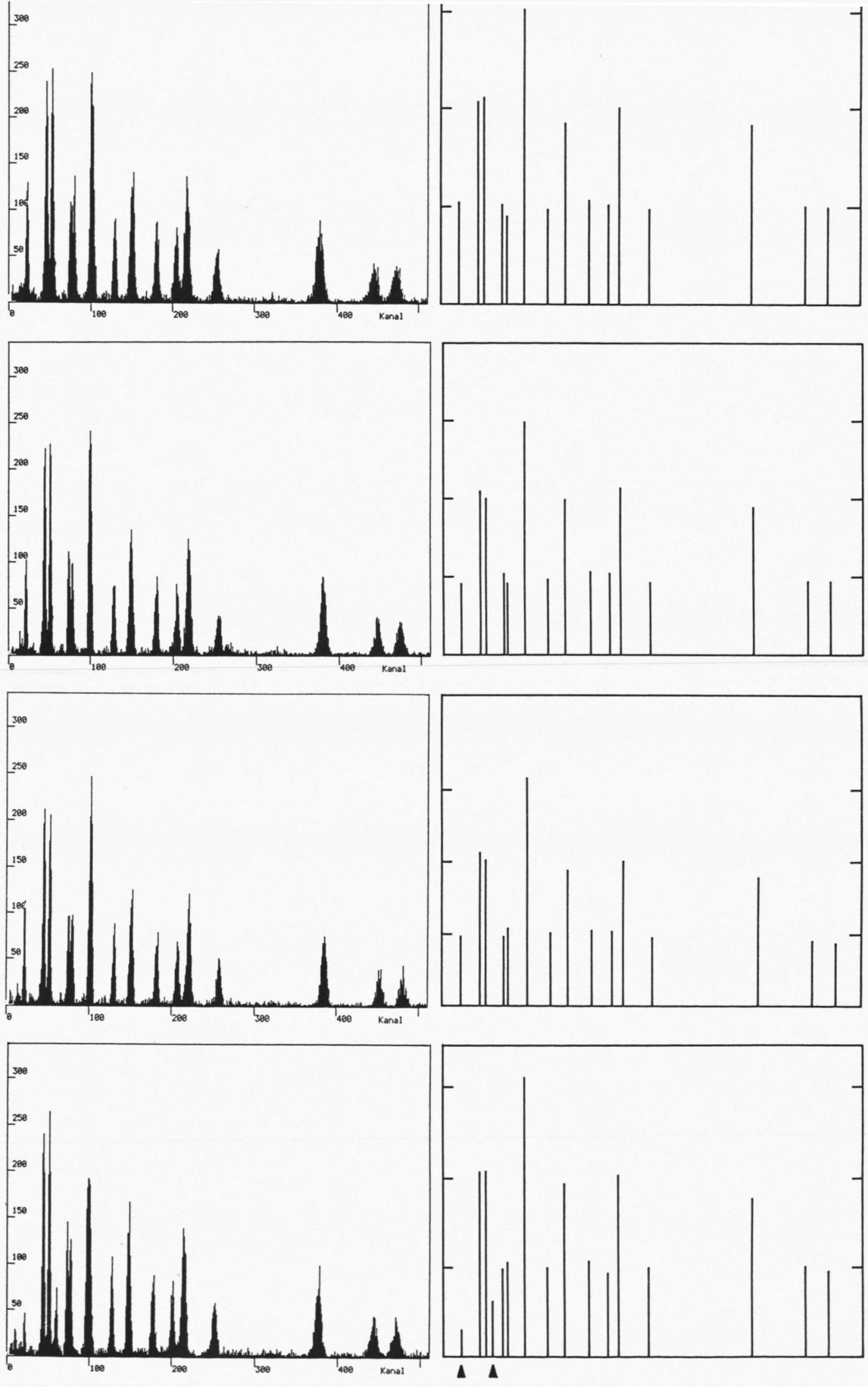

Fig. 3. 4 chromosome histograms out of 12 subcultures grown from a control culture of M3-1 clone H.

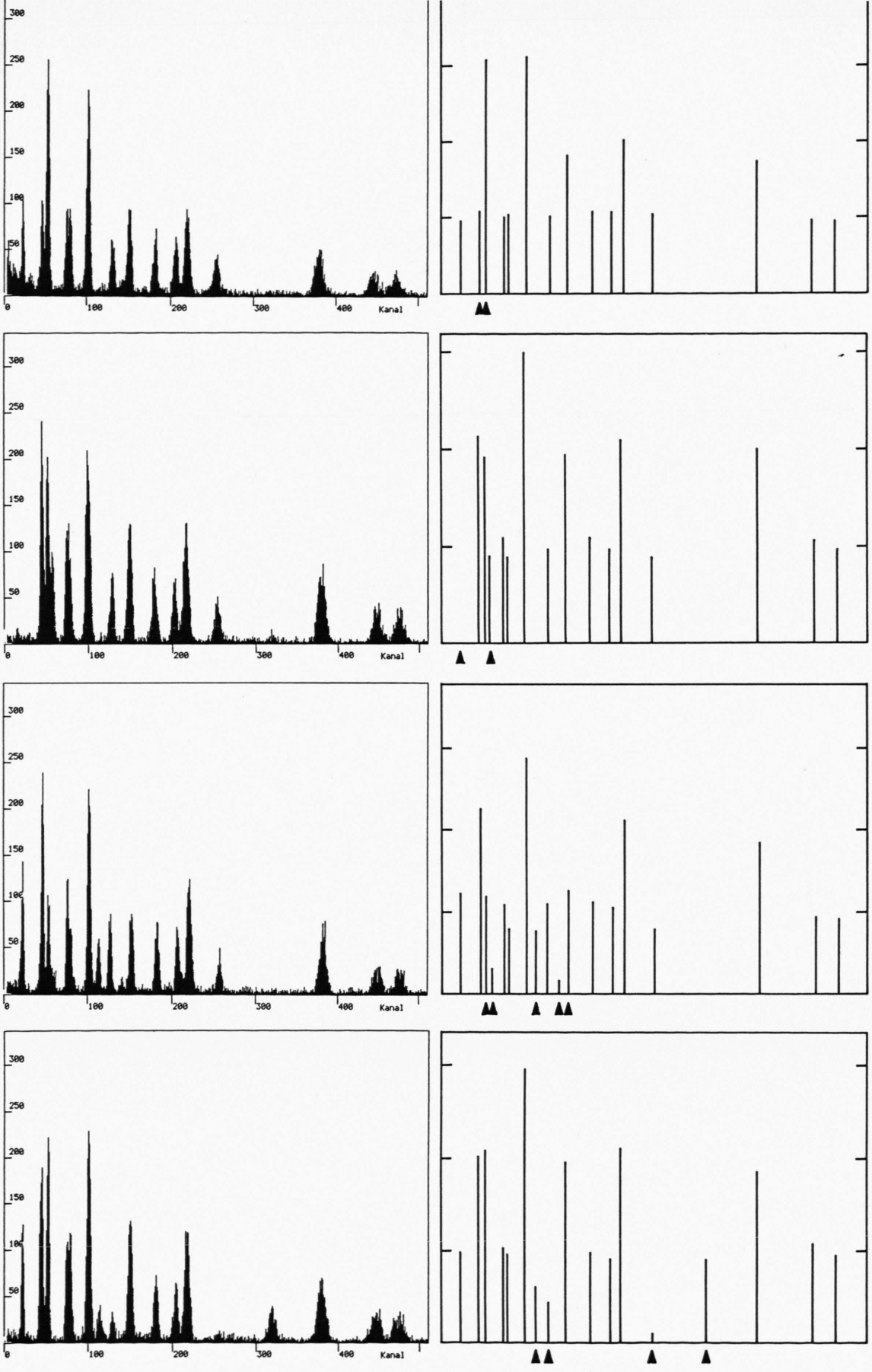

Fig. 4. 4 chromosome histograms out of 9 subcultures grown from a culture of M3-1 clone H treated with MNNG (10 μmol/l).

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