EFFECT OF 6-THIOGUANINE ON MITOTIC COILING OF THE CHROMOSOMES

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Delay in mitotic coiling of chromosomes of Chinese hamster cells after treatment with the guanine analogue 6-thioguanine is described. The combined action of two analogous – 5-bromodeoxyuridine (a thymidine analogue) and thioguanine – on mitotic coiling of the chromosomes also was studied. On the basis of the results, correlation is postulated between the nucleotide composition of DNA in the interphase chromosome and the process of mitotic coiling.

An effect of delay of mitotic coiling of marker (St_1, St_2, St_3) and sex chromosomes in Chinese hamster cells after exposure to the thymidine analogue 5-bromodeoxyuridine (BDU) was described previously [1, 2]. Since delay in coiling was observed in the late-replicating areas of the chromosomes, the segmenting effect of the analogue could be explained by labilization of the phosphodiester bonds of the DNA in areas of its activation. Delay in DNA synthesis by a feedback mechanism evidently took place in these same areas.

The work of Comings [3] showed that the late-replicating fractions of DNA from Chinese hamster chromosomes contain sufficient guanine and cytosine despite its relatively high content of thymidine and adenine. In that case the BDU-analogue of thymidine, if introduced at the end of the S-period, may not reveal

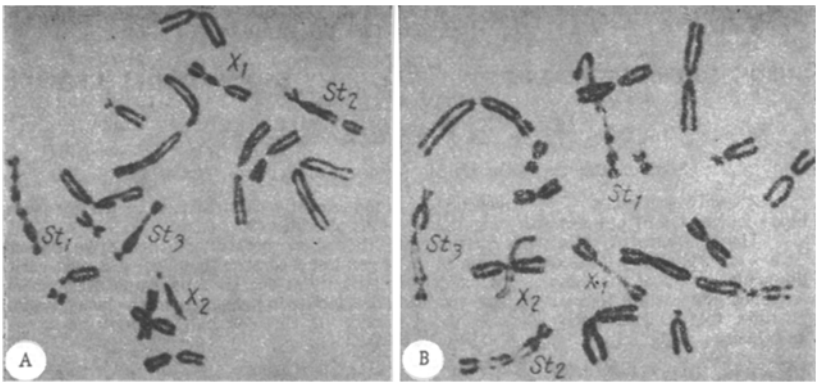

Fig. 1. Metaphase plates of animals exposed to the action of thioguanine and 5-bromodeoxyuridine: a) metaphase plate with marker (St_1, St_2, St_3) and sex chromosomes (X_1, X_2) with a segmental structure as a result of local delay in coiling. Action of TG. Metaphase plate after combined exposure to TG and BDU; b) marked delay in coiling in marker and sex chromosomes. Azure-eosin, 1000×.

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TABLE 1. Effect of TG and BDU Concentration on Coiling of Chromosomes

Concentration of analogues	Percent of metaphases with uncoiled chromosomes	Percent of metaphases with marked delay in coiling
20 μg/ml TG + 200 μg/ml BDU	78	10
8 μg/ml TG + 200 μg/ml BDU	82	22
10 μg/ml TG + 10 μg/ml BDU	60	10
5 μg/ml TG + 10 μg/ml BDU	75	5
2 μg/ml TG + 4 μg/ml BDU	34	1

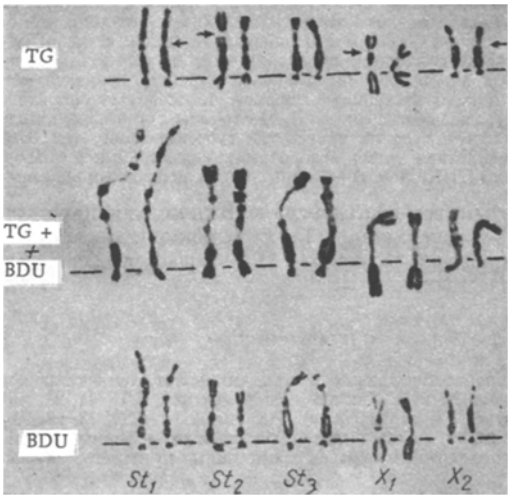

Fig. 2. Marker and sex chromosomes of a Chinese hamster with characteristic segmental pattern after exposure to TG and BDU separately and together.

the whole set of late-replicating areas of the chromosomes. Within the coiled segments there could be areas with a high content of G-C pairs. In that case further treatment with the structural analogue of guanine leads to subsegmentation, more especially because the G-C base pair, linked together with three hydrogen bonds, is more important for the stability of the DNA molecule than the A-T pair with its two hydrogen bonds.

Experiments were carried out to study the effect of the guanine analogue 6-thioguanine (TG) and the combined action of TG and BDU on mitotic coiling. As was shown earlier [4], TG is incorporated in large quantities into the DNA molecule.

EXPERIMENTAL METHOD

Cultures of fibroblast-like Chinese hamster cells (original line B 11 d-ii-FAF 28, clone No. 237) were used. Just as in experiments with BDU [1, 2], the analogues were added at the end of the S-period (3 h before fixation). BDU (8, 10, 20, and 200 μg/ml) and TG (2, 4, 8, 10, 16, 20, 30, and 60 μg/ml) were used. Both analogues were obtained from Calbiochem (USA). The procedures for fixation, preparation, and staining of the chromosome specimens were described earlier [1, 2].

EXPERIMENTAL RESULTS

The Action of TG. Addition even of minimal quantities (2 μg/ml) TG to the culture was accompanied by the appearance of many cells (27%) containing segmented marker and sex chromosomes (Fig. 1a). With an increase in the concentration of the analogue in the medium to 16 μg/ml, an increase in the number of these cells to 65% occurred. A further increase in concentration (30 μg/ml) did not increase the yield of cells with segmented chromosomes (66%), and a concentration of 60 μg/ml actually reduced this percentage to 40. It was observed previously [1] that the "segmenting" action of BDU begins with a concentration of the analogue of 25 μg/ml. The action of small quantities of TG on chromosome morphology may reflect the "structural" importance of G-C pairs indicated above.

The general pattern of segmentation of the chromosomes after treatment with TG was similar to that after treatment with BDU. However, the constrictions on the St_1, St_2, X_1, and X_2 chromosomes after exposure to TG (Fig. 2, arrows) were so pronounced as to resemble "gaps" in character, which is extremely rare after exposure to BDU. These points possibly correspond to late-replicating areas of DNA, rich in G-C pairs.

The Cytological Effect of the Combined Action of TG and BDU. The results of analysis of the combined action of the two analogues are given in Table 1. Among the cells analyzed there were many (from 34 to 82%) that contained marker and sex chromosomes with delay in coiling. The appearance of these cells can be explained by assuming that one analogue acted on one cell. Besides these cells there were others containing marker and sex chromosomes with a higher degree of delay of coiling (Fig. 1b). The number of these cells varied within wide limits (from 1 to 22%) and, as the table shows, was almost independent of the concentration of analogues in the medium. Presumably the appearance of these chromosomes was due to the simultaneous action of both analogues on the replicating area of the chromosome.

Careful study of the morphology of chromosomes with well-marked delay in coiling reveals a considerable increase in length of the uncoiled areas (Fig. 2), the appearance of small subsegments, and, in some cases, for example in the X_2 chromosome, loss of the segmental structure. This can be regarded as an extreme degree of subsegmentation. These observations may be evidence of chemical heterogeneity within the coiled segment, brought to light by the combined action of two analogues.

It can be postulated from the results of these experiments that correlation exists between the nucleotide composition of DNA in the interphase chromosome and the process of mitotic coiling.

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