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THE RADIOSENSITIVITY OF MARCHANTIA THALLI AS RELATED TO NUCLEAR BNL 9611
AND INTERPHASE CHROMOSOME VOLUMES*

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INTRODUCTION

Studies on the comparative radiosensitivities of plants have shown that the size of the nucleus and/or the chromosomes is an important factor in determining tolerance. Initially, chromosome size and metaphase length were recognized as related to radiosensitivity (1-5). Later, a quantitative relationship between the nuclear volumes and the radiosensitivities of 23 chronically irradiated species was demonstrated (6). Further work indicated that the volume of the chromosome at interphase - -viz., the interphase nuclear volume divided by the chromosome number - - is also related to a plant's radiosensitivity and is a better index of radiosensitivity than nuclear volume (7-9). However, the radiosensitivity of 12 species of seeds from 10 families has been shown to be better correlated with nuclear volume than chromosome volume (10-11). Also, the radiosensitivity of 20 species from the mustard family has been shown to be correlated with nuclear volume (12). Thus, there can be little doubt that the volume of the nucleus or of its contents is related to radiosensitivity (13-17).

The present study was undertaken in an attempt to determine the radiosensitivity of cells within multicellular structures, the gemmae and thalli of Marchantia polymorpha L., a thalloid liverwort, $n = 9$ (18). The gemmae are asexual reproductive propagules produced by the haploid thallus within a gemmae cup. The bryophytic capacity for cellular totipotency is well known (19-21) and the gemmae and thalli of Marchantia have this capacity for proliferation; it appears that if an intact cell has not lost its reproductive integrity it will produce a vegetative outgrowth in the absence of other inhibitory factors.

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There are three basic types of cells in a gemma; apical, parenchymatous, and rhizoid initial cells, but only the last appears incapable of forming a thallus. Normally the apical cells produce the thalli but if the apical cells do not function or are killed the parenchymatous cells may regenerate new thalli. Likewise, the thalli have apical and non-apical vegetative cells which are capable of forming thalli. Thus the gemmae and thalli of Marchantia are convenient structures for studying differential cell survival within a multicellular plant structure.

MATERIALS AND METHODS

Gemmae cups containing large numbers of gemmae were placed inside plastic vials with a wall thickness of approximately 2 mm and irradiated for varying lengths of time by a Co^{60} source which delivered approximately 580 R/minute. After irradiation the gemmae were sterilized for aseptic culture, plated onto inorganic agar medium (23), and scored one and two weeks later for apical and non-apical survival. Nuclear volumes of representative cells of the gemmae, and energy absorption values were determined at the lethal exposure by previously published methods (7). Approximately 130 gemmae were scored for each treatment, with exposures ranging from 0 to 60 kR.

Mature thalli of Marchantia were also irradiated and scored two and four weeks later for apical and non-apical survival. Approximately 15 thalli were used for each treatment. The thalli were placed on moist vermiculite and given an overnight exposure in the Brookhaven National Laboratory Gamma Field. Irradiated and control thalli were grown in the greenhouses at the Brookhaven National Laboratory according to previously recommended methods (23). Nuclear volumes of apical and vegetative cells were determined. It is possible to present briefly these preliminary results on the radiosensitivity of the thalli and correlations between nuclear and interphase chromosome volumes and radiosensitivity.

RESULTS

Histological preparations of the gemmae and determinations of nuclear volumes from representative cells showed that they are composed of vegetative cells of two sizes: the apical cells have nuclear volumes of $98.9 \pm 4.9 \mu^3$ and the non-apical parenchymatous cells have nuclear volumes of $54.7 \pm 3.3 \mu^3$. There was no apical survival of the gemmae at an exposure of 29.7 kR, but an exposure of 55.6 kR was necessary to eliminate any non-apical parenchymatous cell from producing a viable vegetative outgrowth.

The energy absorbed per nucleus at the 100% lethal exposure was calculated to be $164.9 \pm 8.2 \times 10^6$ eV and $173.0 \pm 14.8 \times 10^6$ eV per nucleus for the apical and parenchymatous non-apical cells, respectively. The energy absorbed per chromosome at the 100% lethal exposure was $18.3 \pm 0.9 \times 10^6$ eV and $19.2 \pm 1.6 \times 10^6$ eV, respectively. There is no significant statistical difference between the figures within each class. (Table 1)

Table 1

Summary of interphase nuclear and chromosome volumes for representative cells of a gemma and calculated amounts of energy absorption.

Cell type	Nuclear volume (μ^3)	Interphase chromosome volume (μ^3)	Lethal exposure (kR)	Energy Absorption at 0% survival ¹	
				per nucleus ($\times 10^6$ eV)	per chromosome ($\times 10^6$ eV)
Apical ²	98.9 ± 4.9	11.0 ± 0.5	29.7	164.9 ± 8.2	18.3 ± 0.9
Non-apical (parenchymatous)	54.7 ± 3.3	6.1 ± 0.3	55.0	173.0 ± 14.8	19.2 ± 1.6

1. Energy absorption calculations based on an average value of 32.5 eV per ion pair and 1.77 ionizations per cubic micron of wet tissue per roentgen [Table 2 in Lea (24)] .
2. Apical cells normally produce the new thallus but the parenchymatous non-apical cells have the ability to produce thalli in the absence of apical proliferation

Preliminary results with the mature thalli show that the cells mainly responsible for vegetative proliferation are of two different sizes: the apical cells have nuclear volumes of approximately $129\mu^3$ and the vegetative cells surrounding the bulbous ends of the rhizoids have nuclear volumes of approximately $12\mu^3$. There is a corresponding inverse relationship for radiosensitivity of these cells: apical survival is eliminated at 10 kR but non-apical survival is eliminated only with an exposure of 55kR or greater. Specific details regarding energy absorption will be presented elsewhere. Suffice it to say here that the energy absorbed per chromosome for the vegetative non-apical cells at the 100% lethal exposure is approximately 4.0×10^6 eV per chromosome.

DISCUSSION

The data presented above on the radiosensitivity of Marchantia gemmae and thalli do not argue for or against nuclear volume (10-11) or interphase chromosome volume (7-9) as a more accurate index of radiosensitivity since only one species was used and the chromosome number was constant.

The data indicate, however, that the nuclear or interphase chromosome volume differences between cells within a single plant are correlated with the radiosensitivities of those cells. The apical cells of a gemma are approximately twice as large as the non-apical vegetative cells -- $98.9 \pm 4.9 \mu^3$ vs. $54.7 \pm 3.3 \mu^3$, respectively. The radiosensitivity of these same cells is related to their nuclear and/or chromosome volumes, with the apical and non-apical proliferation being halted at exposures of 29.7 kR and 55 kR, respectively. However, when energy absorption values are compared for the lethal exposure the apical and non-apical vegetative cells absorbed $18.3 \pm 0.9 \times 10^6$ eV and $19.2 \pm 1.6 \times 10^6$ eV per chromosome, respectively -- thus, actual tolerance is not different. Both types of cells have the same radiosensitivity on an energy absorption basis.

Similar results were observed with the mature, actively growing thalli. The apical cells, with their large nuclear volume of approximately $129 \mu^3$ were much more radiosensitive on a roentgen basis than the non-apical vegetative cells with their smaller nuclear volume of approximately $12 \mu^3$. Comparisons between energy absorption in the apical and non-apical cells at the 100% lethal exposure will be presented elsewhere in detail since it is difficult to determine precisely whether what appears to be an "apical" survivor is indeed a bona fide proliferation of the apical cells and not a reorganization of an apical area from surrounding parenchymatous cells. The precise determination of apical proliferation in thalli is not as easily determined as with the gemmae. However, it is interesting to note that the exposure which resulted in total destruction of the reproductive integrity of the most radioresistant cells in the thallus resulted in a calculated energy absorption of 4.0×10^6 eV per chromosome; this figure is insignificantly different from the amount of energy reported by Sparrow and co-workers (7) at the 100% lethal exposure for actively growing plants. In this respect, then, the radiosensitivity of actively growing Marchantia thalli resembles that of actively growing "higher" herbaceous plants.

Sparrow has compared the radiosensitivity of 16 herbaceous angiosperms having a 22-fold difference in somatic chromosome number, a 40-fold difference in nuclear volume, and a 120-fold difference in tolerance to exposure and found that the energy absorbed per interphase chromosome varied only with a factor of less than four. (7)

Initially it was thought that Marchantia had a radiosensitivity different from that reported for higher plants on an energy absorption basis; the gemmae absorbed an average of $18.8 \pm 1.3 \times 10^6$ eV per chromosome (average of $18.3 \pm 0.9 \times 10^6$ and $19.2 \pm 1.6 \times 10^6$ eV -- Table 1) which is slightly greater than five times more resistant than the average amount of energy absorbed at the lethal exposure for the herbaceous angiosperms (25).

However, the same general type of plant material was not physiologically the same; in one case a dormant reproductive structure was irradiated -- the gemmae -- and in the case of Sparrow's work (7-9) actively growing plants were irradiated. Later, when the actively growing thalli were irradiated and scored for energy absorption at the 100% lethal exposure the obtained value of 4.0×10^6 eV per chromosome was insignificantly different from the value of 3.6×10^6 eV per chromosome at the lethal exposure for "higher" plants. Thus, it seems reasonable to conclude that the radiosensitivity of the actively growing Marchantia thallus is similar to that of actively growing plants. Furthermore, the correlation between radiosensitivity and chromosome volume, which had previously been shown to exist between species, has now been demonstrated within a species.

The difference in energy absorption per chromosome at the 100% lethal exposure between the gemmae and thalli of Marchantia is considerable, and somewhat analogous to the differences in tolerance between seeds and their actively growing plants. Little work has been reported on comparisons between seed and actively growing plant radiosensitivity in terms of actual energy absorption per chromosome but where evidence is available [(10-11 for seeds) and (7-9 for actively growing plants)] the seed appears to be considerably more resistant than its corresponding actively growing plant. Obviously, then, the physiological condition of the particular plant is also very important in determining radiosensitivity.

That energy absorption values at the 100% lethal exposure are similar for cells within the gemmae, and also that the 100% lethal exposure for most radioresistant cells within the thallus is similar to that reported for "higher" plants is further evidence for the idea that it is not the relative number but rather the absolute number of deleterious events which leads to the same end point for cells with different sized nuclei (7).

CONCLUSIONS

1. The nuclear volume of apical cells ($98.9 \pm 4.9 \mu^3$) of Marchantia ^{polymorpha} ~~gemmae~~ was much larger than the nuclear volume of other vegetative cells capable of producing a vegetative outgrowth ($54.7 \pm 3.3 \mu^3$). The lethal ~~radiation~~ exposures for the apical and non-apical vegetative cells of the gemmae are approximately 29 kR and 55 kR, respectively.
2. The energy absorbed by the gemminiferous apical and non-apical vegetative cells at the 100% lethal exposure is $18.3 \pm 0.9 \times 10^6$ eV and $19.2 \pm 1.6 \times 10^6$ eV per chromosome, respectively. Thus actual tolerance is not different. These amounts of energy are approximately 4.7 times greater than the reported average amount of energy absorbed per chromosome for growing herbaceous angiosperms at the 100% lethal exposure.

3. The nuclear volumes for apical and non-apical vegetative cells of *Marchantia thalli* are approximately $129 \mu^3$ and $12 \mu^3$, respectively. Apical and non-apical proliferation in thalli are eliminated by exposures of 10 kR and 55kR, respectively.
4. The energy absorbed at the 100% lethal exposure by the most resistant cells of the thallus was 4.0×10^6 eV per chromosome, which is insignificantly different from the amount of energy absorbed by herbaceous angiosperms irradiated at the 100% lethal exposure.

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