SPONTANEOUS SISTER CHROMATID EXCHANGES IN AFRICAN GREEN MONKEY CELLS IN CULTURE

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The phenomenon of sister chromatid exchanges (SEE) was first found by Taylor by the use of tritiated thymidine followed by autoradiography [10]. However, the detailed study of this phenomenon became possible after Zakharov and Egolina had used 5-bromodeoxyuridine (5-BDU) and reliable methods of differential staining of sister chromatids had been developed [7, 9, 11].

The precise mechanism of SSE formation has not been established, but it has been shown that the frequency of SSE rises under the influence of mutagens, carginogens, and viruses and in some hereditary syndromes [4, 8, 6]. It thus seems likely that the SSE level is an indicator of the stability of the genetic material of the cell.

SSE are usually studied in cells with few chromosomes in their karyotype or in human cells. It is this fact which evidently can explain the absence of data in the literature on the frequency of SSE in African green monkey cells (2n = 60), which are widely used in experimental virologic research and in vaccine production.

In the present investigation the spontaneous frequency of SSE and the character of their distribution among cells were studied in a primary culture of kidney cells, cells of a diploid line, and cells of two spontaneously transformed lines from African green monkeys.

EXPERIMENTAL METHOD

A primary culture of kidney cells from an adult African green monkey, obtained from a production batch which had passed the biological control for the presence of contaminating viruses. Diploid cell line 5018, obtained from a graft of embryonic skin and muscle, was investigated after the 26th passage. Since the carrier type of the diploid line of cells was 60, XY, and since the X and Y chromosomes differ considerably in length, the primary culture of kidney cells was obtained from a male. Spontaneously transformed cell lines 4647 and 455 had a modal number of chromosomes of 60 and 110, respectively. Cell lines 5018, 4647, and 455 were not contaminated with mycoplasmas, as was shown by a fluorescence method with the dye Hoechst 33 258 [5].

The cells were cultured in Eagle's medium with 10% bovine serum. The 5-BDU (from Serva, West Germany) was added in a dose of 10 μg/ml to the primary cell culture for 33 h, and to the transformed cell lines 4647 and 455 for 34 and 36 h respectively, in the stage of active growth. In experiments with the diploid line 5-BDU was added in the same concentration to the culture at the time of seeding and the culture was fixed after 72 h. Treatment with colcemid (from Serva, West Germany) in a dose of 0.05 μg/ml was carried out 3 h before fixation. The chromosome preparations were obtained by the standard air-drying method. Differential staining of the sister chromatids was done by the method of Chebotarev et al. [2].

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TABLE 1. Mean Values of SSC Per Cell for Four Types of Cultures of African Green Monkey Cells

Parameter	Primary kidney culture	Line 5018	Line 4647	Line 455
Geometric mean and confidence intervals	6,9 (6,20—7,68)	9,26 (8,39—10,23)	4,50 (4,03—5,03)	9,76 (8,84—10,77)
$M \pm m$	7,96±1,03	10,46±1,06	5,14±0,49	10,28±0,86

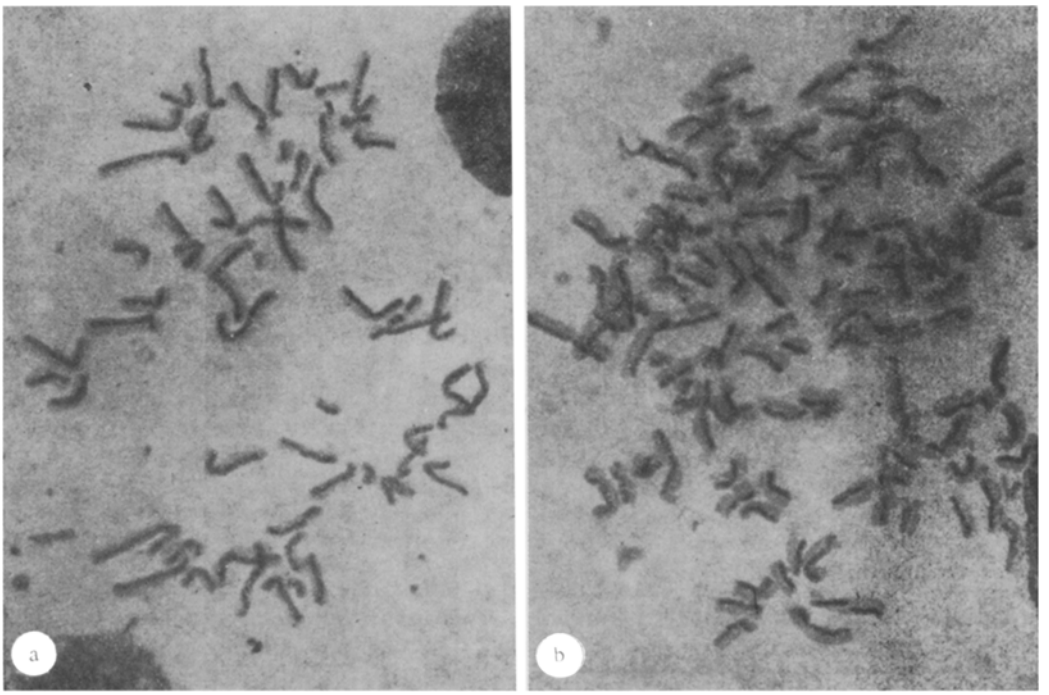

Fig. 1. SSE in African green monkey cells: a) cell from primary kidney culture with eight SSE; b) cells of hypotetraploid line 455 with 14 SSE. Magnification: objective 100, ocular 7.7.

In the primary cell culture and lines 5018 and 4647 100 metaphases were drawn and analyzed in each case, but only 50 cells were studied in line 455. The results were subjected to mathematical analysis on a Hewlett Packard 9830 B computer.

EXPERIMENTAL RESULTS

When the cells were cultured with 5-BDU, at the times indicated above the fractions of cells in the 1st, 2nd, and 3rd mitoses were 11, 71, and 18% respectively for the primary culture, 22, 50, and 28% for line 5018, 12, 76, and 12% for line 4647, and 17, 60, and 23% for line 455.

The frequency of SSE varied from zero in line 4647 cells to 47 in the primary kidney cell culture. Typical metaphase plates with differential staining of sister chromatids are illustrated in Fig. 1.

Since there is no unanimity in the literature on the character of distribution of cells by frequency of SSE, the correspondence of the results to the normal, lognormal, and β- and γ-distributions was studied.

It was found that the distribution of the cells by number of SSE did not obey the normal law in any of the cultures. In the case of the primary culture the relationship was adequately described by a lognormal distribution ($\chi^2 = 13.53$; $f = 7$). For the diploid cell line 5018 agreement was found with the lognormal ($\chi^2 = 11.96$; $f = 9$) and γ-distributions

(χ^2 = 10.88; f = 10). The character of distribution of the cells of the pseudodiploid line 4647 by number of SSE obeyed lognormal (χ^2 = 7.7; f = 4) and γ-distributions (χ^2 = 9.37; f = 5). The values of χ^2 given above for all cultures do not contradict the lognormal distribution with a level of significance of P = 0.05.

The character of distribution was not tested for cells of hypotetraploid line 455, and since good agreement with the lognormal type was found for the previous three types, it was considered *a priori* that the distribution of cells of line 455 by the number of SSE is the same.

Since the graph of a lognormal distribution is shaped like an asymmetrical curve, the geometric mean frequency of SSE per cell was determined and the values are given in Table 1. Since in most investigations of the frequency of SSE it is assumed that the distribution of the cells by frequency of SEE is normal in character, in the bottom part of the table arithmetic mean values calculated for the same sample are given.

Despite the fact that investigations of SSE have been conducted in many laboratories, information on the distribution of cells by the number of SSE is scanty. Yet this is a fundamental problem, important not only for the evaluation of experimental data, but also for the standardization of cell substrates used in the production of virus vaccines. Most workers, when estimating the spontaneous level of SSE, use the arithmetic mean, assuming that the distribution of the cells by frequency of SSE is normal. Calculations of this sort have also been used in experiments to test the effect of various factors on the cell DNA, in which the spontaneous level of SSE was used as the control.

Nevertheless, it has been shown in sufficiently large samples that the distribution of cells by the number of SSE does not obey the normal law [1, 3]. Our own experiments confirmed this fact. In the three types of cultures the distribution of cells by the number of SSE corresponded well to a lognormal distribution, which often describes processes in which factors affecting the frequency of the feature are few in number and the action of each one is small. In the case of spontaneous SSE, their appearance is probably determined by the radiation background, the quality of the culture medium, the surface of the culture vessel, etc.

The mean frequency of SSE per cell was twice as high in the hypotetraploid line as in the pseudodiploid line. This result is perfectly understandable, for the total mass of DNA in the hypotetraploid line is more than twice that in the pseudodiploid line, and this is responsible for the greater liability of the DNA to injury.

The frequency of SSE per genome was significantly lower in the primary cell culture and the transformed lines than in the diploid cell line. This can be explained both by the longer duration of the S-period for cells of the diploid line and by the experimental conditions, in wich 5-BDU was present for a longer time in the medium.

In this investigation on African green monkey cells, with a karyotype of 60 chromosomes, circumstances were encountered which can evidently lead to errors when estimating SSE in cells with a larger number of chromosomes, whose karyotype does not have the standard nomenclature.

One difficulty is that, unless he finds a sufficient number of well spread out metaphases, the investigator is forced to conduct his analysis on cells with several chromosomes superposed. In that case counting errors may lead to overestimation of the number of SSE in a single cell, or else to underestimation, but these are not reflected in the mean frequency of SSE.

Not all the cells in a population analyzed go through two replication cycles, and for that reason some cells will be found which began to utilize 5-BDU sometime after the beginning of the first S-period. Since replication takes place asynchronously between chromosomes, and begins with the telomere, individual chromosomes without differentiatial staining of the ends of their sister chromatids will be met in the metaphases examined. With contrast staining of the sister chromatids and because of the lesser degree of spiralization of the chromatid which has incorporated bromouracil into both DNA strands, the illusion of an SSE will be created in the region of the telomeres, and this could lead to overestimation of the number of SSE in a given cell. To exclude mistakes of the second type, before the frequency of SSE is investigated replication of the chromosomes must be studied and both chromosomes in which it begins before the rest must be distinguished.

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