TELOMERIC FUSION OF CHROMOSOMES IN CELLS TREATED WITH COLCEMID AND 5-BROMODEOXYURIDINE

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After combined treatment of an aneuploid line of Chinese hamster cells for 24 h with colcemid (0.05 μg/ml) and 5-bromodeoxyuridine (50 μg/ml) specific chromosomes with two or more centromeres, not present after treatment of the cells with these substances separately, were found in tetraploid metaphases of the second mitotic division. These chromosomes were the result of telomeric fusion of the chromosomes. The possible link between this phenomenon and telomeric association of chromosomes in the interphase nucleus and also with chromosomal disturbances in the Louis–Bar syndrome (ataxia-telangiectasia) is discussed.

KEY WORDS: cells with micronuclei; colcemid; 5-bromodeoxyuridine; telomeric fusion of chromosomes.

After the addition of colcemid in a concentration temporarily blocking mitosis because of the absence of cytokinesis, to a culture of Chinese hamster cells for a long time multinuclear cells containing micronuclei replicating asynchronously are formed [12]. If the micronucleus moves in advance along the cycle, after completing replication it may induce "premature condensation of the chromosomes" of the retarded micronuclei [10] and the formation of differentially condensed chromosomes [6,12].

To study the mechanisms of this phenomenon a new method was used to analyze the chronology of DNA replication, based on the use of 5-bromodeoxyuridine [5,13]. In the process of the investigation, after simultaneous addition of 5-bromodeoxyuridine and colcemid for 24 h, i.e., for the period of two mitotic cycles, a cytogenetic effect not previously described was discovered: telomeric fusion of the chromosomes.

EXPERIMENTAL METHOD

Clone 237S of line Blld-ii-FAF28 of aneuploid Chinese hamster cells was used. The cells were grown in Eagle's medium with the addition of 10% bovine serum. Colcemid (Calbiochem) in a dose of 0.05 μ g/ml and 5-bromodeoxyuridine (Schwarzbioresearch) in a dose of 50 μg/ml were added to the actively growing culture 18 h after seeding. Chromosome preparations were obtained by the usual method and stained with azure–eosin. The experiment was repeated 4 times.

EXPERIMENTAL RESULTS

It was shown previously that the mitotic cycle of clone 237S lasts 10-12 h [4]. When fixed after 24 h the culture thus contained large numbers of cells with twice the number of chromosomes as the result of the action of colcemid. Most of the tetraploid and hypotetraploid metaphases contained chromosomes which had incorporated 5-bromodeoxyuridine in the course of two replication cycles. The sister chromatids of these chromosomes stained differentially and often had a charactristic sickling [7].

Chromosomes with two centromeres were found in a high percentage of cases (mean 32) in tetraploid and hypotetraploid metaphases with micronuclei. Usually in each such cell there was one dicentric chromosome, but as many as two or three decentrics could be present, with the occasional tricentric (Fig. 1). In control experiments in which colcemid was added without 5-bromodeoxyuridine or in experiments with 5-

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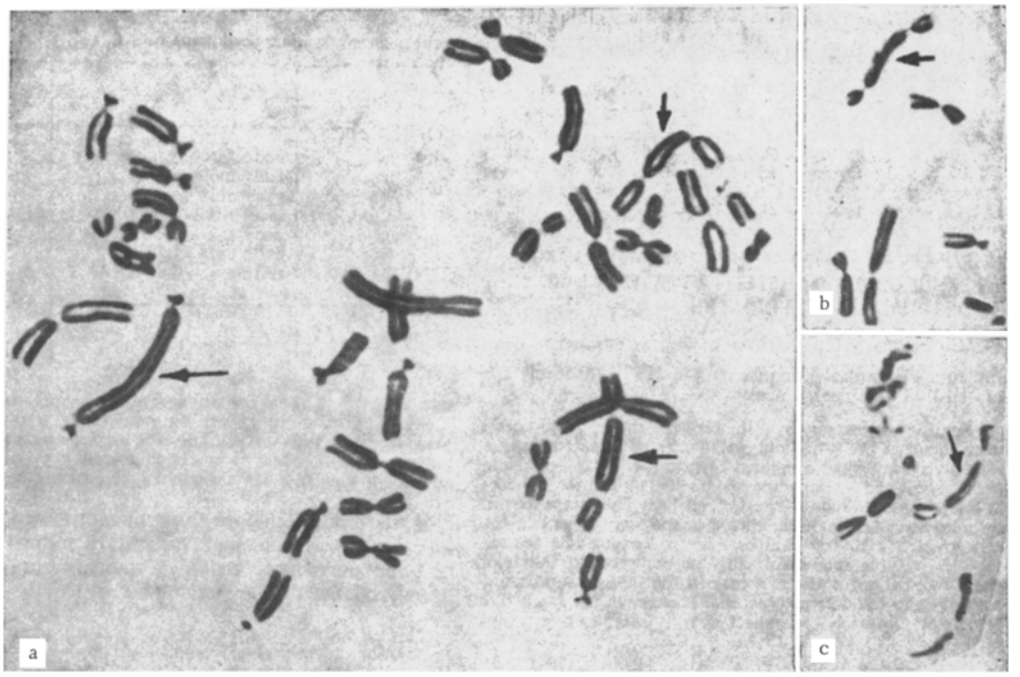

Fig. 1. Specific dicentric chromosomes in cells treated with colcemid and 5-bromodeoxyuridine: a) tetraploid metaphase with three dicentric chromosomes; b, c) fragments of metaphases with dicentrics. Stained with azure–eosin, 770 ×.

bromodeoxyuridine alone no dicentric chromosomes were found.

Chromosomes with two or more centromeres revealed by these experiments cannot be classed as ordinary dicentric chromosomes, which are the result of structural chromosomal aberrations and are formed only if the structural integrity of the chromosomes is disturbed. In aberrations of this sort acentric fragments must be present in the nearest mitosis. The metaphases now analyzed belonged in fact to the second mitosis, as was shown by the different staining of the sister chromatids; however, no acentric fragments were found in any of the metaphases studied. Consequently, the origin of the chromosomes discovered can be more correctly explained by telomeric fusion of two or more monocentric chromosomes. This is confirmed by the discovery in certain cases of telomeric associations, when interchromosomal fibrils were found between the telomeres of two or more chromosomes. The hypothesis that dicentrics are formed by fusion of chromosomes end to end was recently put forward by Hayashi and Schmid [9], on the basis of the results of a cytogenetic analysis of patients with the Louis–Bar syndrome (ataxia-telangiectasia).

In the cytogenetic literature there are descriptions of spontaneous telomeric associations of chromosomes in some species of plants and animals in meiosis and mitosis. The opinion has been expressed that a definite order of arrangement of the chromosomes in the interphase nucleus is produced through the association of their telomeres [3,8]. According to one hypothesis, the activity of the telomeres of chromosomes undergoes cyclic changes [2]. One possibility is that in the experimental model described in this paper the controlled activity of the telomere is disturbed by the action of 5-bromodeoxyuridine and colcemid, as a result of which the breakdown of the telomeric associations is disturbed in individual chromosomes, evidently arising from micronuclei.

A hypothesis can accordingly be put forward to explain the pathogenesis of the Louis–Bar syndrome (ataxia-telangiectasia), an autosomal recessive disease one feature of which is that such patients have cells and their clones with structurally changed chromosomes [1,11], among which specific dicentrics formed by end to end fusion can be found [9]. If the well-known ideas on the regulatory role of genes in the mitotic cell cycle are accepted and the existence of mutations disturbing various stages of this process is taken into consideration, the genetic disturbance of the cyclic activity of the telomeres in the Louis–Bar syndrome can be postulated. As a result of this disturbance the dicentric chromosomes described above would be formed. The dicentrics are unstable chromosomal structures, and on separation of the centromeres into the daughter cells structural aberrations of chromosomal type would be formed.

Now that it is possible, as the writer has shown, to detect telomeric fusion of chromosomes the way is open for the experimental study of the mechanisms and biological role of telomeric interaction between chromosomes in health and disease.

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INDEPENDENT INTEGRATION OF GENES CONTROLLING THE INVASIVE PROPERTIES AND STREPTOMYCIN RESISTANCE OF ENTEROPATHOGENIC STRAIN *Escherichia coli* 0124

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Crossing *Escherichia coli* K12 Hfr AB313 with an enteropathogenic strain of *E. coli* of the serological group 0124 yielded recombinants which had lost their invasiveness. The loss of invasiveness of these recombinants was not due to the acquisition of genes controlling resistance to streptomycin.

KEY WORDS: enteropathogenic strains of *E. coli*; invasiveness; streptomycin resistance; transmission of genes.

One of the factors in the pathogenicity of shigellas and certain serological groups of enteropathogenic escherichias is invasiveness. Investigations [1-3] have shown that replacement of the streptomycin region of invasive strains of shigellas and escherichias by the corresponding streptomycin-resistant region of noninvasive escherichias in conjugation experiments leads as a rule to loss of invasiveness. This suggested the existence of a special gene (or genes) controlling this property. However, the difficulty in the interpretation of these findings is that incorporation of the streptomycin-resistant region can itself cause loss of virulence because of disturbance of synthetic processes.

In this investigation an attempt was made to discover whether the loss of invasiveness is connected with the acquisition of genes of the streptomycin resistant region or of genes outside it.

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