High-Resolution Scanning-Densitometry of Photographic Negatives of Human Metaphase Chromosomes

II. Feulgen-DNA Measurements*

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Summary. In photomicrographic negatives of cytochemically stained human metaphase preparations, images of individual chromosomes were scanned interactively with a Zeiss SMP interfaced to a PDP-12 computer.

By means of the CHROSCAN computer program spot-scanning of selected chromosomes was performed in a direction perpendicular to the length axis, each measured value being corrected for background absorbance taken on both sides of the chromosome image. Plotting of the integrated absorbance values of each transversal scanline results in a graphic representation of the absorbance distribution over the chromosome length. Following this procedure, longitudinal curves were obtained which showed the characteristic patterns obtained after Q or G banding, and, in the case of Feulgen-staining, represented quantitative variations of DNA mass along the individual chromosomes. For Feulgen-stained chromosomes, the total integrated absorbance value and the ratio of integrated absorbance in the long arm over the total integrated absorbance, correspond with the DNA-absorbance and -arm ratio values per chromosome respectively.

The results of investigations concerning the reproducibility and accuracy of cytochemical Feulgen staining and of the photographic procedures are presented, together with total integrated Feulgen-DNA absorbance and arm ratio values for a number of human chromosomes.

For several chromosomes, Feulgen absorbance arm ratio measurements were found to result in values more constant over different metaphases when the long arm was considered to start at the lowest dip in the longitudinal absorbance curve, than when the microscopically observable primary constriction was taken to represent the centromere. The results indicate that the present method allows accurate photometry of naturally absorbing, or of stained or fluorescent objects, with measuring intervals of 0.16 μ. In addition it is shown that the arm ratio values and total DNA content can serve as very constant parameters for karyotype analysis.

Introduction

Since the demonstration of the human chromosome number and the development of methods to prepare metaphase chromosome spreads for routine use (Tjio and Levan, 1956), rapid progress has been made in the field of cytogenetics. Study of the morphology of mitotic chromosomes, identification of chromosomal abnormalities in individuals with different congenital malformations and prenatal karyotyping of cells cultured from amniotic fluid, have become more and more important for the diagnosis of genetic disorders and for genetic counseling.

Apart from the importance of the study of chromosomal abnormalities, quantitative molecular information in topologically well defined areas of metaphase chromosomes is of interest since the structure of the nucleic acid-protein

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complexes in the chromosome play an important role, at least in the higher organisms, in regulating the function of the gene system.

In 1968 a new era for chromosome analysis was opened when Caspersson et al. (1970, 1971) developed a cytochemical staining method with quinacrine derivatives which results in characteristic patterns of bands with different fluorescence intensities along chromosomes.

By use of this method (and of the modified Giemsa-staining methods, giving similar banding patterns) (for a review see Pearson, 1973a) all chromosomes of the normal human karyotype, at least in selected human metaphase spreads, can be identified unambiguously. Approaches in studying the organization of the gene system, chromosome mapping and gene linkage relationships can be expected to progress rapidly now by further development of quantitative chromosome cytochemistry.

For such approaches, the determination of the total DNA content and its distribution along the individual chromosomes will be necessary to provide the basis for a topologically more minute investigation of the molecular composition of each chromosome. Fine structural cytochemical analysis will also be the ultimate goal with respect to a better understanding of chromosome abnormalities. DNA contents and absorbance arm ratio values can also be used for karyotyping purposes.

So far, in relatively few reports concerned with the classification of human chromosomes, the DNA content has been used as an identification parameter. In the present paper are presented the initial results of total integrated Feulgen-DNA absorbance measurements and absorbance arm ratio determinations in a number of human metaphase chromosomes obtained from blood lymphocyte cultures of healthy volunteers.

Material and Methods

Most of the information concerning material and methods has been described in the preceding paper (Van der Ploeg et al., 1974a). Staining of the chromosome preparations with quinacrine ("atebrin"), batch G 29: 0.5% in a McIlvaine citrate-phosphate buffer of pH 4.1) and mounting of the preparations in the buffer was performed as described by Van der Ploeg and Ploem (1973) following the principle of Caspersson's technique. The preparations were examined after atebri-staining with an Ortholux microscope provided with epi-illumination and an HBO 200 W mercury lamp; a BG 38 (4 mm), a Calflex infrared reflecting filter, and an AL 436 exciter filter in the excitation beam (Van der Ploeg and Ploem, 1973). In the vertical illuminator, a dichroic mirror nr. 2 (4H 455) in combination with a K 490 barrier filter was used, and an extra barrier filter K 490 was inserted in the slit above the vertical illuminator.

Overall views were obtained with an oil-immersion 22×/0.65 objective and an oil-immersion objective (Achromat 100×/1.30) was used for photography. The immersion oil (Immersionsoel nach DIN 58884) showed negligible autofluorescence, and was applied in amounts just sufficient to give immersion between objective and slide (Davidson, 1973). For microphotography with the automatic microscope camera equipment (Orthomat), either the filter combination mentioned, or a new filter KP 450 was used (Ploem and Van der

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