LONGITUDINAL DIFFERENTIATION OF HUMAN X-CHROMOSOMES IN CASES OF X-POLYSOMIA

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Longitudinal differentiation of X-chromosomes into segments with different degrees of coiling was demonstrated by means of 5-bromodeoxyuridine in the cells of women with 48 XXXX and 47 XXX karyotypes. All the supernumerary X-chromosomes were distinguished by the greater length of their weakly coiled segments. The pattern of differentiation of these chromosomes was constant in all cells of both women and characteristic of the late-replicating X-chromosome of female diploid somatic cells. The results show conclusively that X-chromosomes can be identified and the late-replicating forms distinguished among them without the use of autoradiographic methods.

Previous investigations in the writer's laboratory showed that the late-replicating X-chromosome can be identified together with its homologue with the aid 5-bromodeoxyuridine, added to a culture of lymphocytes from the peripheral blood of women, without the need to resort to autoradiographic methods. This can be done because of inhibition of the coiling of the chromosomal segments with a late replication time, so that the chromosomes acquire the specific picture of differentiation into segments of different densities [1, 2].

The object of the present investigation was to use this method to demonstrate the pattern of longitudinal differentiation of human X-chromosomes in cases of X-polysomia and to study the functional morphology of these chromosomes when present in a higher than normal number.

EXPERIMENTAL METHOD

Chromosomes of the peripheral blood lymphocytes of two women with chromosome sets 47 XXX and 48 XXXX were investigated. The presence of supernumerary X-chromosomes in these patients was demonstrated before this investigation began as the result of clinical and ordinary cytological tests. Blood was cultured in the usual way. The 5-bromodeoxyuridine was given in the same way as in the previous investigation. Colcemide was added in a concentration of 0.3 μg/ml 1.5 h before fixation to produce accumulation of metaphases. Chromosome preparations were obtained by drying and staining with azure-eosin. The technique of analysis of the preparations was described previously.

EXPERIMENTAL RESULTS

During total analysis of the preparations chromosomes with the specific morphological pattern typical of X-chromosomes were found in almost 70% of cells examined from both patients. In a large proportion of cells, besides the X-chromosomes the autosomes of the set were segmented. In some cases differential delay of coiling was observed only in the X-chromosomes.

* Material for the investigation was obtained from Doctor Yu. I. Filippov (Department of Pathophysiology and Schizophrenia, Institute of Psychiatry, Academy of Medical Sciences of the USSR) and from the Institute of Progenesis [7].

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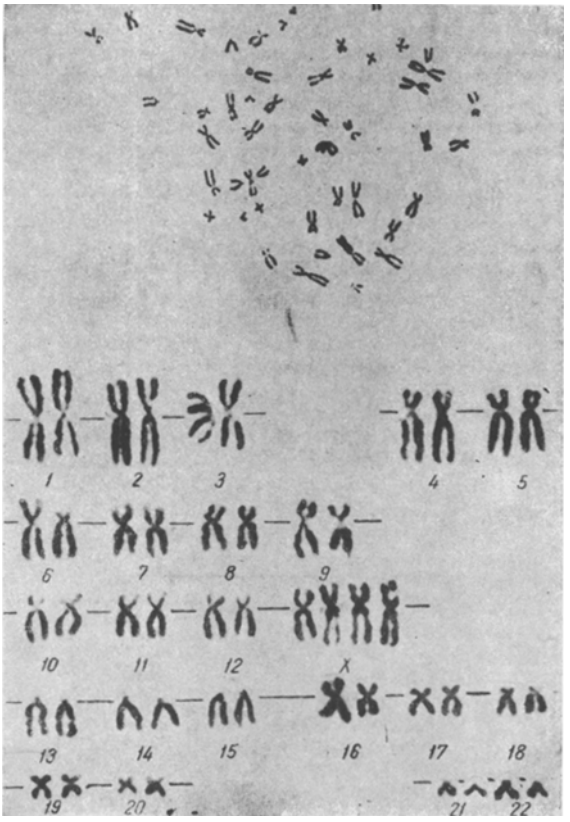

Fig. 1. Chromosome set of the 48 XXXX patient after treatment with 5-bromodeoxyuridine: clear identification of four X-chromosomes in the set, three X-chromosomes are distinguished by the greater length of the weakly coiled segments.

The 48 XXXX Chromosome Set. Altogether 30 metaphase plates showing clear differentiation of X-chromosomes along their length were examined. This particular culture, it will be noted, was distinguished by its extremely slow reaction to 5-bromodeoxyuridine. In most metaphases a weak degree of segmentation was observed along the length of the X-chromosomes and also of the autosomes. Nevertheless in all cells examined it was possible to detect all four X-chromosomes with their characteristic pattern, and the degree of delay of coiling in three chromosomes was more marked (Fig. 1). The character of longitudinal differentiation of these chromosomes remained unchanged from one cell to another, and in three of the X-chromosomes it corresponded to the fine morphological pattern of the late-replicating X-chromosomes of normal diploid cells. These chromosomes were distinguished by having a dense region in both arms near the centromere. Two other densely coiled but smaller segments, separated by a small constriction band, were observed in the distal part of the long arm. The whole of the central part of this arm was occupied by a region with considerable delay in coiling. In the short arm the dense region near the centromere and the equally condensed telomere segment were separated by a not very wide but sufficiently deep constriction band. The fine morphology of the fourth X-chromosome corresponded to the morphology of X_1 in normal diploid cells. Just as in the previous investigations, analysis of the morphology of the X-chromosome from different cells revealed some variability in longitudinal differentiation of the supernumerary X-chromosomes, but this variability did not alter the basic pattern of segmentation, but merely reflected differences in the degree of delay of coiling along the length of these chromosomes.

Comparison of the three chromosomes described above in each cell revealed certain differences in the fine morphology of one chromosome by comparison with the other two. They were manifested as the lower density of the segments near the centromere and telomere in one of the three homologues (Fig. 2).

The 47 XXX Chromosome Set. In 30 metaphase plates it was easy to identify three chromosomes with the characteric pattern of longitudinal segmentation of X-chromosomes (Fig. 3). The longitudinal differentiation pattern of two of these, because of the lower density of coiling in individual segments, corresponded fully to the pattern of the late-replicating X-chromosome of normal cells. These chromosomes were iden-

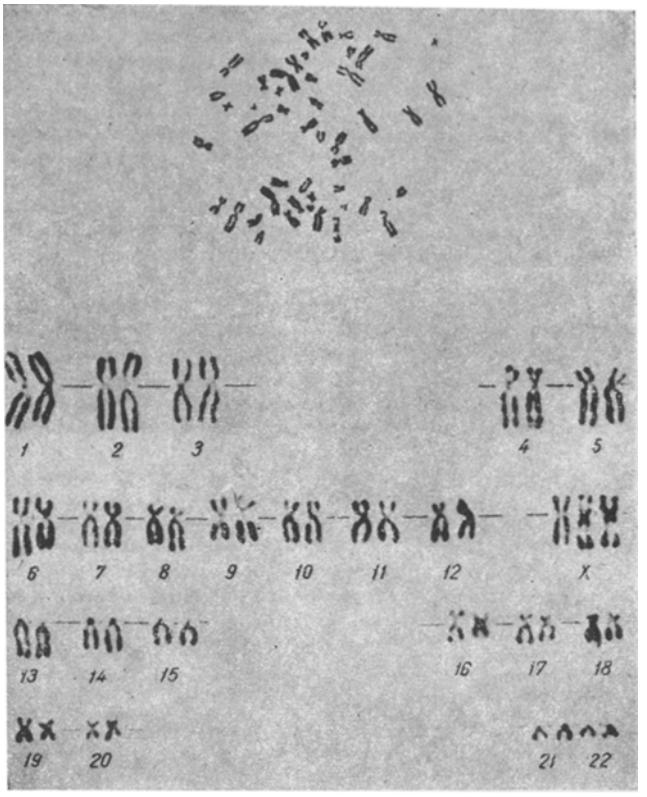

Fig. 2. Examples of pattern of longitudinal differentiation of X-chromosomes from the cells of women with 48 XXXX and 47 XXX sets of chromosomes.

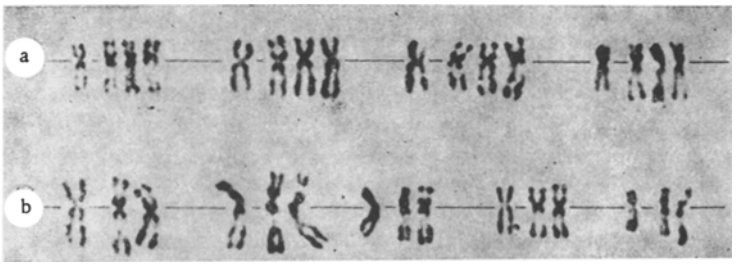

Fig. 3. Chromosome set of a 47 XXX patient after treatment with 5-bromodeoxyuridine: clear identification of three X-chromosomes, delay of coiling in two X-chromosomes is more marked.

tified as supernumerary X-chromosomes in the XXX system. The fine morphological pattern of these chromosomes was exactly the same as that described above, and in this case the delay of coiling between these chromosomes was only slightly asynchronous. The third X-chromosome, by its morphology, could be classed as the early-replicating homologue (Fig. 2).

The results of the investigation show conclusively that the method of differential delay of coiling using 5-bromodeoxyuridine can be used to identify with considerable reliability not only the early-replicating homologue, but all the supernumerary late-replicating X-chromosomes in the set without the need to resort to autoradiography. The new methods used nowadays to detect longitudinal differentiation of chromosomes make it possible to identify the X-chromosomes in the set, but without the use of autoradiography it is impossible to distinguish the late-replicating homologue among them [3, 4, 11, 12].

Analysis of the fine morphology of the X-chromosomes in the cases described above showed that the segmentation pattern was identical in all the X-chromosomes, but coiling of one chromosome (X_1) was sub-

ject to less delay in each cell studied from both patients. The three X-chromosomes in the 48 XXXX set and two in the 47 XXX set were distinguished by their particularly marked delay of coiling in the middle part of the long arm. Complete agreement between the segmentation pattern along the length of these X-chromosomes and the pattern obtained in the late-replicating X-chromosome of normal diploid cells suggested that they were included among the late-replicating chromosomes of the set. The preliminary autoradiographic findings have confirmed the validity of this identification. Numerous autoradiographic studies have shown that all the supernumerary X-chromosomes in cases of X-polysomia always incorporate the label intensively at the end of the S-period [6, 8, 9].

It was shown previously that the degree of delay of coiling along the length of the chromosomes gives indirect evidence of the replication time of some of the segments relative to the others [1, 2]. Analysis of the distribution of label along the length of the morphologically normal late-replicating X-chromosome differentiated by means of this method showed that it contained clearly localized segments of early replication, which corresponded to the densely coiled regions of this chromosome. The presence of analogous segments in the supernumerary X-chromosomes is indirect evidence of the incompleteness of inactivation of the heterochromatinized X-chromosome when changes are found in its number.

The asynchronous character of the delay in coiling of the late-replicating X-chromosomes, observed in both cases, even though very slight in degree, is particularly interesting. Some degree of interchromosomal asynchrony of replication of the supernumerary X-chromosomes has been described [5, 10]. It is possible that in this case there was a true difference between the supernumerary X-chromosomes in their time of DNA replication, which may possibly reflect differences in their degree of genetic inactivation.

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