CHANGES IN THE CHROMOSOME PROFILE AS A RESULT OF CYTOLOGICAL TREATMENT

V. A. Benyush and A. I. Ibraimov

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Aspects of the morphology of chromosomes as three-dimensional structures detectable by optical methods were examined. Treatment of specimens with certain solutions and fixatives containing water, and homogeneous or differential staining were found to produce characteristic changes in the chromosome relief. The reversibility of the changes in relief is interpreted as the conversion of the chromosonal material into a state of greater or lesser density, accompanied by changes in its consistency of the sol-gel type. On the basis of experiments on the transformation of differential staining into homogeneous it is postulated that the differentiation revealed is due to structural features of the body of the chromosome.

The main purpose of this investigation was to study the morphology of the chromosome as a three-dimensional structure. Using special illumination the relief of the chromosomes was investigated and changes in that relief in the course of experimental procedures were analyzed.

EXPERIMENTAL METHOD

Preparations of a culture of human lymphocytes obtained by the standard method were used: hypotonic solution 0.075 M KCl, fixative methanol/acetic acid (3:1), drying without incineration. An aqueous solution of azure and eosin (0.02 and 0.01%, respectively) was used for the homogeneous staining. Differential staining by Giemsa's method was carried out as described elsewhere [2]. The Ultrarot III microscope (Opton, West Germany), with dry 80 × 0.95 epiobjective, was used for the microscopic analysis and photography.

EXPERIMENTAL RESULTS

The relief of the chromosome becomes visible in the microscope if light falling from one side at an angle to the plane of the preparation is used. By closing down the aperture diaphragm of the light source to a minimum and shifting it to one side of the optical axis, it is possible to illuminate the preparation at various angles from the desired side. These manipulations, combined with refocussing for the height of the object enabled the character of the relief to be demonstrated sufficiently clearly. In its general features the pattern of relief of the chromosomes can also be transferred to photomicrographs, although these illustrations are less informative than direct microscopic analysis.

The appearance of the unstained chromosomes is shown in Fig. 1a and c. Each chromatid in this case appears as a convex half-cylinder of roughly equal height throughout its length. This picture is unchanged after subsequent treatment of the preparation with fixatives (alcohol, alcohol and acetic acid). However, water and salt solutions (for example, physiological saline and phosphate buffer) lead within a few minutes to a decrease in height of the chromatids along their axial line. The intensity of these changes increases with an increase in pH, but at the same time it depends on the specific nature of the specimens

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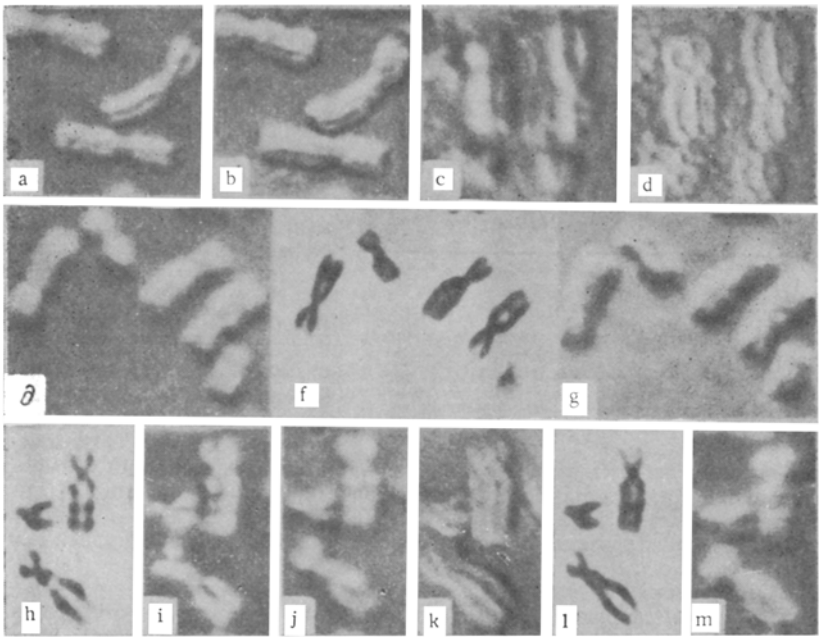

Fig. 1. Chromosomes after various procedures: a, b) fragment of unstained preparation before and after incubation for 20 min in water at room temperature; c, d) another preparation treated under similar conditions, with a more marked effect; e) preparation before staining; f, g) after staining with azure-eosin (f – transmitted light); h, i) differential staining by Giemsa's method (h – transmitted light); j) the same chromosome washed with alcohol; k) the same chromosome after washing out the dye with water; l, m) the same chromosome with restoration of the uniform distribution of dye after repeated staining with azure-eosin (l – transmitted light).

and is evidently connected with the nature of their preparation and keeping. Differences in the reactions of two specimens to treatment with neutral water at room temperature for 15 min are illustrated by Fig. 1b and d. In all the cases examined only the height of the middle part of the chromatids was appreciably reduced, whereas the height of their external contour remained unchanged, thus leading to the formation of a relief resembling a concave half-cylinder.

The relief of the homogeneously stained chromosomes did not differ significantly from that found originally although their height was increased if the staining was deep enough (Fig. 1: f and g). After removal of the dye with alcohol, the convex relief remained. Washing with water or a weak solution of alcohol led to collapse of the middle part of the chromatids (Fig. 1b and d).

The essential feature was that the changes in the relief of the chromosomes were largely reversible. For example, the "concave" relief could be made "convex" again if, having stained the preparation again, the dye was removed with alcohol. The connection between the accumulation of dye and the character of the relief could be seen particularly clearly as the result of differential staining by the Giemsa method. In that case the relief of the chromatid had the appearance of a chain of nodules corresponding precisely to areas of dense staining (Fig. 1h and i). Just as in the previous case, washing with alcohol did not affect the picture obtained (Fig. 1j) but washing with water led to the formation of a "concave" relief (Fig. 1k). In addition, visual analysis showed that where there was more dye the segments sank rather more, and as a result small craters appeared against the general background of the "concave" relief. It is important to note that differential staining can be retransformed into homogeneous, during which the relief also undergoes the characteristic changes. These changes took place after a series of repeated decolorizations and stainings with azure-eosin. After the first procedure the differential pattern and relief were reproduced sufficiently clearly, but after 3 or 4 repetitions the typical picture of homogeneous staining appeared (Fig. 1l and m).

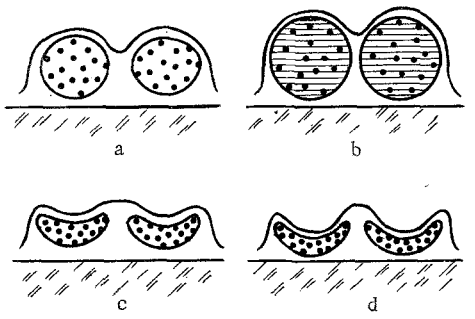

Fig. 2. Schematic illustration of transverse section through a chromosome on a cytological preparation: a) unstained chromosome; b) stained chromosome; c) unstained chromosome after incubation in water; d) chromosome after removal of dye with water.

The special features of the changes in chromosome relief and the reversibility of these changes suggest that the chromosomal material occupies a greater volume in the cytological preparation and is in a more porous state than in the intact chromosome. The increase in volume takes place during hypotonic treatment, while the fixative, replacing the water, evidently reinforced the internal structures so that the volume of the chromosome remains increased even after drying of the preparation. In a dry state and also, evidently, in an alcoholic medium, the chromosomal material has sufficient rigidity, whereas in water, especially in an alkaline solution, it softens and becomes gel-like in consistency. With evaporation of the water, the body of the chromosome may become denser because of removal of the pores formed previously, accompanied by a decrease in height of the relief. In addition, the formation of the characteristic relief of the concave half-cylindrical type together with various other observations indicate that the chromosomes in these preparations are embedded in a dense layer of aromatic substance, probably formed from residues of nuclear and cytoplastic material. A transverse section through a chromosome after treatment under various conditions is shown schematically in Fig. 2. Section A corresponds to the unstained chromosome, section C to a chromosome which has been incubated in water or in salt solutions. After intensive staining the section of the chromosome acquires the form B, and this state is fixed if the dye is extracted with alcohol. After removal of the dye with water the picture illustrated by section D appears. After differential staining, the densely and weakly stained segments correspond to sections C and A, and after removal of the dye with water, to sections D and B.

In conclusion some observations may be made about the factors responsible for the appearance of the differential staining. Two possible explanations of this phenomenon are discussed in the literature. According to one hypothesis, during staining by Giemsa's method the dye is bound chiefly with double-stranded DNA, and it reveals only those regions where the stained substrate is in the native (or renatured) state [3]. The starting point for the second hypothesis is that under the conditions created for obtaining differential staining, some proteins are extracted from certain regions of the chromosome but the dye is bound differently where the DNA/protein ratio differs [5]. The transformation on differential staining into homogeneous described above does not accord with either of the existing hypotheses, for no processes similar to the renaturation of DNA of the restoration of the lost protein could take place during restaining. The factors concerned in revealing differentiation on the chromosome are evidently the character of the chemical composition or the architectonics of the individual segments forming the chromosome. It can be postulated on the basis of the observations on relief that, just as the chromosome as a whole responds differently to a certain procedure (treatment with water, alkali, alcohol, etc.), so also do its individual segments behave differently during the combined action of several agents. This behavior could be expressed, for example, as a varied degree of swelling, changes in the mutual arrangement, density, and conformation of the elementary structures, and the consequent facilitation or prevention of access of the dye to the staining substrate. In turn, the hypothetical heterogeneity in the chromosome structure is connected with the fact that the differentially stained segments are as a rule those which differ in their reproductive function both in the stage of DNA synthesis and in the period of condensation of the mitotic chromosome [1, 4].

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