SOMATIC ASSOCIATION IN BHK_{21} HOMOSYNKARYONS

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1. Introduction

Homologous chromosomes have been shown to associate in somatic cells in several organisms. This phenomenon was described by Metz (1916) in males of *Drosophila melanogaster* and was subsequently related to the somatic crossing-over occurring in this insect (Stern, 1936). King (1970) has suggested that the high frequency of mitotic recombination in *Drosophila* may be due to the existence of somatic association. Recently somatic association of homologous chromosomes has been reported in a variety of plant and animal cells (Ohno *et al.*, 1961; Kitani, 1963; Bennett, 1966; Feldman, Mello-Sampayo and Sears, 1966; Chaunan and Abel, 1968; Avivi *et al.*, 1969; Kelly and Almy, 1969; King, 1970). In cultures of human and other mammalian cells when such association is observed only a few pairs of homologous chromosomes are involved.

In this report we describe BHK_{21} homosynkaryons with a high frequency of chromosome pairing and discuss the use of somatic cell hybridisation for the study of this phenomenon.

2. Materials and methods

Cell fusion was obtained by the technique as described by Harris *et al.* (1966). Four cell clones were isolated and studied in the 3rd *in vitro* subculture. One of the clones was also studied in the 10th and 15th *in vitro* passages.

Chromosome preparations were made by the air-drying technique after colchicine treatment. Homology was determined morphologically (plate I). In several instances the chromosomes were identifiable and were matched (*e.g.* short metacentrics or submetacentrics). In other cases their arm ratio and relative lengths were measured. Chromosome forms were established according to the karyotype of syrian hamster elaborated by Haemmerli *et al.* (1966). Two or more homologous chromosomes were considered associated when they were separated by no more than one other chromosome (Kelly and Almy, 1969) (plate II, 1, 2, 3, 4, 5).

In order to test if the association results from chance juxtaposition of chromosomes, distances were measured between easily identifiable chromosomes. Distances between short metacentrics, between short submetacentrics and between both of them, were measured. The distribution of chromosomes was calculated following the method used by Feldman *et al.* (1966).

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Observed distributions were compared with the theoretical by means of the Kolmogorov-Smirnoff one-sample test for goodness of fit (Siegel, 1956).

3. Results and discussion

The distribution of chromosome numbers in 50 cells of each of the four clones studied are presented in table 1. In clone 1 the chromosome numbers remained fairly constant in the 3rd, 10th and 15th subcultures. The chromosomes of each cell were associated as is shown in fig. 1.

Table 1

Chromosome numbers in the clones studied

	Chromosome numbers	
	Range	Most frequent numbers
Clone 1	87-218	near-octoploid
Clone 2	93-197	near-octoploid
Clone 3	76-104	near-tetraploid
Clone 4	62-107	near-tetraploid

Chromosomes were often arranged in pairs or triplets and in many cases the centromere seemed to be involved in the association (plate II, 1, 4). In other cases the telomeres appeared to be involved (plate II, 2, 3) and also other regions of the chromosomes (plate II, 5). Certain chromosome pairs may not be homologous although morphologically similar. No absolute criteria were found for avoiding such difficulties.

The distribution of nonhomologous chromosomes, was not significantly different from a random distribution within the cell. The mean distance between homologous chromosomes was significantly different ($P < 0{\cdot}01$) (fig. 2) indicating somatic association of chromosomes in these cells.

Suppression of somatic association by colchicine has been described by Avivi *et al.* (1969) and it has been suggested that spindle disruption might play an important role in suppressing chromosome pairing. Although colchicine was used in our experiments, the frequency of somatic association remained high. This could mean that colchicine was not effective, but as the frequency of metaphase plates was normal, this is extremely unlikely. Perhaps, therefore, chromosomes associate through a different mechanism, as reported for other systems (Metz, 1916; Watkins, 1935; Ohno *et al.*, 1961; Bennett, 1966; Wagenaar, 1969). Thus chromosome extraction by mechanical means during prometaphase suggests that there are connections among chromosome pairs other than those between spindle fibres and chromatids (Diacumakos *et al.*, 1971).

Recent work has shown that the chromatin of the centromere regions (Pardue and Gall, 1970) and of some telomeric bands contains repetitive DNA (Eckhardt and Gall, 1971). This could provide the basis for the association of chromosomes and also for chromosome rearrangements (*e.g.* plate II, 6) through a fusion of common regions.

Other cases of somatic association in diploid and polyploid mammalian cells have been reported (Bennett, 1966; Kelly and Almy, 1969). But in diploid cells the number of chromosomes associated is low, and in polyploid

cells although the frequency of association is higher, the occurrence of endomitosis or endorreduplication where daughter chromosomes tend to lie close to each other cannot be ruled out.

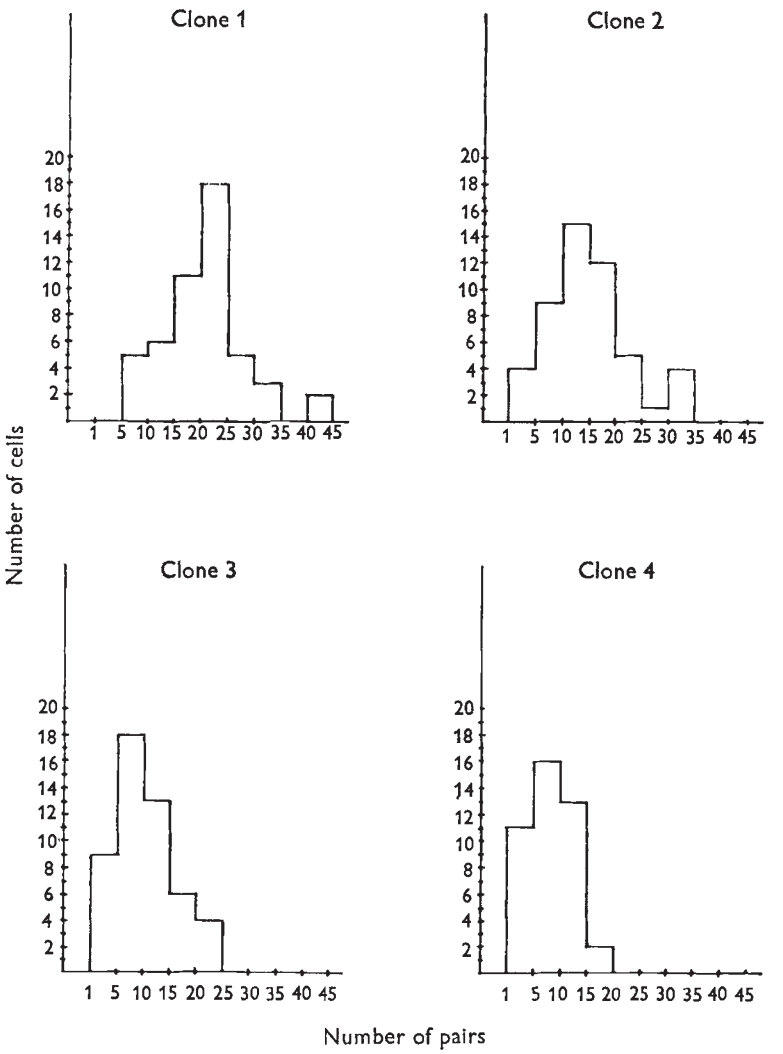


FIG. 1.—Histograms of the distribution of pairs of associated chromosomes in the clones studied.

The chromosome association we observed is reminiscent of endomitosis (Geitler, 1939) or endorreduplication (Levan and Hauschka, 1953). A systematic study of cells in the 3rd, 10th and 15th subcultures, however, showed that all phases of the mitotic cycle are present and that chromosome numbers remain constant or are in some cases lower. Therefore it seems unlikely that the chromosome association observed was a direct result of endomitosis or endorreduplication.

Somatic association might be expected to facilitate chromosome breakage

and exchange between homologous chromosomes. In the cultures studied 5 per cent of the cells were seen to possess unequal exchanges, which could be homologous in origin. They were of the dicentric type in which different meta and submetacentric chromosomes seemed to be involved. This relatively high frequency of interchange could therefore be due to the high frequency of somatic association in this system. One would also expect the

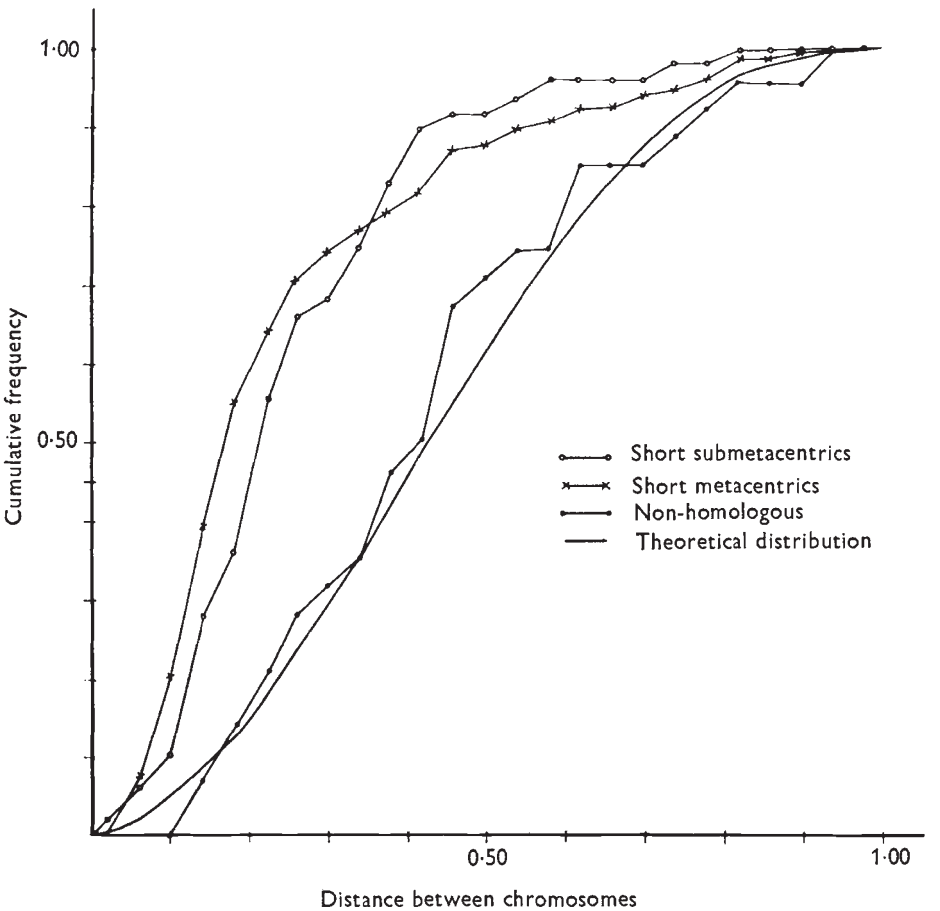


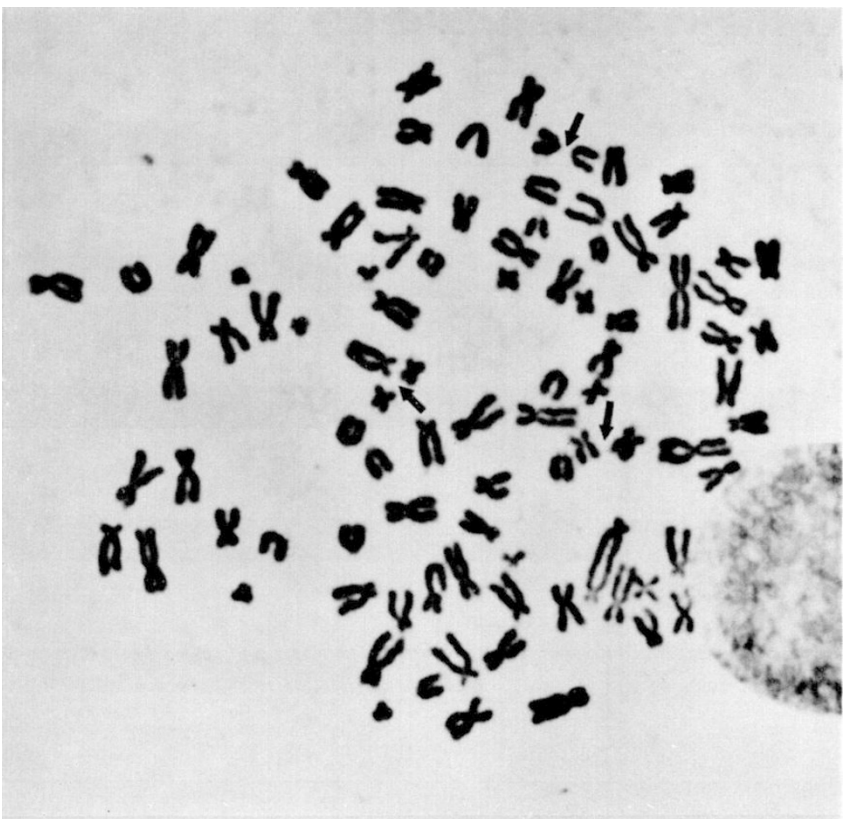
FIG. 2.—Observed and expected cumulative frequencies of distances between chromosomes.

frequency of mitotic recombination, which is caused by equal homologous exchanges, to be raised by somatic association as is the case in *Drosophila* (King, 1970).

The cells studied here show a high frequency of somatic pairing in the 3rd and the 15th subcultures. Other observations in our laboratory of different cell systems (*e.g.* fused by Syrian hamster fibroblasts) indicate that this phenomenon occurs in other types of fused cells. Consequently the method may be useful for the study of somatic association in mammalian cells.

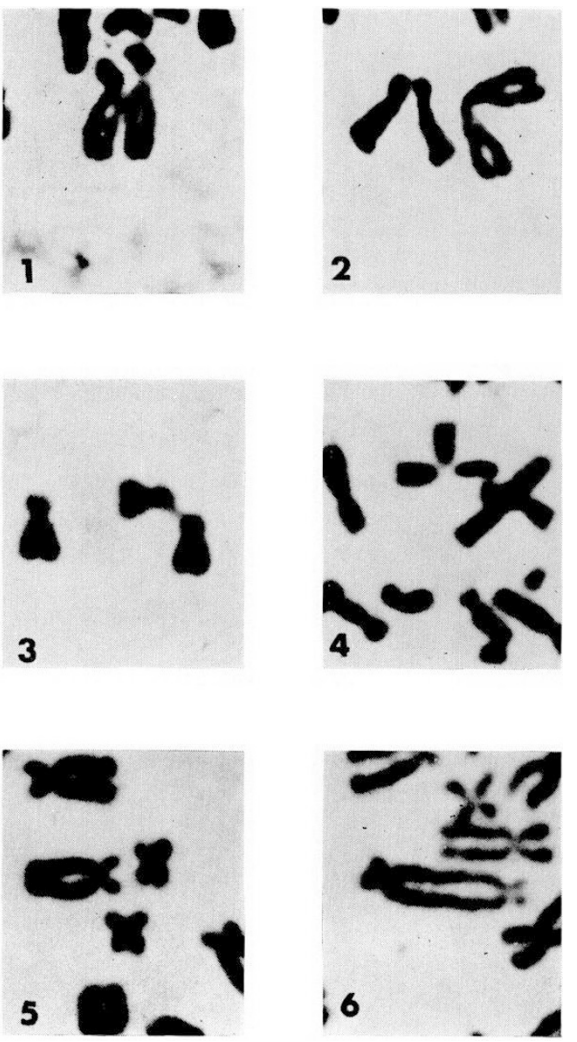
Furthermore, it has been possible to demonstrate that the elimination of chromosomes from hybrid cells is followed by disappearance of the gene

Plate I



Metaphase of a BHK_{21} hybrid cell showing examples of association of homologous chromosomes (arrows).

Plate II



(1, 2, 3, 4, 5) Different types of chromosome association.
(6) Chromosome rearrangement.

effects conferred by the eliminated chromosome (Weiss and Green, 1967). The use of such elimination could help to locate possible " association-controlling " genes in mammalian cells, such as were found in wheat, where there is evidence that the association of chromosomes is under the genetic control of certain chromosome segments (Feldman, 1966; Feldman and Mello-Sampayo, 1966; Mello-Sampayo, 1969). The method of somatic cell hybridisation could give some new insight into the mechanism of chromosomal pairing still poorly understood in mammalian cells.

4. Summary

1. Homosynkaryons obtained from BHK_{21} cells fused by Sendai virus were cloned and maintained *in vitro*.

2. The chromosomes in these cells showed a high frequency of pairing with arrangement in pairs and triplets.

3. The centromeres, telomeres and other chromosome regions seemed to be involved in the association.

4. A high frequency of chromosome rearrangements was observed.

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