Selection of Hybrids from Matings of Fibroblasts in vitro and Their Presumed Recombinants

Abstract. *When two clonal lines of mouse fibroblasts, each containing a drug-resistant marker, are grown together for 4 days, hybrid cells can be detected by selective conditions. These hybrid cells are presumed to be the result of mating. By the same method evidence can be obtained which suggests that mating may be followed by segregation.*

Barski, Sorieul, and Cornefert (*1*) first demonstrated that fusion can occur between cultured mouse cells; Ephrussi and Sorieul (*2*) have since studied this phenomenon extensively by karyotype analyses, having in mind its possible utility for the genetic analysis of mammalian cells. Gershon and Sachs (*3*) recently confirmed the occurrence of fusion, using mouse cells with different histocompatibility antigens. In the study reported here, the detection of far fewer hybrid cells than were found previously has been possible through the use of two clonal lines of mouse fibroblasts (L cells), each containing a drug-resistant marker.

Cell culture and analytical methods were the same as those used previously (*4*); the assay for thymidine kinase activity in cell extracts was a modification of that for guanylic acid–inosinic acid pyrophosphorylase (*4*). Cells of the A3-1 line lack pyrophosphorylase and are resistant to 3 μg of 8-azaguanine per milliliter (*4*), and those of the B34 line lack thymidine kinase and are resistant to 30 μg of 5-bromodeoxyuridine (BUDR) per milliliter (*5*, *6*). These markers do not appear to be linked (*7*). Cultures of the B34 line contain a small number of revertant cells (*6*) and therefore are maintained in the presence of BUDR. For the mating experiments the cells were washed to remove BUDR and resuspended in medium containing 10 μg of thymidine per milliliter. Then an equal number of these cells and A3-1 cells were grown together for 4 days in a suspension culture. Four or more replicate petri dishes containing 5×10^6 cells from this culture, and dishes containing the same number of B34 cells (similarly washed and grown with thymidine) or A3-1 cells, were incubated with medium containing $4 \times 10^{-7}M$ aminopterin, $3 \times 10^{-6}M$ glycine, $1.6 \times 10^{-5}M$ thymidine, and $1 \times 10^{-4}M$ hypoxanthine, in which only cells with both enzymes can survive to form colonies (*4*, *7*, *8*). The medium was replenished after 1, 3, and 6 days, and the petri dishes were examined for colonies after about 14 days.

In three experiments no colonies were present in the dishes containing A3-1 cells, while in the dishes containing B34 cells the frequency of cells (presumably revertant) which survived to form colonies averaged 8×10^{-7} (3×10^{-7}, 7×10^{-7}, and 14×10^{-7}). In the mixed cultures the frequency of viable cells averaged 5.6×10^{-6} (2.6×10^{-6}, 7.0×10^{-6}, and 7.2×10^{-6}), or 14 times the number of revertant B34 cells which would have been present in the mixed cultures.

Clonal lines were established from six such colonies from the mixed cultures. One (M3) was similar morphologically to B34, and in all other respects appeared to be a revertant of B34. Another (M11) contained two types of cells, and has not been studied extensively. The other four (M1, M4, M12, and M13) were composed of large cells similar to A3-1 morphologically, and mostly mononuclear; they contained approximately double the usual amount of DNA, RNA, and protein per cell. Chromosome counts on these lines are given in Fig. 1, and the activities of thymidine kinase and guanylic acid–inosinic acid pyrophosphorylase per cell are shown in Table 1. These data indicate that M1, M4, M12, and M13 contained about twice the usual number of chromosomes and both enzymes, presumably through the fusion of an A3-1 cell and a B34 cell.

If cell fusion is to be useful for the genetic analysis of mammalian cells, as is a comparable process in fungi (*9*), the hybrid cells must segregate to form cells of lower chromosome number. Although perhaps due to artifacts, the occasional low chromosome counts in the fused cell lines (Fig. 1) suggested segregation, as suspected also by Ephrussi and Sorieul (*2*). Furthermore, by selection with 8-azaguanine or with 8-azaguanine and BUDR it has been possible to detect in the M4 culture two cell types which might have arisen by segregation. (For technical reasons BUDR alone could not be used as a selective agent in petri dishes). In two experiments the frequencies of 8-azaguanine–resistant cells in the M4 culture were 5.9 and 7.8×10^{-4}, and the frequencies of doubly resistant cells, which grew quite slowly, were 2.6 and 3.8×10^{-6}. Some of the 8-azaguanine–resistant cells might have arisen by mutation of M4 cells (*4*), and studies of the chromosome numbers of such cells will help to answer this question. The frequency of the doubly resistant cells is considerably more than might have been expected (about 10^{-10}) from coincidental mutations in the M4 cells (*10*). Thus it seems probable that the doubly resistant cells are due to segregation with recombination. If this process can be validated, perhaps methods will be found to increase its frequency, as well as the frequency of fusion, and it may become possible to extend these techniques to diploid cultures.

Table 1. Enzymatic activities in cell extracts. Results expressed as arbitrary units per cell.

Line	Thymidine kinase	Guanylic acid–inosinic acid pyrophosphorylase
A3-1	100	1
B34	3	100
M1	98	172
M4	62	96
M12	81	109
M13	117	167

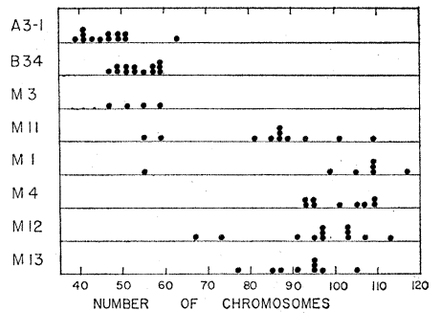

Fig. 1. Chromosome counts on the parental (A3-1 and B34) and the "mated" cell lines. No count greater than 64 was found in the parental lines.

JOHN W. LITTLEFIELD
John Collins Warren Laboratories of the Huntington Memorial Hospital of Harvard University, Massachusetts General Hospital, Boston

References and Notes

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10. The frequency of 10^{-10} was estimated from previous work (*4*) and unpublished studies.
11. Supported by USPHS grant CA-04670-05. This is publication No. 1173 of the Cancer Commission of Harvard University. We acknowledge the assistance of Susan J. Whitmore.

4 May 1964

Seed Radiosensitivity: A New Constant?

Abstract. *Dormant seeds of different species tolerate varying amounts of ionizing radiation, species having smaller nuclei in the apical meristem generally withstanding greater exposure. Nuclear volume (in μ^3) multiplied by radiation exposure (in roentgens) equals a constant, estimated from 12 species to be $(10.14 \pm 1.17) \times 10^6$. From nuclear volumes alone, predictions of radiation response for two unknown species were made; experimental values in both cases fell below the 95 percent but within the 99 percent confidence intervals of the predictions.*

Sparrow *et al.* (*1*) observed that sensitivities of 16 actively growing plant species to acute x- or γ-irradiation may vary up to 125-fold as measured by total exposure, but only 4-fold when the criterion is energy absorbed per chromosome at the lethal exposure. The implication is that a similar quantity of energy is absorbed for a similar amount of nuclear damage regardless of total radiation exposure. This striking concept was hinted at in earlier papers (*2*) describing high positive correlations of nuclear volume (or DNA content) with sensitivity of growing plants to chronic irradiation and with frequencies of somatic mutation and chromosome aberration. Similar correlations exist for the sensitivity of dormant (seed) embryos to acute irradiation (*3*). We here derive a constant with which seed radiosensitivity can be predicted from nuclear volume of certain embryonic cells.

Controlling important modifiers such as seed moisture (*4*), we have found that most interspecies differences in radiosensitivity are attributable to nuclear volumes in the apical initial cells of shoot meristems (*5*), although eight additional measurements are required to account for all genetic variability (*6*). For comparisons between species, we calculated the maximum radiation exposure tolerated by seeds before the dry weight of seedlings grown in controlled-environment rooms was reduced by 50 percent ("50 percent exposure"). When growth values were transformed to probits, an almost linear response was obtained with the logarithm of the radiation exposure. Nuclear volumes were determined for dormant seeds stored at 35 to 60 percent relative humidity; volume has been found to remain unchanged within this range (*4*). Embryos were excised from dormant seeds, fixed in chrom-acetic-formalin, infiltrated with tertiary butyl alcohol, and embedded in paraffin. They were then sectioned at 10 μ, stained with warm safranin, and counterstained with fast green in clove oil. Cells in the apical meristem region were examined at $\times$ 930 with an ocular micrometer, and two measurements at right angles were made for each nucleus: the longer axis was designated "*a*" and the other "*b*." After all nuclei of the meristems of several embryos were measured, "*a*" and "*b*" values were averaged and average nuclear volume, *V*, was computed from the ellipsoid formula:

$$V = \frac{4}{3}\pi\left(\frac{a}{2}\right)\left(\frac{b}{2}\right)\left(\frac{a+b}{4}\right)$$

Chromosome numbers were obtained from Darlington and Wylie (*7*) except for *Festuca elatior*, which was determined in our laboratory from root tips.

The nuclear measurements, 50 percent exposures, and calculated values for energy absorption for 12 species from 10 botanical families are listed in Table 1. The second column from the right is comparable to the pertinent numbers of Sparrow *et al.* (*1*), except for our using the 50 percent rather than the lethal exposure. In our computations, however, a spread of more than 11-fold was obtained despite a range of only 10-fold in tolerance as measured in radiation units (kr, third column from the right). When comparisons were made on a per-nucleus rather than a per-chromosome basis (right-hand column), a spread of about 3.3-fold was found.

The data from reference (*1*) were used to determine energy absorbed per nucleus at the lethal exposure, and a 28-fold range was found; however, the range of the nine polyploid species was only 8-fold and that of the seven diploid species was less than 5-fold. The apparent lack of concordance between the two series of experiments may be ascribed to the facts that, in the experiments of Sparrow *et al.*, actively growing meristems were irradiated to the lethal point and most species—9 out of 16—were polyploid while, in our experiments, dormant meristems were irradi-

Table 1. Test of the hypothesis that in dormant seeds of quite different sensitivity, as measured by total radiation exposure, similar or identical sensitivity exists as measured by energy absorbed per chromosome or per nucleus. (SE, standard error.)

Plant group and chromosome number	Average nuclear volume ($\mu^3 \pm$ SE)	Energy per chromosome per roentgen (ev)*	Energy per nucleus per roentgen (ev)	50% exposure (kr $\pm$ SE)†	Energy per chromosome at 50% exposure (Mev)	Energy per nucleus at 50% exposure (Mev)
1. *Cucumis sativus* (14)	117 $\pm$ 2.2	502.9	7,041	46.3 $\pm$ 0.21	23.28	326.0
2. *Trifolium incarnatum* (14)	126 $\pm$ 1.4	541.6	7,583	135.0 $\pm$ 3.74	73.12	1023.7
3. *Brassica napus* (38)‡	125 $\pm$ 0.2	198.0	7,522	142.2 $\pm$ 8.11	28.16	1069.6
4. *Linum usitatissimum* (30)	164 $\pm$ 3.8	329.2	9,870	71.3 $\pm$ 5.91	23.47	703.7
5. *Lycopersicon esculentum* (24)	193 $\pm$ 5.5	483.8	11,615	47.5 $\pm$ 2.01	22.98	551.7
6. *Lactuca sativa* (18)	193 $\pm$ 0.3	645.1	11,615	47.3 $\pm$ 3.66	30.51	549.4
7. *Arachis hypogaea* (40)‡	249 $\pm$ 2.9	224.5	14,985	29.3 $\pm$ 0.82	6.58	439.1
8. *Festuca elatior* (42)‡	435 $\pm$ 7.1	623.5	26,178	14.0 $\pm$ 0.69	8.73	366.5
9. *Hordeum vulgare* (14)	467 $\pm$ 2.1	2,007.6	28,104	25.9 $\pm$ 1.99	52.00	727.9
10. *Allium cepa* (16)	901 $\pm$ 21.0	3,388.7	54,222	13.0 $\pm$ 0.38	44.05	704.9
11. *Gossypium arboreum* (26)	435 $\pm$ 3.6	1,006.8	26,178	16.8 $\pm$ 0.27	16.91	439.8
12. *Daucus carota* (18)	114 $\pm$ 1.8	379.7	6,830	61.8 $\pm$ 2.32	23.47	422.1
				(Averages)	(29.44)	(610.4)

* Based on 1.77 ionizations per cubic micron of tissue per roentgen and 34 ev per ion pair; for the computations it is assumed that nuclei are composed entirely of chromosomes. † Maximum exposure to seeds causing 50 percent reduction in seedling dry weight. ‡ Polyploids.