VARIABILITY OF FRAGILE X CHROMOSOME EXPRESSION IN CONSECUTIVE CELL GENERATIONS

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In men with a syndrome of X-linked mental retardation, whose X chromosome has an isochromatid gap in the distal region of the long arm [fra (X) (q27)], this morphological change, usually called a fragile region, is never found in all cells of the body, but is observed as a rule in fewer than 50% of the cells. There are two possible explanations of this phenomenon: The first is that there exist in the body two independent cell populations, the cells of one of which have specific fragility of the X chromosome and inherit it strictly in cell generation. where cells of the other do not have this change; the second explanation is variability of expression of a fragile X, which is present in all cells, exists. It has been postulated that the corresponding morphological change in the interphase nucleus is present in all cells, but in metaphse it is preserved in only some of them [6]. Some observations have been made in support of the second explanation. The fragile region is brought to light as a rule when the cells are cultured in medium deficient in folic acid. The frequency of appearance of Fra (X) is affected by the pH of the medium and the duration and other conditions of culture of the cells. Direct proof of possibility of a change in the expression of the fragile region in cell generations has been obtained by clonal analysis: in all cell clones obtained from hemizygotes, the X chromosome in some of the cells has a fragile region, in other cells it does not [2, 3, 5].

A direct answer to the question of the stability of inheritance by the cell of the morphological state of the X chromosome could be obtained by analyzing the morphology of this chromosome in two or more consecutive cell divisions. A suitable method for studying this problem could be to prevent the cell division after the first cycle of reproduction and to analyze the X chromosome in any tetraploid or endoreduplicated cells produced.

The aim of this investigation was to study the morphology of the fragile X chromosome in two consecutive cell cycles.

EXPERIMENTAL METHOD

Peripheral blood lymphocytes obtained from a 9-year-old oligophrenic boy, with the diagnosis of imbecility, served as the test object. Cytogenetic analysis undertaken by the writers previously showed that during culture of the patient's lymphocytes on medium 199 the fraction of cells with a fragile X chromosome was 30%, whereas during culture on Eagle's medium containing 5-fluorodeoxyuridine, the fraction was 35%.

In the present investigation the patient's peripheral blood lymphocytes were cultured for 120 h on medium 199 with low folic acid concentration (0.01 mg/ml) in the presence of 5% bovine serum. To obtain tetraploid cells the method in [4] was used with certain modifications: after 54 h of culture, colcemid (from "Serva" West Germany) was added to the nutrient medium in a concentration of 0.5 μg/ml, and after incubation for 2 h the cells were washed, and colcemid (0.1 μg/ml) was again added and remained until fixation. The cells were fixed and specimens prepared by the standard method. The specimens were stained with Giemsa's stain (from Merck, West Germany), and this was followed by morphological analysis of the X chromosome in diploid and tetraploid cells. Chromosomes of the C group with a specific morphological fragile region were identified by restaining the analyzed specimens by the G method.

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TABLE 1. Characteristics of Expression of the Fragile Region of the X Chromosome in Diploid and Polyploid Cells

Ploidy of cells	Number of cells	Number of cells with expression of fra(X) (q27)	
		absolute	%
Diploid	110	25	22,7
Tetraploid	27	15	55,5
Endoreduplication	4	1	25,0
Total	31	16	51,6

Legend. In tetraploid cells, of the 2 X chromosomes expression of the fragile region was observed in all cases in one X chromosome.

EXPERIMENTAL METHOD

The results of chromosome analysis are given in Table 1. Among 110 diploid metaphase plates studied, 25 (22.7%) of the plates contained an X chromosome with a distinct isochromatid gap in segment q27. In these same specimens 31 tetraploid cells were analyzed, 4 of which were endoreduplicants, whereas in the rest the reduplicated chromosomes were spatially separated (Fig. 1). On careful analysis of the X-chromosome morphology in the tetraploids, two striking features were found. First, an X chromosome with a distinct fragile region was contained by more than half of all the tetraploids, and second, there was not more than one fragile X chromosome in any of the 16 such cells analyzed. On the basis of these two facts an unequivocal conclusion can be drawn as regards expression of the fragile region in two consecutive generations of dividing lymphocytes. In the case when this particular X chromosome was preserved and reproduced its characteristic morphology in two reproduction cycles (Fig. 2), the fraction of cells with an fra (X) chromosome would be the same among diploid and tetraploid metaphases, but in the latter, both X chromosomes would contain a fragile region. In fact, none of these conditions held good. The fraction of cells with a fragile X chromosome was increased twofold among the tetraploids, whereas in all cells the fragile region appeared in only one of the two reduplicated chromosome. In other words, in each such cell the fra X (q27) region was not expressed in one of the two chromosomes.

Both facts are in favor of the extraordinary lability of expression of the fragile site in the X chromosome. Arising from the state of the morphology of the original chromatid and the possibility of appearance or disappearance of the fragile site in two reproduction cycles, four different versions of the change in expression can be submitted, which lead to the same morphological picture: the presence of one fra (X) in a tetraploid cell (Fig. 2). The fact that sister chromatids, replicating in a completely identical metabolic medium, behave in a diametrically opposite manner as regards expression of the fragile site, makes it significantly more difficult for us to understand the factors determining the appearance or disappearance of this structural change.

Data on the X chromosome are evidence of the extreme variability of expression of fra (X) (q27) in consecutive cycles of reproduction, do not rule out the possibility that this state of the q27 segment may be preserved in consecutive cell generations. This is indicated by the discovery of a specific fragile site fra (q26) in two autosomes 6 in one of the tetraploid cells. As we know, this region is also folate-sensitive and may be exhibited in autosome 6 independently of the state of the X chromosome [1].

The results, adding to those of previous investigations by clonal analysis, thus leave no doube that the change in morphology of the X chromosome, in the form of an isochromatid gap, observed in X-linked mental retardation, is extremely variable in its manifestation, even in consecutive cell generations. This state of affairs makes it even more difficult for us to understand the mechanism of appearance and biological significance of this cytologic

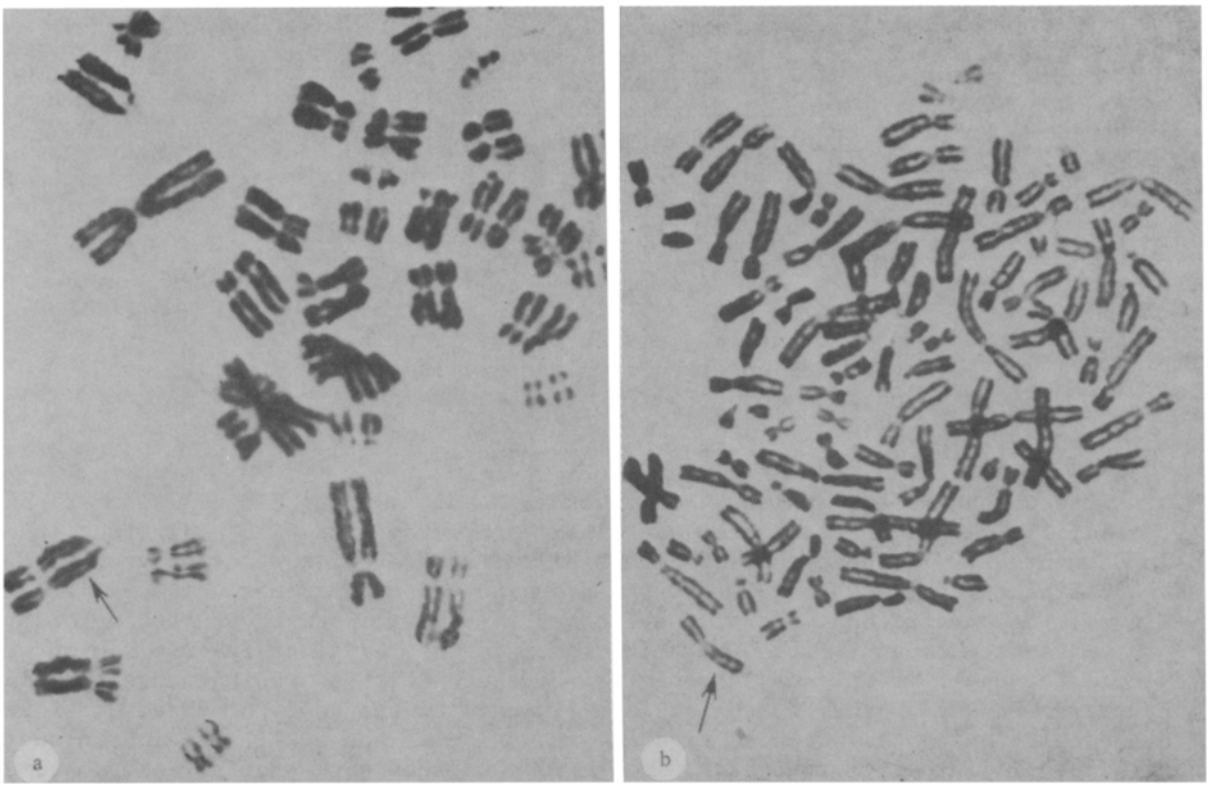

Fig. 1. Fragment of tetraploid cells. a) endoreduplicated chromosomes lie side by side; b) reduplicated chromosomes are spatially separated. Arrows indicate fra (X). Homogeneous Giemsa staining. Magnification: objective 100×, ocular 10×.

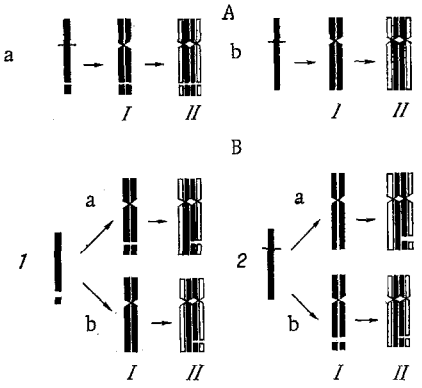

Fig. 2. Scheme showing transmission of state of fragile region of X (q27) in cell generations. A) stability of state of fragile region in first (I) and second (II) cycles of reproduction. X chromosome with expression of fragile site (a) or without expression (b); B) variability of state of fragile region in first (I) and second (II) cycles of reproduction. Variant 1: expression of fragile site in original chromosome; a) its preservation in cycle I and disappearance in one of the daughter chromosomes of cycle II; b) its disappearance in cycle I and appearance in one of the daughter chromosomes in cycle II. Variant 2: absence of fragile site in original chromosome; a) expression absent in cycle I, and appears in one of the daughter chromosomes of cycle II; b) expression of fragile site in cycle I and its disappearance in one of the daughter chromosomes of cycle II.

cytologic phenomenon. Further investigations are needed to explain how this phenomenon is linked with the basic monogenic defect.

LITERATURE CITED

1. L. I. Baranovskaya, Kh. A. Bedel'baeva, and A. F. Zakharov, Genetika (in Press).
2. G. Barbi, P. Steinbach, S. Bauer, and W. Vogel, Human Genet., 69, 106 (1985).
3. E. M. Bryant, G. M. Martin, and H. Hoehn, Cytogenet. Cell Genet., 35, 223 (1983).
4. B. Herreros and Giannelli, Nature, 216, 286 (1967).
5. P. Steinbach, G. Barbi, S. Bauer, and A. Wiedenmann, Human Genet., 63, 404 (1983).
6. G. R. Sutherland, Int. Rev. Cytol., 81, 107 (1983).

DYNAMIC AND STATIC CHROMATIN

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In papers published in May, 1984, by Worcel and co-workers [8, 13] the concept of the existence of two types of chromatin — dynamic and static — in cells of eukaryotes was introduced. In the same month the present writers published their own data [3], which also suggested the existence of the same two types or two states of chromatin [3]. The grounds for introduction of this concept by ourselves and by Worcel's group were different, but they are mutually complementary and provide a firmer basis for views on the dynamic nature of active and potentially active chromatin and the static nature of transcriptionally inactive chromatin [3, 4, 8, 13].

In our opinion, the most important criterion for differentiation between dynamic and static chromatin is the character of relations between their main components — DNA and histone, which can easily be tested by the presence of absence of exchange of chromatin histones with free histones. Some preparations of chromatin did not show this kind of exchange, even if histone was added to them in a 200-fold excess. After ultrasonic treatment of chromatin the fraction of histones H2A + H2B, exchanged with the more competitive H3 + H4, increased, and exchange was recorded when total histone was added in only a fivefold excess [3, 4]. This weakening of the DNA-histone bond cannot be explained by a decrease in the fraction of masked (inaccessible for free histones) nucleosomes due to destruction of interfibrillary junctions in chromatin (an increase in the degree of dispersion of chromatin), for histone H1 is completely displaced in all chromatin preparations if the ratio between added histone to chromatin DNA is more than unity. This proved that all nucleosomes are accessible and that in each of them histone H1 interacts dynamically with DNA. We postulated that static relations of histones H2A + H2B with DNA are determined by the presence of fibrils with circular superstranded DNA (cssDNA) [3, 4] in chromatin preparations, in which the dynamics of the nucleosomal nuclei is limited (fluctuation unwinding), and histone exchange is thereby blocked. Linearization of cssDNA by ultrasound ought to facilitate the dynamics of nucleosomal nuclei and the dynamics of relations between their histones and DNA.

To test the hypothesis, in the investigation described below the possibility that cssDNA may be present in chromatin preparations was studied, using the phenomenon of extremal dependence of viscosity of solutions of covalently closed circular superstranded DNA on concentration of the intercalator [9, 12], which is exhibited also in cell lysates [1, 10] containing cssDNA in nuclear nucleoids not mechanically destroyed, as the criterion.

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