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The oncogenic transforming potential of the passage of single α particles through mammalian cell nuclei

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Estimation of risk from exposure to radon is based on epidemiological data in miners, and extrapolated to the low exposure levels present in homes.

Bronchial epithelial cells in miners may have been traversed by several particles in a short period.

For the individual in a domestic radon situation, it is highly unlikely that any cell will be traversed by more than one alpha particle in an entire lifetime.

Objectives:

- Investigate the oncogenic effects of exactly one alpha particle.
- Compare the effects of exactly one to a Poisson mean of one alpha particle.
- This is a check on whether the cells receiving more than one particle dominate the response.
- “Control” study: comparison of exactly four with a Poisson mean of four particles.

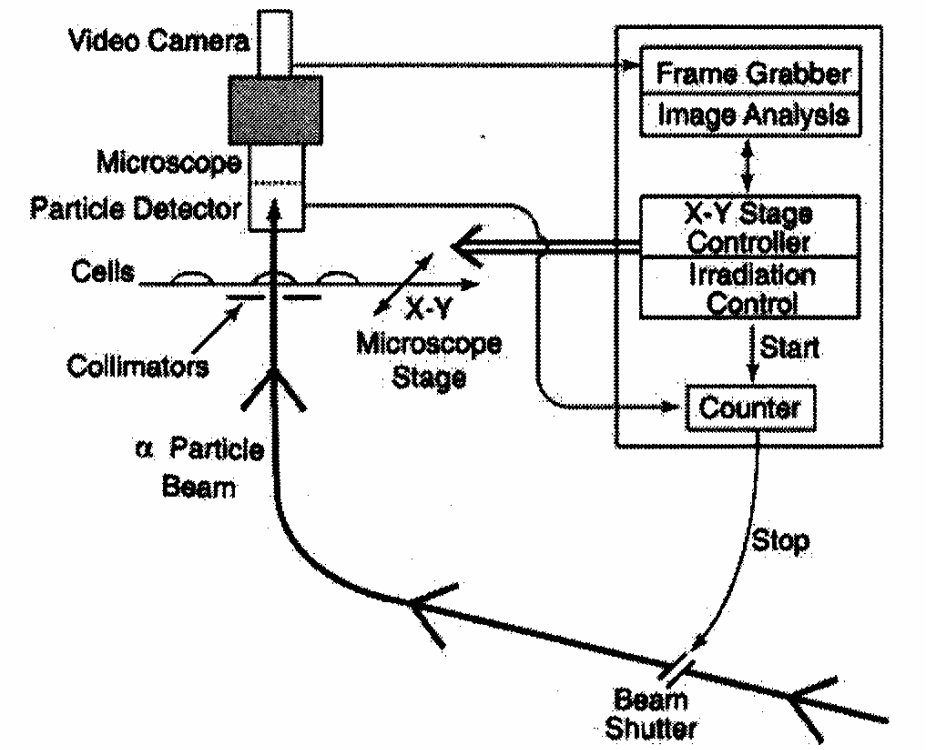

FIG. 1. Schematic of microbeam system.

The microbeam facility at Columbia.

Continuous improvements in automation make it possible to irradiate ~ 3000 cells per hour.

“Broad beam” studies produce the statistical Poisson distribution of particle traversals.

For a mean of 1 particle:

- 37% will see 0 traversals
- 37% will see 1 traversals
- 26% will see 2 or more traversals

The average dose will be the same as the group that all received **exactly one** traversal.

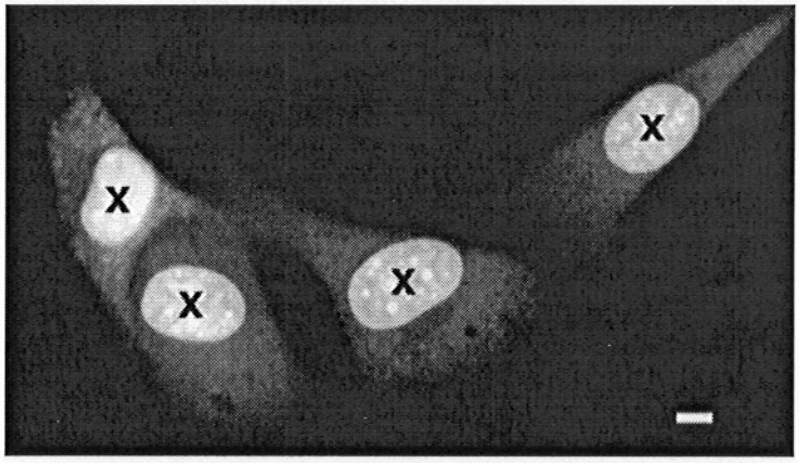

FIG. 2. Detail of C3H10T½ cells attached to the polypropylene surface of the mini-well, as detected by the automated microbeam image analysis system. The cells were stained with a low concentrations of Hoechst 33342 fluorescent dye as described in the text, which is preferentially taken up by the cell nucleus. The cell dish is moved under computer control such that the centroid of every cell nucleus as determined by the image analysis system (marked by the image analysis system as crosses) is sequentially situated under the collimated microbeam for irradiation with a predetermined number of α particles. For illustrative purposes, the cells shown here also were stained with orange fluorescent probe (CellTracker orange CMTMR, Molecular Probes), which is preferentially taken up in the cytoplasm and is used when microbeam irradiation of only the cytoplasm is required. The bar illustrates the overall spatial precision of the α particle microbeam of ± 3.5 μm. (Bar = 7 μm.)

Cells are stained with a Hoechst dye to visualize the nucleus for targeting. Broad beam groups are also stained as a control.

Transformation was judged by microscopic inspection of colonies after 7 weeks of growth. Methods previously reported. Such transformed cells produce tumors when injected into animals.

Transformed cells lose contact inhibition.

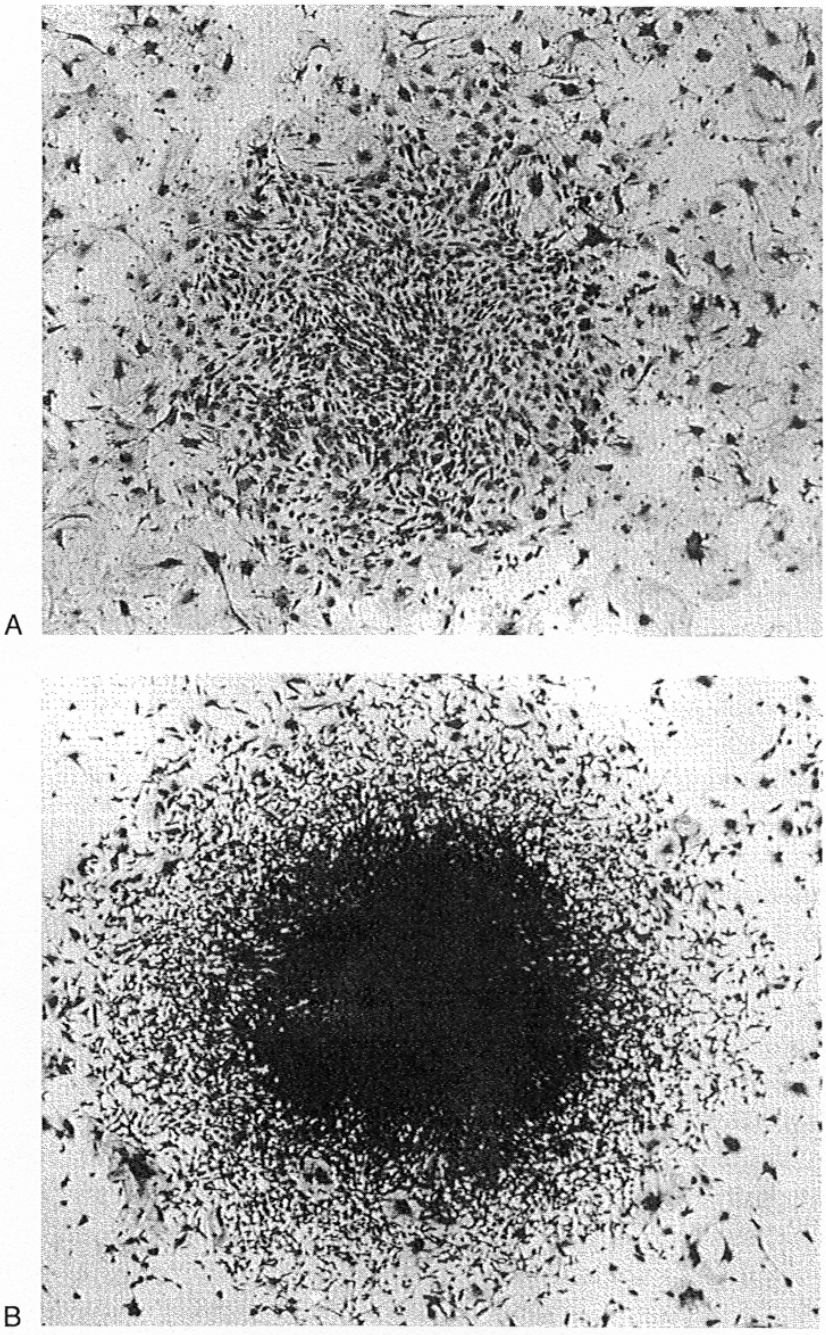

Figure 16.10. A: A normal untransformed colony of hamster embryo cells. The cells are orderly and show contact inhibition. **B:** A colony of radiation-transformed hamster embryo cells. Note the densely stained, piled up cells and the criss-cross pattern at the periphery of the colony.

[Hall, 2000]

Transformed cells lose anchorage dependence.

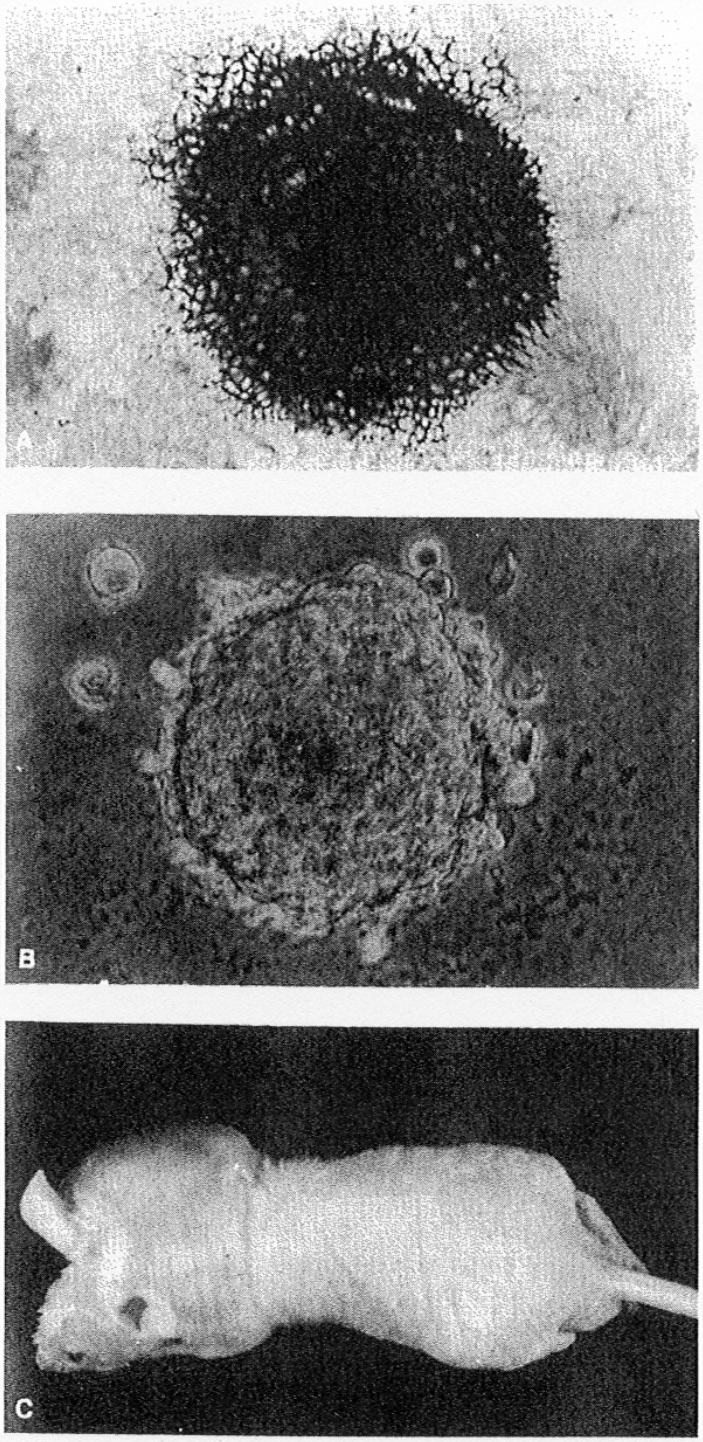

Figure 16.11. A: A type III transformed focus of C_3H 10TI/2 cells induced by the hypoxic cell sensitizer etanidazole (SR 2508). Note the multilayered growth and the criss-crossing of cells at the periphery of the clone over a contact-inhibited background of nontransformed cells. **B:** Cells from the above focus were plucked, expanded in culture, and plated into semisolid medium; they formed colonies, indicating that they had lost anchorage dependence. This is an indication of malignancy. **C:** The ultimate test of malignancy is whether cells from a type III transformed clone injected into a suitably prepared animal produce a tumor (a fibrosarcoma) that eventually kills the animal.

[Hall, 2000]

Table 1. Clonogenic survival rates, numbers of viable cells exposed in transformation studies, number of transformed clones produced, and transformation frequencies for microbeam (exact number of α particles) and broad-beam (Poisson-distributed number of α particles) irradiations

Irradiation	Exact or mean no. of α particles	Clonogenic surviving fraction (plating efficiency)	Number* of viable cells exposed/10^4	Number of transformants produced	Transformation frequency/ 10^4 surviving cells
Microbeam	0 (control)	0.60	4.62	4	0.86
	1	0.83	4.27	5	1.2
	2	0.64	1.22	7	5.8
	4	0.41	0.66	5	7.6
	8	0.16	0.38	5	13.2
Broad-beam	0 (control)	0.33	14.37	6	0.42
	1	0.85	12.42	38	3.1
	2	0.77	11.06	51	4.6
	4	0.46	3.76	20	5.3
	6	0.28	5.06	31	6.1
	8	0.18	7.05	66	9.4

*Estimated, accounting for plating efficiency and clonogenic survival.

Transformation frequency/10^4 surviving cells.

- Control rates are similar.
- Broad beam results are greater than microbeam results only at 1 particle.
- At 2, 4, and 8 particles the microbeam results are the same (within the error bars) as the broadbeam results.

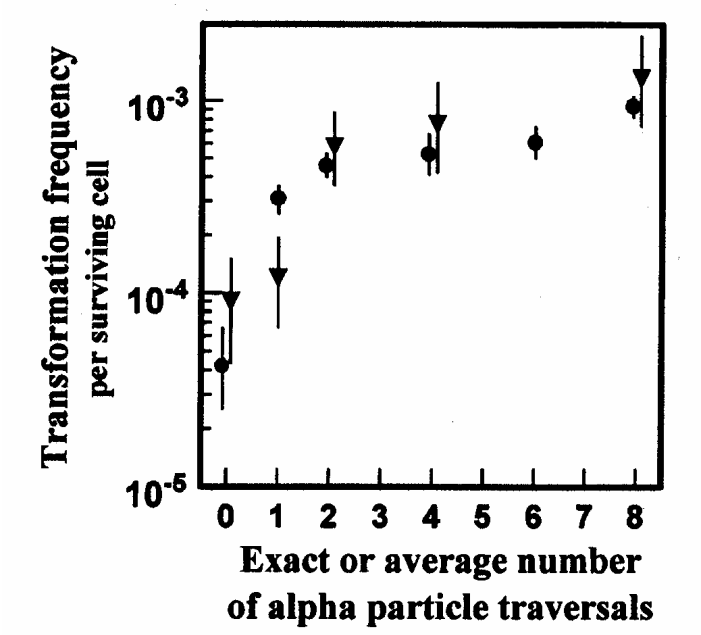

FIG. 3. Yield per surviving C3H10T½ cell of oncogenically transformed cells produced by nuclear traversals by 5.3-MeV α particles. ▼ represents exposure of cell nuclei to exact numbers of α-particle traversals by using the microbeam system. ● represents exposure to a Poisson-distributed number of α-particles traversals with a given average traversal number. Standard errors ($\pm$1 SD) were estimated by assuming an underlying Poisson distributed number of transformed cells (13).

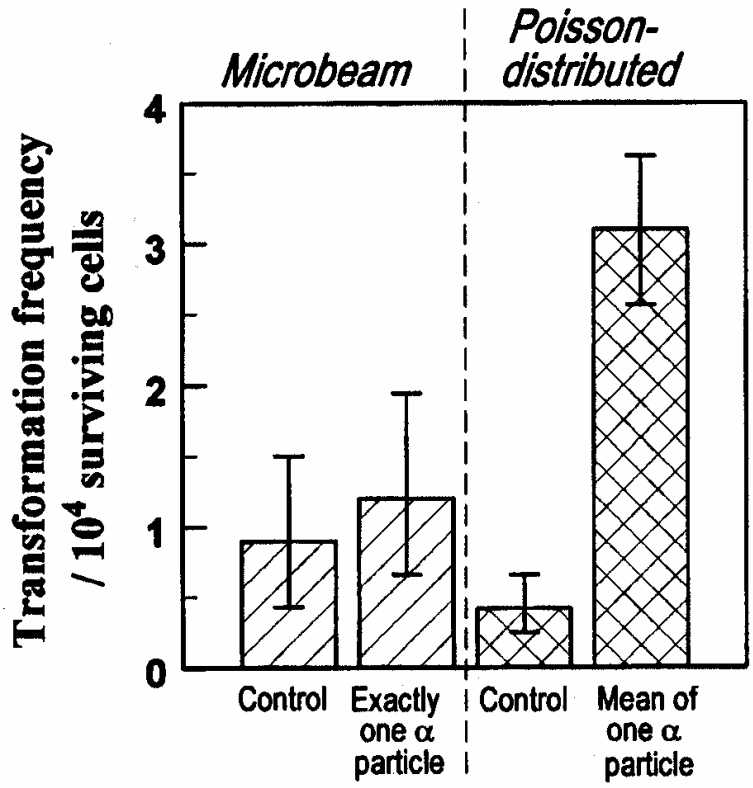

FIG. 4. Yield per surviving C3H10T½ cell of oncogenically transformed cells produced by nuclear traversals by exactly one (microbeam irradiation) or a Poisson mean of one (broad-beam irradiation) 5.3-MeV α particle. Standard errors (± 1 SD) were estimated assuming an underlying Poisson distributed number of transformed cells (13).

- Cells traversed by exactly one alpha particle show a lower risk than those receiving a mean of one.
- Exactly one alpha particle is not significantly different than controls receiving zero!!
- This implies that the majority of transformed cells resulting from a mean of one traversal must come from the subpopulation that received more than one traversal.
- If traversal by only one particle does not significantly raise the risk of oncogenic transformation, estimations by extrapolation from higher doses received by miners will overestimate the risk.

Induction of a bystander mutagenic effect of alpha particles in mammalian cells

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Previous work by this group with this cell line

- Exact numbers of alpha particles: survival and mutation endpoints.
- Cytoplasmic irradiation.

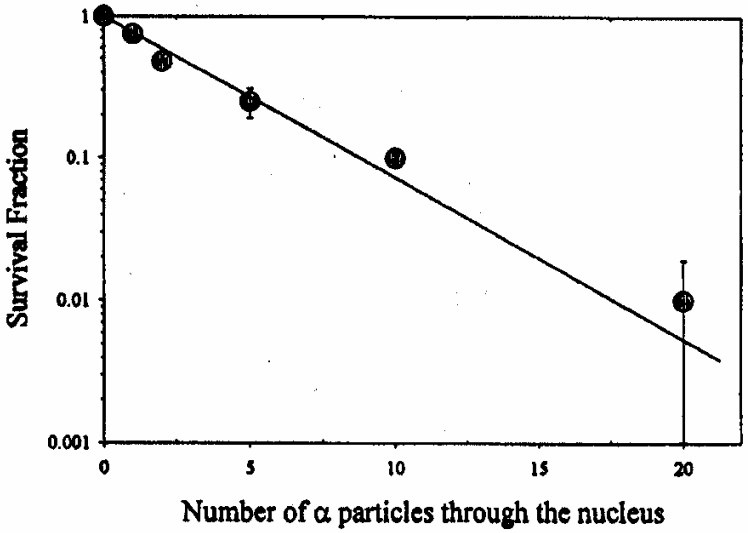

Fig. 1. Survival of A_L cells irradiated with an exact number of alpha particles in the nucleus. Data were pooled from three to four independent experiments. Error bars represent ± SEM.

- Now carried out to 20 particles per nucleus.
- D_0 is approximately 3.6 alpha particle traversals.
- Survival after 20 particles through the nucleus is ~ 1%.

A_L cells: hamster with one human chromosome that expresses cell surface markers for complement inactivation.

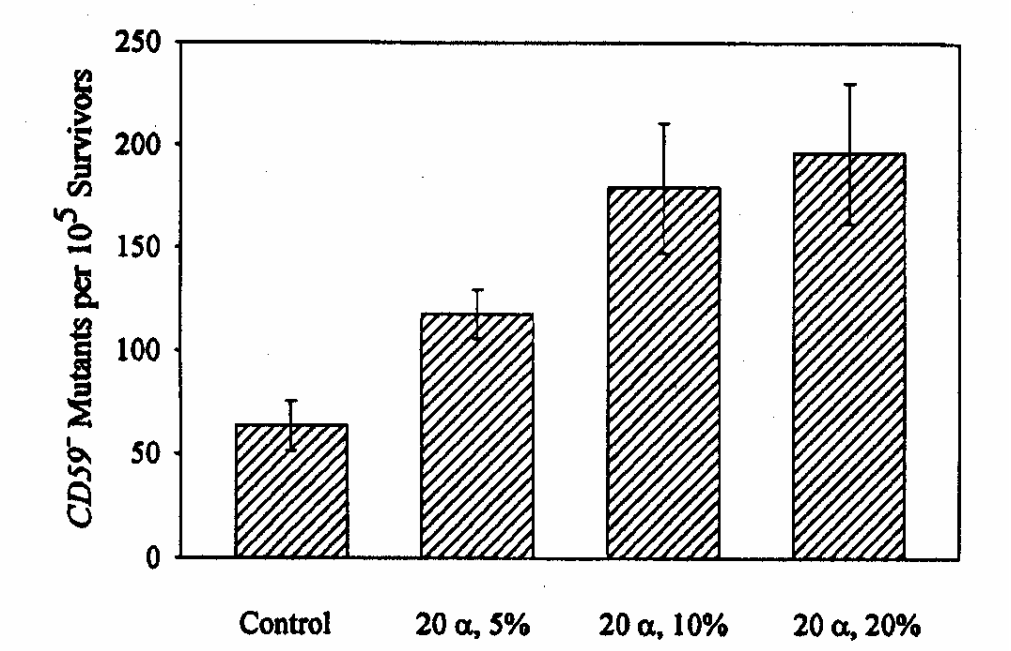

Fig. 2. Mutant fraction obtained from populations of A_L cells in which 0, 5, 10, or 20% of whose nuclei were traversed by 20 alpha particles. Data were pooled from three to eight independent experiments. Error bars represent ± SEM.

- ~500 cells plated in irradiation dish
- At random, 5, 10 or 20% of the cells in the dish irradiated with 20 alpha particles each
- >99% of the surviving cells are unirradiated, but the mutation rate is 3 times higher than control.
- Bystander effect in cells not traversed by alpha particles.
- The effect appears to “saturate”.

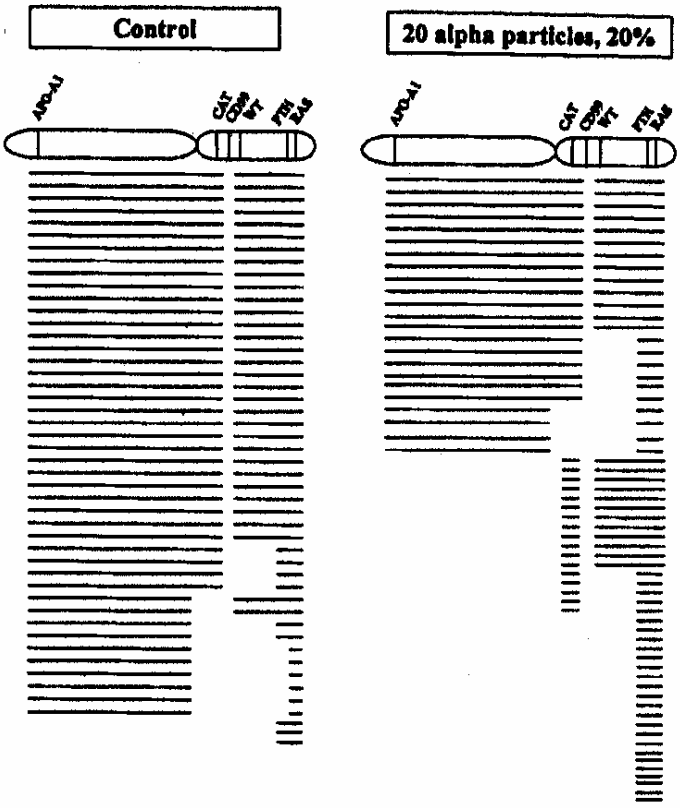

Fig. 3. Mutational spectra of *CD59*⁻ mutants isolated from unirradiated populations or from populations in which 20% of the cells had been irradiated with 20 alpha particles through their nuclei. Each line depicts a single mutant. Blank spaces depict missing markers on chromosome 11 as determined by multiplex PCR.

- For the population where 20% of the cells were exposed to 20 alpha particles, 99.8% of the progeny are calculated to be from unirradiated cells.

- The mutational spectra are clearly different from those that occur spontaneously.

Table 1. Effects of the free radical scavenger DMSO on mutant yield in A_L cells in which 20% of them were irradiated with 20 alpha particles each through their nuclei

Irradiation	DMSO, %	Mutant fraction per 10^5 survivors
0	0	63 ± 20
0	0.2	41 ± 12
0	8	61 ± 10
20 α, 20%	0	210 ± 30
20 α, 20%	0.2	203 ± 27
20 α, 20%	8	224 ± 39

DMSO when used at 0.2% was added to the cells 24 hr before irradiation and was removed after 7 days of incubation. DMSO, 8%, was present for 20 min, 10 min before and 10 min after irradiation (14). Data were pooled from three independent experiments.

- The bystander mutagenicity was not affected by addition of DMSO.
- The effect is not due to a free radical ROS.

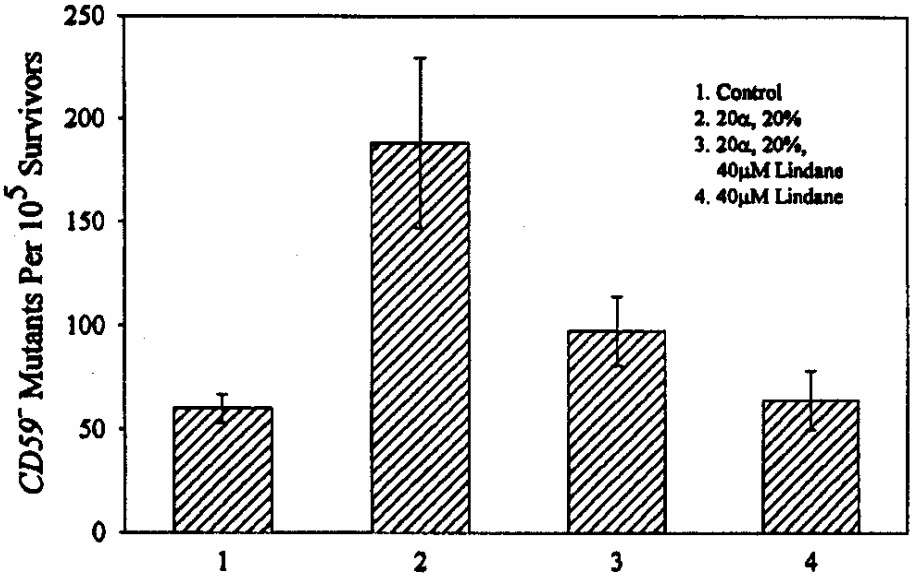

Fig. 4. Effect of lindane treatment (40 μM, 2 hr before and 3 days after irradiation) on mutant yields in A_L cells 20% of which had been irradiated with 20 alpha particles through their nuclei. Data were pooled from three independent experiments. Error bar represents ± SEM.

- Lindane blocks gap junctions and reduces the bystander effect.
- Cell-cell contact plays an important role in this effect.