NATURE OF THE LONGITUDINAL DIFFERENTIAL STAINING OF METAPHASE CHROMOSOMES

A. F. Zakharov, A. I. Ibraimov, and V. S. Demintseva

UDC 576.312.32.086.15

Patterns of differential longitudinal spiralization of chromosomes induced by the addition of 5-bromodeoxyuridine (200 μg/ml) for 5-7 h before fixation of the cells were compared with the differential staining of normally spiralized chromosomes with Giemsa stain in chromosomes of lymphocytes in human blood cultures. The patterns of differentiation of both types were found to coincide for all chromosomes of the normal karyotype; areas of incomplete spiralization corresponded to areas staining intensively with the Giemsa stain. The significance of these results in connection with the mechanisms of differential staining of metaphase chromosomes is discussed.

KEY WORDS: metaphase chromosomes; differential staining; differential spiralization.

The ability of metaphase chromosomes to give longitudinal differential staining by the use of certain fluorochrome or Giemsa's stain has recently been discovered [4, 6, 7, 11]. Because of the constancy and specificity of the pattern of this staining in individual chromosomes the corresponding methods have become widely used for chromosomal identification. However, the nature of this phenomenon is still unexplained. Initially differences in the ability of areas of the chromosome to bind the dye were explained by differences in the content of repeating nucleotide sequences of DNA. This explanation met with several difficulties; in particular, it ignores the possible role of the protein component of the chromosome in this phenomenon and relations between the chromosomal DNA and proteins [1, 5, 8].

The writers have shown previously that different parts of the chromosomes pass through mitotic spiralization at different times and this unsynchronized spiralization in mitosis is closely connected with the unsynchronized longitudinal replication of the chromosome [12].

The comparative study of patterns of differential staining and differential spiralization in the same chromosomes is of great scientific interest. The basic results of such a comparison conducted on human chromosomes and their importance for the elucidation of the nature of differential staining of chromosomes in the metaphase of mitosis are the subjects of this paper.

EXPERIMENTAL METHOD

Chromosomes of peripheral blood lymphocytes of normal persons of both sexes, cultivated by the usual method, were investigated. Chromosome preparations were made by drying. Differential spiralization was detected by treating the chromosomes with 5-bromodeoxyuridine (BDU) by the method described earlier [2]. Two variants of the so-called G-technique were used for differential staining [10]. In the first, Giemsa's stain was used in a dilution of 1 : 50 with phosphate buffer, pH 6.8 [3]. The second form differed from the first in that trypsin (0.25%) was added to the solution of dye in buffer in a final dilution of 1 : 50.

Laboratory of General Cytogenetics, Institute of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Kraevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 78, No. 7, pp. 106-108, July, 1974. Original article submitted September 10, 1973.

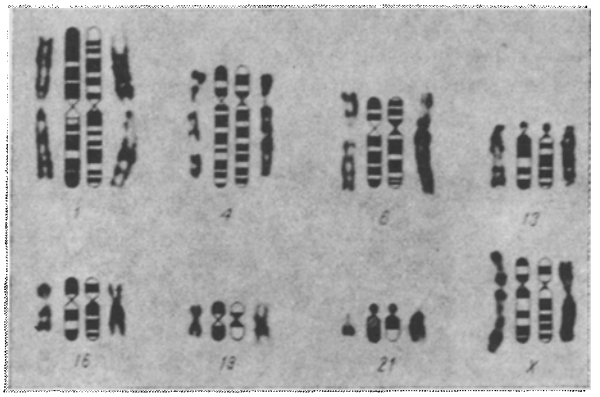

Fig. 1. Longitudinal differentiation of individual human chromosomes (1, 4, 6, 13, 16, 19, 21, X) detected with the aid of BDU (on the left of schemes) or by G-staining (on right of schemes) and schematic interpretation.

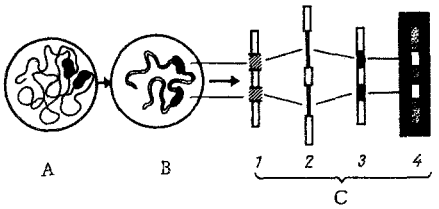

Fig. 2. Scheme of hypothetical connection between differential condensation and longitudinal differential staining of the chromosome: A) interphase cell nucleus; B) prophase; C) metaphase; 1) late replicating segments corresponding to densely condensed areas in A and B; 2) segments differentially spiralized under the influence of BDU; 3) differentiation by G-staining; 4) differentiation by Q-staining.

EXPERIMENTAL RESULTS

Compared with the picture of differential spiralization induced by BDU, longitudinal differentiation of the chromosomes detected by the G-technique was richer in details and in successful preparations it affected all chromosomes of a given metaphase plate. Beside these differences, during careful comparison of the pictures of BDU-induced irregular spiralization and of differential G-staining a basic similarity was found in the number, size, and mutual arrangement of the principal segments in each chromosome of the human karyotype. This state of affairs is illustrated in Fig. 1 for chromosomes 1, 4, 6, 13, 16, 19, 21, and X. Areas of delayed spiralization (i.e., of late replication) correspond to areas staining intensely with Giemsa dye. Because of coincidence between these areas and the actively fluorescent areas of the chromosome when stained with quinacrine or quinacrine mustard [10], the patterns of differential spiralization and of differential fluorescence can also be said to correspond.

It was shown previously that the replication time of a chromosomal segment and the time of its mitotic spiralization correlate closely [1, 2, 12]. There is every reason to suppose that both these processes are determined, in turn, by the state of condensation of the chromosomal segment in the interphase cell nucleus [9]. Consequently, it can be concluded from these results that the behavior of the chromosomal segment toward staining with Giemsa dye corresponds to its state in the interphase nucleus: segments in a condensed state in the interphase period are deeply stained (Fig. 2). The present results agree closely with recent observations indicating the decisive role of chromosomal proteins or their interaction with DNA in the phenomenon of differential staining [5, 8]. They also help to explain why different segments of the metaphase chromosomes could differ depending on the type of chromosomal proteins or on their relations with DNA. The metaphase chromosome evidently preserves many of the features of organization that preexist in the interphase nucleus, that are connected with differential function of the chromosome segments, and on which interaction between protein and nucleic acid macromolecules must be based. Consequently, our observations raise the question that differential staining of metaphase chromosomes is determined primarily by structural and chemical features distinguishing the organization of the chromosome in the interphase nucleus and which lie at the basis of differential longitudinal condensation of the chromosome and their differential activity with respect to the functions of reproduction and transcription associated with it.

LITERATURE CITED

1. A. F. Zakharov, Vestn. Akad. Med. Nauk SSSR, No. 1, 44 (1973).

2. A. F. Zakharov, L. I. Baranovskaya, V. S. Demintseva, et al., Tsitologiya, 15, 508 (1973).
3. Yu. V. Seleznev, Byull. Éksperim. Biol. i Med., No. 4, 122 (1972).
4. T. Caspersson, L. Zech, C. Johansson, et al., Chromosoma (Berlin), 30, 215 (1970).
5. D. E. Comings, E. Avelino, T. A. Okada, et al., Exp. Cell Res., 77, 469 (1973).
6. M. E. Drets and M. W. Shaw, Proc. Nat. Acad. Sci. (Washington), 68, 2073 (1971).
7. B. Dutrillaux and J. Lejeune, Compt. Rend. Acad. Sci. (Paris), 272D, 2638 (1971).
8. H. Kato and K. Moriwaki, Chromosoma (Berlin), 38, 105 (1972).
9. T. Kuroiwa, Exp. Cell Res., 69, 97 (1971).
10. Birth Defects, Original Article Series (New York), 8, No. 7 (1972).
11. A. T. Sumner, H. J. Evans, and R. A. Buckland, Nature, 232, 31 (1971).
12. A. F. Zakharov and N. A. Egolina, Chromosoma (Berlin), 38, 341 (1972).