DELAYED DISRUPTION OF TELOMERIC LINKS BETWEEN CHROMOSOMES IN POLYKARYOCYTES FROM HUMAN–CHINESE HAMSTER CELL HYBRIDS

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The phenomenon of delayed disruption of telomeric links between chromosomes was discovered in 1978 [1]. The essence of this phenomenon is that chromosomes with two centromeres or more are found in first division metaphases of polykaryocytes induced by colcemid during incorporation of 5-bromodeoxyuridine (BUdR) in the course of two replication cycles. The phenomenon was discovered in experiments with Chinese hamster cells: clone 237S, line B11d-ii-FAF28.

In the present investigation the possibility of reproducing this phenomenon experimentally in human–Chinese hamster somatic cell hybrids was studied.

EXPERIMENTAL METHOD

Experiments were carried out on two hybrid cell clones MOM-8-1 and MOM-8-3 at the 62nd and 72nd passages. The hybrid cells were generously provided by V. G. Chernikov (Institute of Medical Genetics, Academy of Medical Sciences of the USSR). MOM-8-1 and MOM-8-3 cells were obtained by fusion of a variant of clone 237 of strain B11d-ii-FAF28 with human myoblasts. The model number of chromosomes of MOM-8-3 and MOM-8-1 cells was 48 and 50, respectively. The karyotype of the MOM-8-3 cells contained the following human chromosomes: 1, 8, 9, 13, 17, 18, and two each of 19 and 20; the karyotype of the MOM-8-1 cells contained human chromosomes 8, 10, 12, 13, 15, 16, 21, and 22 and two each of 19 and 20. The hybrid cells were grown in rectangular 0.5-liter flasks on Eagle's medium with 10% bovine serum. Colcemid in a dose of 0.08 μg/ml was added to the actively growing culture after 30 h of cultivation. Simultaneously with colcemid, BUdR also was added in a dose of 20 μg/ml. The cells were fixed in a mixture of methanol and acetic acid (3:1). Chromosome preparations were obtained by the standard air-dried method and stained with azure–eosin.

EXPERIMENTAL RESULTS

In about half of all metaphases of both lines suitable for analysis, chromosomes with two centromeres and more than dicentrics were found (Fig. 1). In 25% of cases only one dicentric chromosome was present, two or three dicentric chromosomes in 50% of cases, and four dicentrics or more in 25% of cases. Dicentric chromosomes, once they had begun to break up, could not be reliably tested. In the control, namely cells cultured with colcemid alone or with BUdR alone, no dicentric chromosomes were found. The length of the dicentric chromosomes and the position of their centromeres varied, suggesting that chromosomes of the whole set, both Chinese hamster and human chromosomes, took part in their formation. Since the chromosomes took up BUdR during two replication cycles, differential staining in order to identify the chromosomes forming the dicentric was impossible. Often micronuclei or material of "premature condensation of chromosomes" were present in metaphases together with dicentrics, and usually this was a case of "pulverization of chromosomes." On the whole the pictures observed corresponded to those found during work with cells of clone 237S of line B11d-ii-FAF28.

Incidentally, the karyotype of clones MOM-8-1 and MOM-8-3 is not convenient for the interpretation of this phenomenon, for it contains several acentric minichromosomes (double minutes). However, the absence of dicentrics in the control tests and of radials in the experi-

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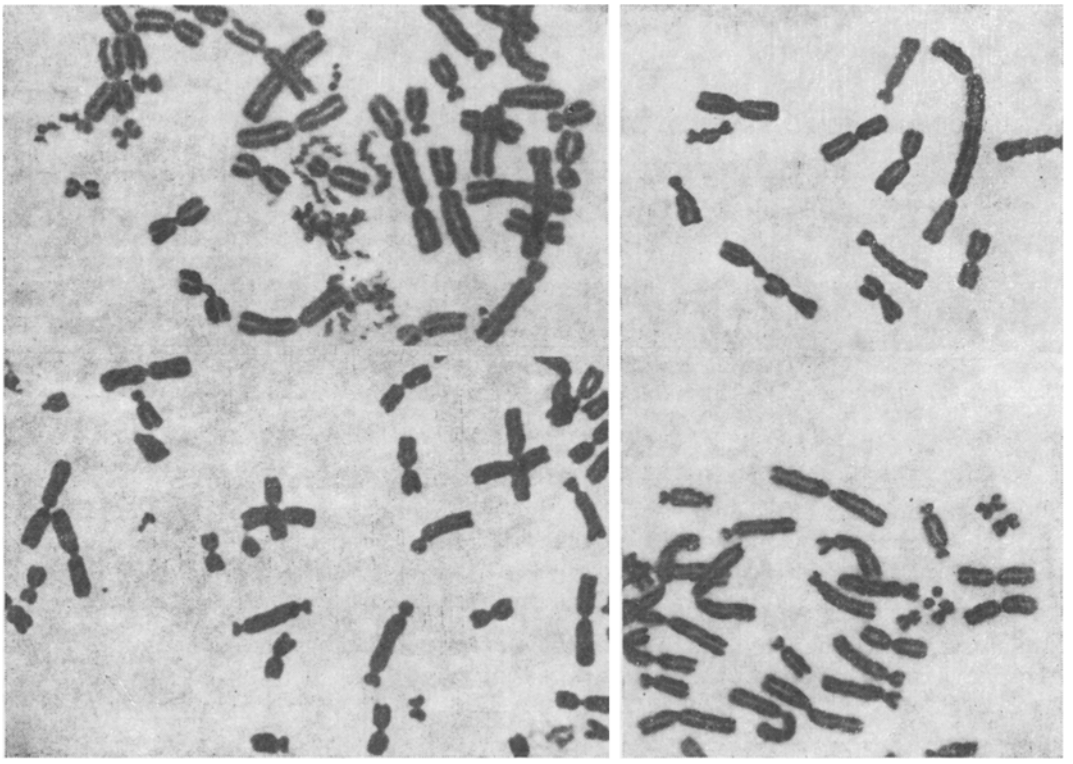

Fig. 1. Dicentric chromosomes incorporating BUdR in polykaryocytes consisting of micronuclei. Metaphase fragments. Azure–eosin, 770 ×.

mental tests is evidence that this was in fact a phenomenon of delayed disruption of telomeric links between chromosomes and not chromosomal aberrations.

This phenomenon is difficult to reproduce. Previously the writer made several unsuccessful attempts to induce it in human diploid fibroblasts and in transplantable African green monkey cells of line 4647.

Another phenomenon of polykaryocytes consisting of micronuclei, namely chromosomes with regions of delayed spiralization, was absent in the experiments with clones MOM-8-1 and MOM-8-3. Consequently it can be tentatively suggested that induction of the phenomenon of delayed disruption of telomeric links between chromosomes in human–Chinese hamster cell hybrids is dominant, unlike the phenomenon of chromosomes with regions of delayed spiralization.

Human–Chinese hamster hybrid cells MOM-8-1 and MOM-8-3 are thus the second object in which the phenomenon of delayed disruption of telomeric links between chromosomes has been reproduced.

LITERATURE CITED

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