Hybrids Formed by Fusion of X- or γ-irradiated and Non-irradiated Mouse Cells

J. Hofmanová and V. Spurná

Institute of Biophysics, Czechoslovak Academy of Sciences, Královopolská 135, 612 65 Brno, Czechoslovakia

Abstract. Hybrid cells were obtained by fusion of irradiated and non-irradiated mouse cells of two different lines; they differred from the parent lines and from the hybrid cells of non-irradiated parents in their morphological, growth and karyological properties. The frequency of their occurrence was lower than in hybrids from non-irradiated cells, and unlike the irradiated cells of the parent line, these hybrid cells were capable of permanent proliferation in vitro. Chromosomes of the irradiated parent line were preferentially eliminated from the karyotype of the hybrids.

Key words: Cell hybrids — X-or γ-irradiation — Growth characteristics — Chromosome number

Introduction

In our previous works we have studied interaction and fusion capacity of mouse cells of two different lines with one or both of them having been irradiated with ionizing radiation. The ability to synthetize DNA in the nuclei of the resulting heterokaryons was found to be positively affected, though in a population of irradiated cells as such, DNA synthesis was considerably inhibited (Hofmanová and Spurná 1981; 1983).

These results prompted us to study the possibility of hybridization of irradiated and non-irradiated cells with the aim of determining the proliferation capacity and selected cytogenetical properties of the resulting hybrids. We considered the fact that mammalian cells irradiated with high doses of ionizing radiation resulting in a complete loss of their colony forming ability, can form viable hybrids with both non-irradiated and irradiated cells (Jullien and Lawrence 1976; Megumi 1976; Jullien et al. 1978; Donald et al. 1981); this way, they offer a suitable model for the study of interaction of genomes of irradiated and non-irradiated cells.

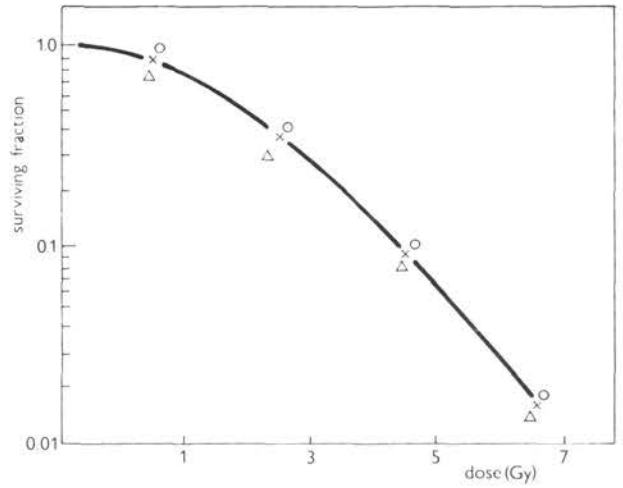

Fig. 1. Survival curve of LB 10 AG mouse fibroblasts following X-irradiation. The values were established on the basis of the plating efficiency of cells exposed to 1—7 Gy, three experiments in each case. For each value eight parallel samples were run.

Materials and Methods

Parental cells: Cells of a heteroploid mutant line of mouse fibroblasts (HGPRT⁻ = hypoxanthin-guaninephosphoribosyltransferase⁻) resistant to 8-azaguanine (strain LB 10 AG), and cells of a diploid highly malignant mouse lymphosarcoma, LS/BL (Jurášková 1982) were used. The conditions of cultivation have been described elsewhere (Hofmanová and Spurná 1981).

Irradiation: Prior to fusion, LB 10 AG cells were transferred from a monolayer into suspension in a balanced salt solution (BSS) (3×10^6 cells/ml) and X-irradiated at a dose of 5 Gy (TUR, 180 kV, 15 mA, filter 0.5 Cu plus 0.5 Al). This dose was selected on the basis of the surviving fraction of cells exposed to doses of 1—7 Gy (Fig. 1).

LS/BL cells cultured in the peritoneal cavity of C57 BL mice were subjected to long-term continuous irradiation with gamma rays (^{60}Co) in vivo (LS/BL-CIR) for 4 years (exposure rate 0.048 Gy/hr; total dose about 1.77 kGy) (Jurášková 1982).

Fusion: The method of cell fusion and isolation of hybrids has already been described (Spurná and Nebola 1973). The experimental schedule is shown in Fig. 2. Hybrids were obtained in two ways:
I. Fusion of irradiated LB 10 AG cells with non-irradiated LS/BL cells obtained from the peritoneal cavity of mice.
II. Fusion of non-irradiated LB 10 AG cells with LS/BL-CIR cells irradiated continuously in vivo.
Controls were hybrids arising from the fusion of non-irradiated parental lines. To check any revertants, non-irradiated and irradiated LB 10 AG cells were separately set in the selection medium HAT.

Identification of the presence of HGPRT: The activity of the enzyme HGPRT in the parent and/or hybrid cells was determined autoradiographically after the incorporation of ^{3}H-hypoxanthine (activity 74 kBq/ml; specific activity 74×10^3 MBq/mmol.l^{-1}; emulsion Ilford K2; exposure 3 days). Preparation of karyotypes: Metaphases were prepared by the method described previously (Nebola et al. 1981).

Growth curves: Growth curves of both the control and experimental hybrids and of the parental line LB 10 AG were obtained by assaying cell numbers in Müller flasks (capacity 50 ml) in sampling

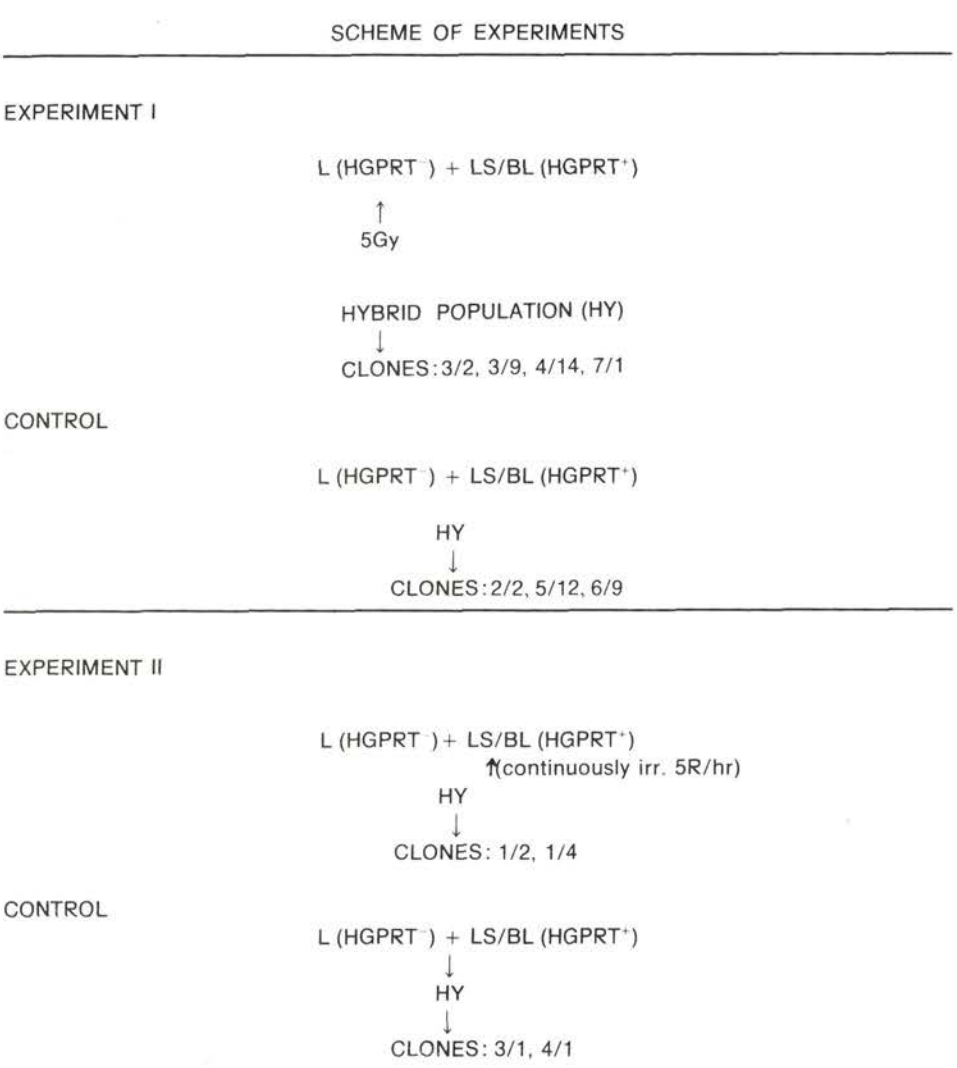

Fig. 2. Scheme of experiments to obtain hybrid cells after the exposure of one of the parents (LB 10 AG or LS/BL cells) to ionizing radiation.

periods during an 8—10-day cultivation; 2×10^5 cells were set in each flask. The cells were shaken down into 1 ml of trypsin solution, and assayed in a Bürker chamber.

Results

The occurrence of viable hybrid cells in populations with one irradiated parental line in the systems I and II, respectively, was about five times lower than in the

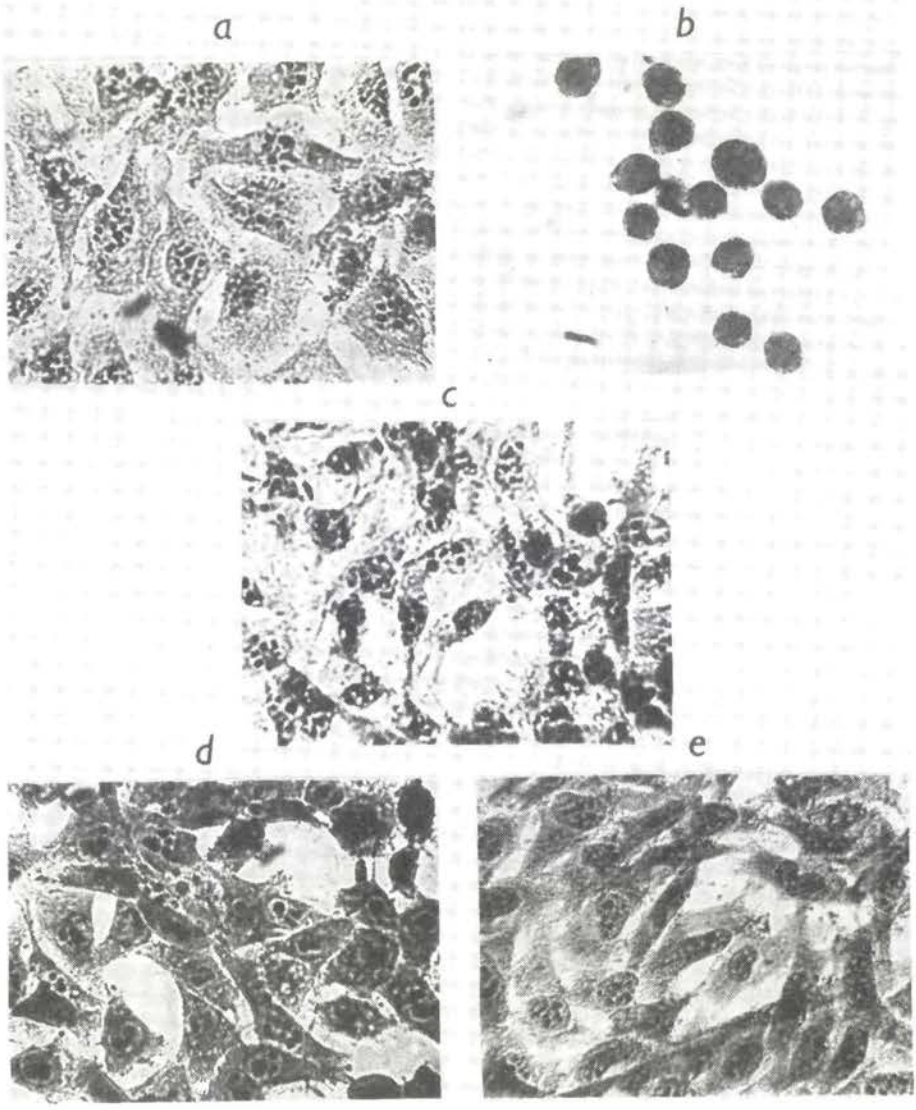

Fig. 3. Parental cells LB 10 AG and LS/BL, and hybrids arising from their fusion:
a) LB 10 AG cells
b) LS/BL cells
c) a control clone of hybrid cells arising from the fusion of non-irradiated parent cells
d) an experimental clone of hybrid cells arising from a fusion of LB 10 AG cells exposed to X-rays (5 Gy) with non-irradiated LS/BL cells
e) an experimental clone arising from a fusion of non-irradiated LB 10 AG with LS/BL-CIR cells continuously exposed to γ-radiation.

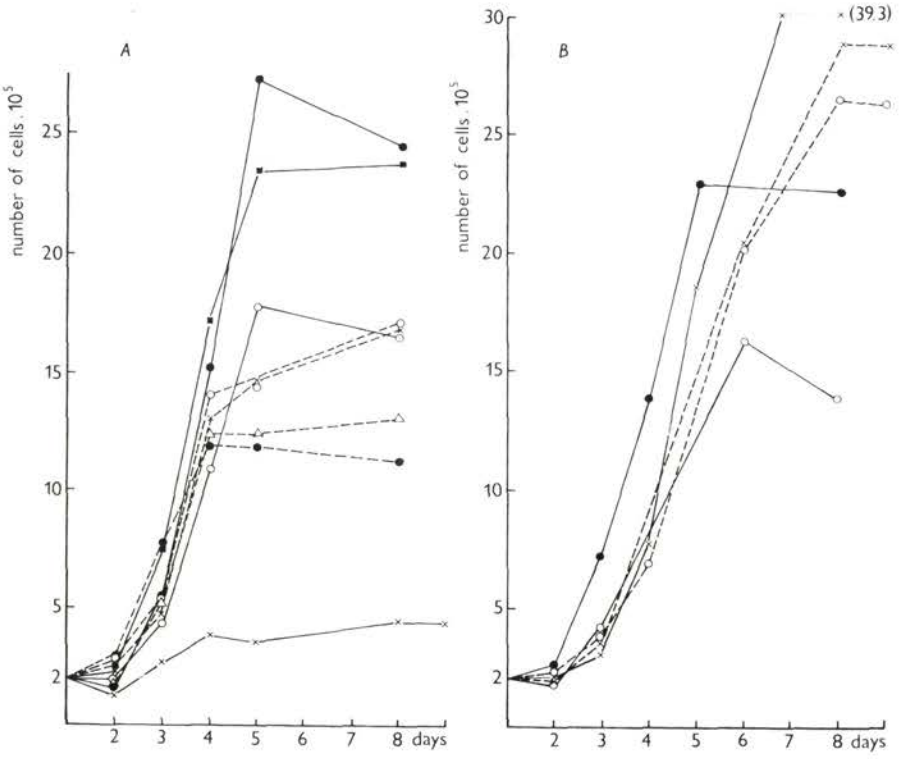

Fig. 4. Growth curves of the parental line LB 10 AG (non-irradiated or irradiated with ionizing radiation) and hybrid clones after a fusion with irradiated (LS/BL-CIR) or non-irradiated LS/BL cells.

a) ×——× parental line LB 10 AG exposed to 5 Gy

——— control clones of hybrids arising from a fusion of non-irradiated parental cells LB 10 AG × LS/BL: 2/2 (○), 5/12 (■), 6/9 (●).

– – – – clones of hybrids arising from a fusion of irradiated LB 10 AG × non-irradiated LS/BL cells: 3/2 (×), 3/9 (○), 4/14 (●), 7/1 (△).

b) ×——× parental line of non-irradiated LB 10 AG cells

——— control clones of hybrids arising from a fusion of non-irradiated parental cells LB 10 AG × LS/BL: 3/1 (○), 4/1 (●)

– – – – clones of hybrids arising from a fusion of non-irradiated of LB 10 AG × irradiated LS/BL-CIR cells: 1/2 (○), 1/4 (×).

2×10^5 cells were set and five parallel samples evaluated in each sampling.

control populations with non-irradiated cells. While in the control population in HAT medium hybrid colonies appeared, after three weeks, in experimental populations, colonies of hybrids appeared as late as after 5—8 weeks. Both non-irradiated and irradiated LB 10 AG cells did not form any revertants in the selection HAT medium. Hybrid cells of all the experimental and control clones

incorporated ^{3}H-hypoxanthine, showing complementation of HGPRT activity of the two parent cells in the hybrid genome.

Morphological and growth characteristics of hybrids:

Clones of hybrid cells differred from each other and from their parental cells in both, their morphology and growth on a monolayer (Fig. 3). The proliferation capacity was higher in all the experimental and control hybrid clones as compared with irradiated parental LB 10 AG cells; it was however lower than in non-irradiated LB 10 AG cells. LB 10 AG cells irradiated with 5 Gy, were growing very slowly, they did not form monolayers: by the eighth post-setting day the number of cells was only 2.2 times the initial one (2×10^5 cells) (Fig. 4*a*). Hybrid cells arising from a fusion of irradiated LB 10 AG cells with non-irradiated LS/BL cells (system I: clones 3/2; 3/9; 7/1; 4/14) were growing more rapidly, and they were capable of permanent proliferation in vitro. Compared with the control hybrids from non-irradiated cells, and with the experimental hybrids in system II (irradiated LS/BL cells), respectively, they entered the stationary phase of growth 1—3 days earlier, without forming continuous monolayers (Fig. 4*a*,*b*).

Karyological properties:

Table 1 shows modal numbers of chromosomes of parental lines and those of hybrid clones obtained after the fusion of the formers. Chromosome numbers of the parental line LB 10 AG remained unchanged after the exposure to 5 Gy. The total number of chromosomes in both the control and experimental hybrid cells was on average lower than the expected sum of chromosomes of the two parents. The only exception was the clone 2/2 with a modal number of 110 chromosomes, probably arising from a fusion of both two genomes of LB 10 AG cells and one genome of an LS/BL cell. In the experimental hybrid clones arising from a fusion of irradiated and non-irradiated cells (in systems I and II), the modal numbers of chromosomes were lower than in the control clones arising from a fusion of non-irradiated cells. In both systems, not only a decrease in the modal number of chromosomes after the fusion of irradiated and non-irradiated cells, could be observed but a narrowing of the variation range, and a shift to lower values (Fig. 5) as well.

In analysing karyotypes, a particular loss of bi-armed chromosomes of the irradiated line LB 10 AG was found in the experimental clones in system I (irradiated LB 10 AG × non-irradiated LS/BL). As compared with the original modal number of 23 bi-armed chromosomes, maintained in the control clones, this number fell to 16—21 in the experimental clones.

On the other hand, in system II (non-irradiated LB 10 AG × irradiated

Table 1. Modal chromosome number of the parental lines LB 10 AG and LS/BL, and of hybrids arising from their fusion.

	Lines (clone)	Irradiation	Chromosome number (modal)		
			Total	Bi-armed	Telocentric
Parental lines	LB 10	–	50	23	27
	AG	+	50	23	27
	LS/BL	–	40	–	40
	LS/BL −CIR	+	39	–	39
Hybrid clones 'L × LS/BL	2/2	–	110	35	75
	5/12	–	85	23	60
	6/9	–	83	23	60
	3/2	+	77	19	54.61
	3/9	+	78	21	59
	4/14	+	79	21	59
	7/1	+	78	16	56
L × 'LS/BL	3/1	–	97	23	77
	4/1	–	86	22	64
	1/2	+	70	23	48
	1/4	+	69	22	47

For each line (clone) 30—50 mitoses were evaluated: × — irradiated parental line

LS/BL-CIR), the modal number of bi-armed chromosomes remained the same in both the experimental and control clones. However a considerable decrease in the modal number of telocentric chromosomes could be observed: 47 and 48 telocentric chromosomes were counted in the experimental clones, respectively, as compared with the original 64 and 77 in the control clones.

Discussion

The finding that the appearance of heterokaryons after the fusion of mouse fibroblasts and mouse lymphosarcoma cells is not inhibited by ionizing radiation (Hofmanová and Spurná 1981; 1983) led us to study the hybridization of irradiated and non-irradiated mouse cells. Although the results of experiments with heterokaryons showed a certain stimulation of fusion of cells irradiated with ionizing radiation, the fusion of the nuclei in heterokaryons and the appearance of hybrid cells were not stimulated. The frequency of the formation of hybrids was considerably less after the fusion of irradiated and/or non-irradiated cells as

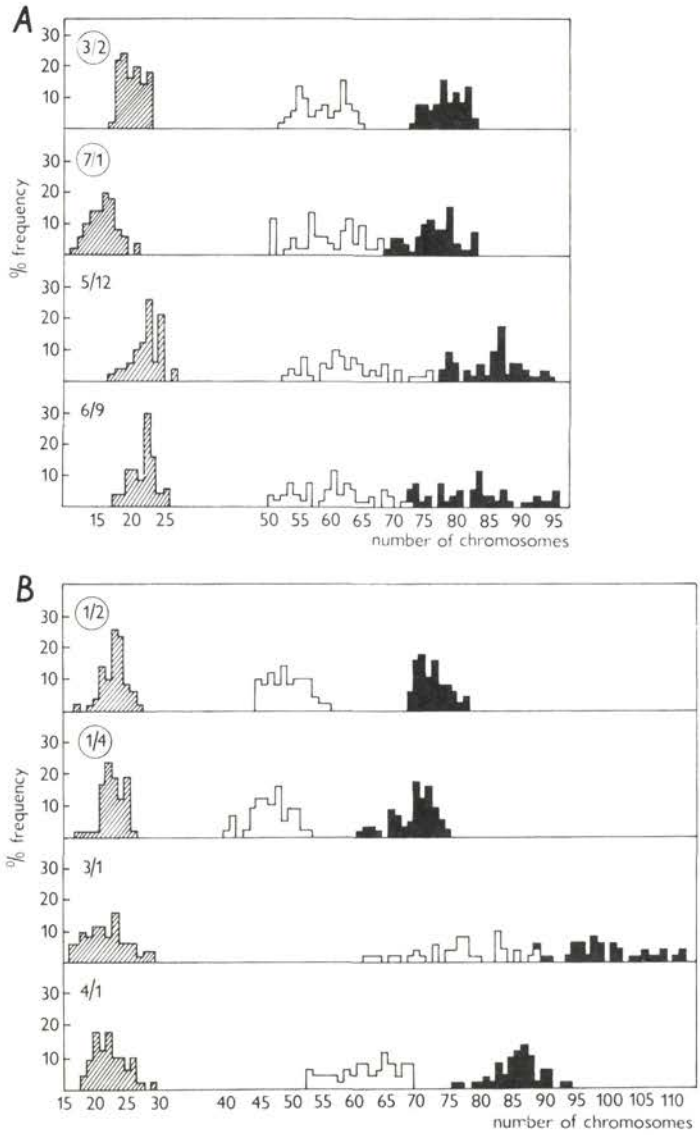

Fig. 5a, b Chromosome number in hybrids of non-irradiated LB 10 AG × LS/BL cells (5/12, 6/9, 3/1, 4/1), irradiated LB 10 AG × non-irradiated LS/BL cells (3/2, 7/1) and non-irradiated LB 10 AG × irradiated LS/BL-CIR cells (1/2, 1/4). Stripped squares — bi-armed chromosomes; □ — telocentric chromosomes; ■ — total number of chromosomes.
Modal number of chromosomes: LS/BL:40 (39—41); LS/BL-CIR:39 (35—42); LB 10 AG:50 (43—54); and LB 10 AG (exposed to 5 Gy): 50 (40—56). 30—50 mitoses were evaluated from each sample.

compared with control cultures of non-irradiated cells. This may partly be due to the fact that, in view of the formation of polykaryons and of disturbances to mitosis, proliferating hybrids may be formed from a limited number of heterokaryons only (Muryayma and Okada 1970). After an exposure of parental cells to ionizing radiation, which causes further breakdowns in the mitotic apparatus of the cell and leads to the formation of gigantic polykaryons (Hurwitz and Tolmach 1969; Joshi et al. 1982), the ability to form viable hybrids is even further decreased.

In our system we were able to obtain hybrids which proliferated well even with one of the parental cells previously exposed to a single in vitro dose of ionizing radiation (LB 10 AG), or to long-term continuous irradiation in vivo (LS-/BL-CIR). Although the frequency of the occurrence of hybrids was about five times less as compared with hybrids formed by the fusion of non-irradiated parental cells, the selection system in HAT medium was suitable to select and isolate hybrids. All the isolated hybrid cells proliferated in HAT medium and incorporated radioactive hypoxanthine, showing after fusion a complementation of $HGPRT^+$ and $HGPRT^-$ cells.

Unlike some other authors who could obtain proliferating hybrid cells even after an exposure of one or even both parental lines to very high doses of radiation (tens of Gy) (Harris 1972; Megumi 1976; Jullien and Lawrence 1976; Boyle et al. 1976; Jullien et al. 1978), we were able to isolate hybrid cells only with one parental line exposed (5 Gy or continuous irradiation). Colonies of hybrid cells appeared in the culture as late as after 5—8 weeks following the fusion and they were first growing very slowly. Megumi (1976) and Donald et al. (1981) noted in an intraspecific system of L cells and in an interspecific system of human and hamster cells, respectively, a marked delay in the initial formation and a slow growth of colonies of hybrid cells. One of the causes of the slow growth of hybrids may be the prolongation of the generation time resulting from the irradiation of the parental cells (Walters and Petersen 1968; Kimler et al. 1981).

The decreased frequency of the formation of hybrids from irradiated and non-irradiated cells may also be brought into connection with the influence of the cell cycle on the ability to fuse (Stadler and Adelberg 1972) and also with the variable sensitivity of cells to irradiation during their cell cycle (Fidorra and Linden 1977; Kimler and Henderson 1982). Another cause of differences in results may be the cell lines used, the methods of irradiation and cell fusion, the composition of nutrient media, and cultivation conditions.

The results of our experiments have suggested that interactions between irradiated and non-irradiated cells may result in a certain repairing of the damage caused to their genomes. The proliferation capacity of these hybrid cells was modified as compared with the control hybrids. The hybrid clones differred in growth from each other as well as from the growth capacity of irradiated or non-irradiated LB 10 AG cells themselves (Fig. 4*a*, *b*). The proliferation capacity

of irradiated LB 10 AG cells was very low and their growth slow, while the hybrid clones formed by their fusion with non-irradiated LS/BL cells proliferated well; they did not, however, achieve the same saturation density as the control hybrids from non-irradiated parental cells, or the experimental hybrids arising from a fusion of non-irradiated LB 10 AG with irradiated LS/BL-CIR cells at the given interval.

In addition to various expression of phenotypical traits, the hybrid cells differred in their karyotype composition. Hybrids arising from a fusion of irradiated and non-irradiated cells showed a loss of a greater number of chromosomes than control hybrids of non-irradiated cells (Table 1). Based on analysis of the numerical composition of the karyotype it can be assumed that there was a preferential elimination particularly of chromosomes from the genome of the irradiated parental cells LB 10 AG (in system I, particularly bi-armed chromosomes) or LS/BL-CIR cells (in system II, particularly telocentric chromosomes) (Fig. 5). It may, however, be that, in the hybrid genome, chromosomes of the non-irradiated partner may be influenced secondarily, as observed, e. g., by Graves (1980) and Donald et al. (1981). These authors also mentioned that the decrease in the chromosome number in the hybrid cells would be directly dependent on the radiation dose applied to the parental cells. These data are in accordance with the original findings of Pontecorvo (1971) who used various doses of ionizing radiation to control the elimination of the parental cell chromosomes from the hybrid genomes. From this point of view, it may be necessary to study in particular the dependence between the occurrence of chromosomes of irradiated and non-irradiated parental cells and their segregation in hybrids, and their proliferation and morphological characteristics. A certain dependence between the occurrence of loss of a certain chromosome, or group of chromosomes, from the irradiated parental cell may be assumed; this may modify, e. g. the rate of growth of the hybrid (Donald et al. 1982), or the anchorage dependence (Marshall et al. 1982). The fact that viable hybrids are formed even after an exposure to doses resulting in a very low or no probability of survival of parental cells, indicates that the occurrence of hybrids is a relatively radioresistant process (Jullien et al. 1978). In hybrid cells there is a certain damage repair based on the interaction of the two parental genomes, with the non-irradiated intact cell offering the irradiated one, e. g., its enzyme apparatus of certain metabolites. Processes such as recombinant repair may be supposed to take place on the molecular level, where radiation damage to the irradiated cells can also be repaired (Chadwick and Leenhouts 1978). In this case, biosynthetic mechanisms and functions, which are blocked in the irradiated cell, may be maintained in the hybrid cell.

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