HUMAN CHROMOSOME METHODOLOGY

Second Edition

Edited by

Jorge J. Yunis

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SECOND EDITION

Edited by Jorge J. Yunis, M.D.

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DEPARTMENT OF LABORATORY MEDICINE AND PATHOLOGY
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Preface

A decade has elapsed since the appearance of the first edition of "Human Chromosome Methodology." In the interim, the field of cytogenetics has dramatically changed from a pure cytological art to a field in which the structure of chromosomes and the molecular organization of the genome are rapidly merging into the new field of molecular cytogenetics. Breakthroughs in the understanding of constitutive heterochromatin, repetitive DNA, and the structure of chromosomes have had an unprecedented impact on biology and medicine. The discovery of chromosome banding and of the Y body as well as their application to clinical medicine were natural results of these studies. Because of these advances, the new edition is a completely new version in which only the familiar style and general approach of the first edition have been retained. Various chapters cover each phase of laboratory work from preparation of materials for the X and Y bodies, chromosome banding, and culture techniques for blood, bone marrow, skin, tumor tissue, and amniotic cells to chromosome identification, autoradiography, and dermatoglyphics. Included also are sections on the structure and molecular organization of chromosomes, advances in the automation of chromosome analysis, and a thorough review of the clinical manifestations of chromosome disorders.

This edition should serve as an authoritative guide to cytogenetic techniques for research workers and particularly for members of the medical profession involved in medical genetics.

JORGE J. YUNIS

Preface to First Edition

Recent advances in human cytogenetics have stimulated widespread interest among many investigators in the medical and biological sciences. As a direct result of this interest, a genuine need has been felt for an authoritative and up-to-date treatise which would serve as a text and reference. Readily comprehensible chapters are offered covering each phase of laboratory investigation from the preparation of materials for sex chromatin and chromosome techniques for bone marrow, blood, skin, and gonadal specimens to the subject of autoradiography and chromosome identification. Included also are guides to microscopy and photomicrography as well as an up-to-date treatment of chromosomes in disease. It is hoped that this volume will serve as an adequate guide to laboratory techniques and their applications for research workers, students of genetics, and members of the medical profession involved in setting up a laboratory of cytogenetics.

JORGE J. YUNIS

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Structure and Molecular Organization of Chromosomes

JORGE J. YUNIS

In recent years a number of important developments have taken place in the field of cytogenetics. These include (1) the finding of highly repetitive satellite DNA in constitutive heterochromatin (Yasmineh and Yunis, 1969) and its localization in metaphase chromosomes by *in situ* hybridization (Pardue and Gall, 1970; Jones, 1970) and (2) the visualization of characteristic banding patterns within metaphase chromosomes by the use of various dyes and denaturing agents which have helped greatly in the identification of chromosomes (Caspersson *et al.*, 1969, 1970; Arrighi and Hsu, 1971; Yunis *et al.*, 1971; Sumner *et al.*, 1971). Although it is known that satellite DNA is the component that is generally localized within centromeric regions (Yunis and Yasmineh, 1971, 1972), the nature of the components responsible for banding by the various stains is just beginning to be ellucidated (McKay, 1973; Sumner and Evans, 1973; Yunis and Sanchez, 1973). The use of chromatin denaturation and reassociation in the C-banding techniques (Arrighi and Hsu, 1971; Yunis *et al.*, 1971), as well as the earlier suspicion that chromosomal dyes may bind differentially to heterochromatin and repetitive DNA, led several investigators to visualize chromosome banding by a modified Giemsa technique (Sumner *et al.*, 1971; Schnedl, 1971; Drets and Shaw, 1971; Patil *et al.*, 1971; Dutrillaux and Lejeune, 1971). Most recently, however, it has been shown that such a banding pattern can be ellicited without the need of any pretreatment (Fig. 1) and that chromosome bands primarily represent a conformational feature of chromosomes (Yunis and Sanchez, 1973; McKay, 1973). This is indeed confirmed by the fact that fluorescent quinacrine dyes show chromosome

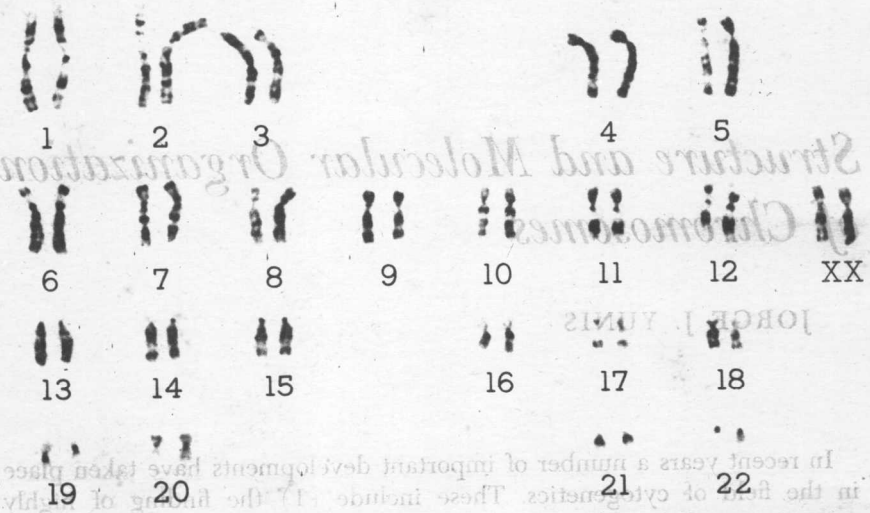

Fig. 1. G-banding pattern of a normal female karyotype from a freshly made chromosome preparation stained with Giemsa diluted 1/100 with 0.13 *M* phosphate buffer, pH 6.7 for 30 minutes. Approximately 300 bands per haploid set can be counted in mid-metaphase cells such as this (from Yunis and Sanchez, 1973). × 375.

banding with the use of the standard chromosome technique and by the recent visualization of similar chromosome banding by electron microscopy (Fig. 2) using water-treated and unstained chromosomes (Bahr *et al.*, 1973).

In mammals, the most convincing evidence suggesting that there may also be differences in chemical content and/or arrangement of chromatin comes from *Microtus agrestis*, in which the bulk of the giant chromosomes are heterochromatic and late replicating (Schmid, 1967; Lee and Yunis, 1971a,b), display thick and closely associated G-bands (Cooper and Hsu, 1972), and are enriched in repetitive DNA (Yasmineh and Yunis, 1973; 1974). In man, suggestive evidence comes from: (a) staining differences between Q- and G-banding, particularly in centromeric regions (Ganner and Evans, 1971) which are also known to be enriched in satellite DNA (Jones, 1973); (b) the striking differences in color and intensity of the centromeric region of chromosome 9 when stained with Giemsa at pH 7 and 11 (Bobrow *et al.*, 1972; and (c) the general relationship observed between Q- and G-bands and late replicating regions (Ganner and Evans, 1971). Most recently, a general correlation between the major Q- and G-bands and the intermediate repetitive DNA of man has been observed (Fig. 3) using c-RNA to

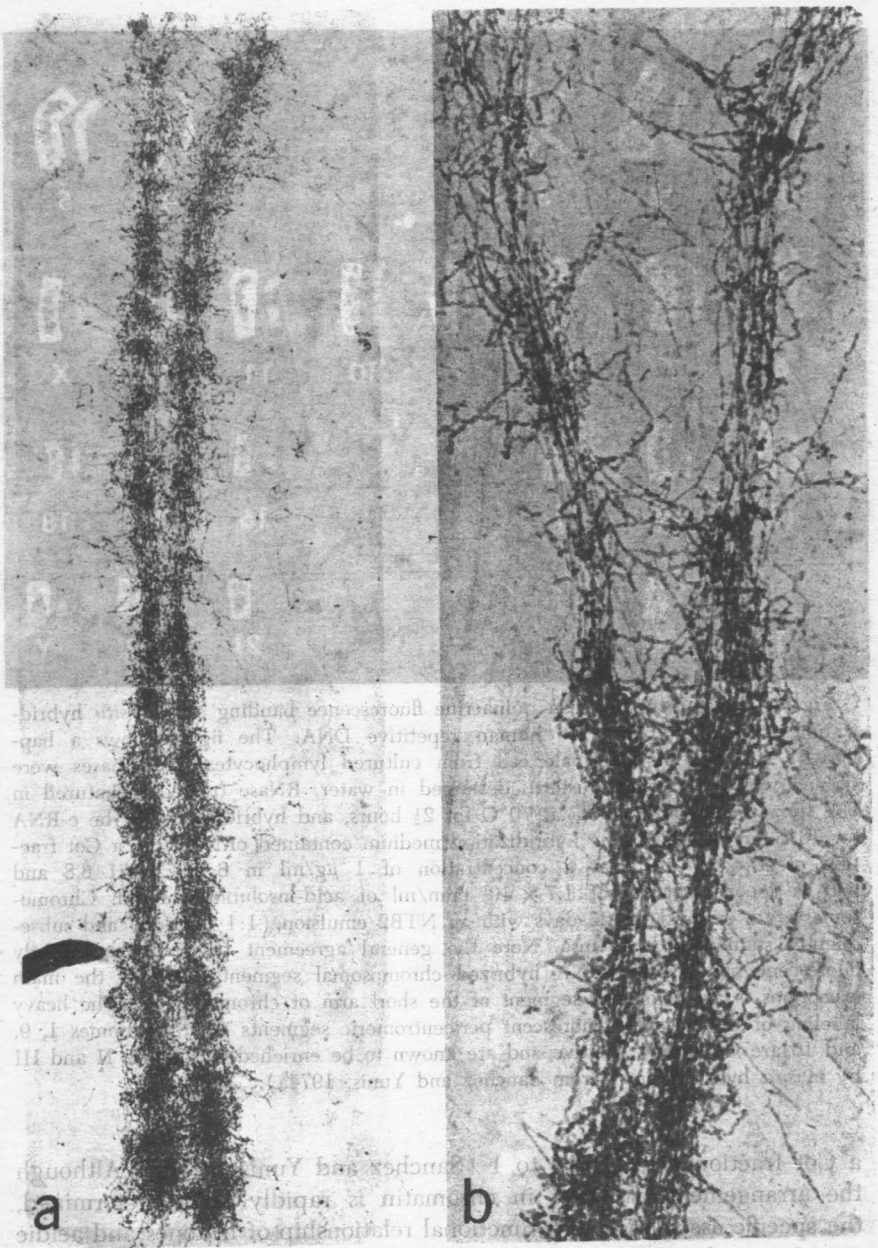

Fig. 2. (a) Whole-mount electron microscopy of a human chromosome showing dense chromatin bands primarily composed of kinky and packed horizontal loops and light chromatin bands or interbands made up of a looser arrangement of predominantly longitudinal fibers. × 20,000. (b) Larger magnification of a chromosome segment showing details of this arrangement. × 28,000. With this technique, approximately 678 bands per haploid set can be counted on metaphase chromosomes (from Gunter Bahr and Walter Engler, with kind permission).

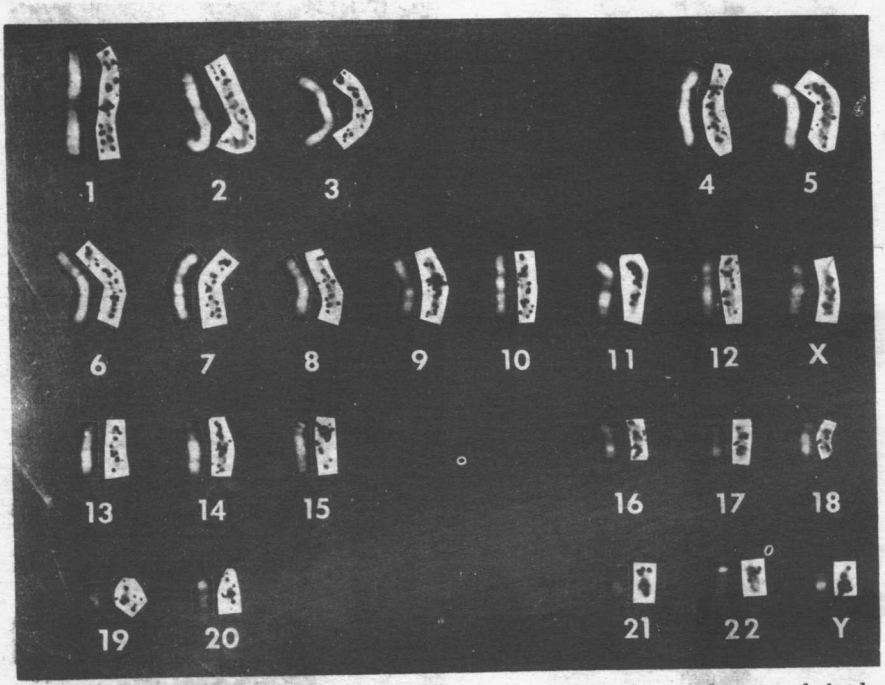

Fig. 3. Comparison between quinacrine fluorescence banding and *in situ* hybridization with cRNA made to human repetitive DNA. The figure shows a haploid-XY set of a normal male cell from cultured lymphocytes. Metaphases were stained with quinacrine mustard, destained in water, RNase treated, denatured in 95% formamide—0.01 × SSC at 70°C for 2½ hours, and hybridized with the c-RNA for 18 hours at 65°C. The hybridization medium contained cRNA from a Cot fraction of 4×10^{-3} to 1 at a concentration of 1 μg/ml in 6 SSC, pH 6.8 and with a specific activity of 1.7×10^{7} cpm/ml of acid-insoluble material. Chromosomes were exposed for 22 days with an NTB2 emulsion (1:1 dilution) and subsequently stained with Giemsa. Note the general agreement between the brightly fluorescent bands and c-RNA hybrized chromosomal segments. One of the main exceptions is the telomeric segment of the short arm of chromosome 1. The heavy labeling of the negative fluorescent pericentromeric segments of chromosomes 1, 9, and 16 are C-banding positive and are known to be enriched in satellite II and III by *in situ* hybridization (from Sanchez and Yunis, 1974a).

a Cot fraction of 4×10^{-3} to 1 (Sanchez and Yunis, 1974a). Although the arrangement of DNA in chromatin is rapidly being determined, the specific association and functional relationship of histones and acidic proteins to the DNA of the various kinds of chromatin remains to be ellucidated (Comings, 1972).

It is now possible to design a chromosome model (Fig. 4) that takes into account the folded chromatin fiber model of DuPraw (1970),

Fig. 4. Chromosome model in which the fine structure of chromosomes, the G-banding pattern, and the localization of repetitive DNA are combined to illustrate various structural elements of the chromosome. A, perinucleolar and pericentromeric constitutive heterochromatin, rich in the highly repetitive satellite DNA; B, secondary constriction of nucleolar organizer, site of 18 and 28 S rRNA cistrons; C, primary constriction or centromere; D,E, minor and major dark G-bands, rich in intermediate repetitive DNA, spacing vital genes such as tandem gene duplicates for 5 S rRNA, 4 S tRNA, and 9 S histone IV; F, light G-bands, where the bulk of the structural genes spaced by intergenic segments are believed to be localized.

the ultrastructural visualization of chromosome bands in humans by Bahr *et al.* (1973), the presence of approximately 40% of repetitive DNA in the diploid nuclei of eukaryotes (Britten and Kohne, 1968), the localization of satellite DNA in centromeric regions, and the preferential localization of the intermediate repetitive DNA in the darkly staining chromosomal bands of *Drosophila* (Jones and Robertson, 1970; Gall *et al.*, 1971) and man (Sanchez and Yunis, 1974a). This hypothetical model also makes use of the widely accepted concept (based on gene mapping and mutational rates) that the number of essential structural genes may not exceed 5000 in *Drosophila* (Müller and Prokofyeva, 1935; Lefevre, 1971; Judd *et al.*, 1972) and 3×10^4 in mammals (Müller, 1967; Ohta and Kimura, 1971), inspite of the presence of DNA amounts that could theoretically code for 15×10^4 genes in *Drosophila* and 3×10^6 genes in mammals. In this model, mammalian chromosomes are believed to be composed of long chromatin fibers the bulk of which approximately 80%) is arranged in packed and predominantly horizontal loops (Bahr, 1973) which are generally visualized as deeply staining bands in the light or fluorescent microscope and are rich in repetitive and other nongenic DNA. The remaining DNA is made up of genic and intergenic DNA that is arranged in a looser and somewhat vertical fashion and is generally localized in the noncentromeric and lightly stained chromosomal bands. Pericentromeric and perinucleolar bands are rich in highly repetitive satellite DNA's and correspond to the classic constitutive heterochromatin (expressed as chromocenters) of interphase nuclei (Heitz, 1934). The dark bands on the chromosome arms are enriched in intermediate repetitive DNA and correspond in part to the intercalary heterochromatin (expressed as heterochromatic fibers with varying degrees of folding and packing) of interphase nuclei (Lee and Yunis, 1971a,b). This DNA replicates during the second half of the DNA synthetic period, is nontranslational (largely nontranscriptional), and plays vital roles in chromosome structure and cell regulation (Yunis and Yasmineh, 1971; Walker, 1971; Yasmineh and Yunis, 1973; Yunis, 1973) (Table I). The presence of a large amount of nongenic DNA in mammals, coupled with the evidence that the chromosomes of *Drosophila* contain approximately 5000 bands with an average of only one essential gene per band–interband region (Judd *et al.*, 1972), makes it possible to predict chromosome band defects with moderate to no phenotypic effects. Indeed, since the discovery of the new banding techniques it has been possible to observe chromosomal breaks and rearrangements involving the majority of the chromosomes of man with a propensity for chromosomal regions rich in nongenic DNA (such as centromeres