PRELIMINARY ANALYSIS OF PROTON RADIATION DAMAGE RESPONSE IN DEFECTIVE, GAMMA-RADIATION-SENSITIVE CELLS

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Proton radiation emanating from solar events poses a serious problem for prolonged earth-orbiting space projects. A comprehensive understanding of the differences between cellular responses to gamma radiation and proton radiation, and possible differences in the mechanisms of repair, is required to develop radiation protection or resiliency. We have isolated and characterized 37 mutants of the mushroom *Coprinus cinereus* that are sensitive to gamma radiation. These mutants define at least 10 complementation groups for survival of gamma damage. Four of these genes are also required for normal progression through meiosis. It is the objective of the current investigation to determine the extent of overlap of the subsets of mutants exhibiting defects in proton sensitivity, gamma sensitivity, and meiotic defect.

To establish the validity of mutant variation, the proton sensitivity characteristics of the congenic wild type *C. cinereus* (Java 6 strain) were examined. Mitotically derived, haploid spores termed oidia, were obtained by mechanical scraping of hyphae. The cells were counted and spread on growth medium at a cell number previously determined to produce sufficient viable colonies for scoring following irradiation. Identical samples were plated and irradiated by either of two methods within 24 hours. This time frame had been determined previously to be well within acceptable viability limits.[1] The number of viable colonies arising from the surviving oidia were scored for 7 days following irradiation and the results were plotted as per cent survival of the un-irradiated control sample vs. dose (Fig. 1). At 1.67 krads/min, little difference was noted between the survival of gamma irradiated oidia and proton irradiated oidia when they were exposed to equal total doses of radiation. Small differences in the shapes of the shoulders for the two curves may indicate subtle differences in repair processes. These experiments will be repeated at lower dose rates that will emphasize these differences, although maintenance of viability will be more difficult.

Because dose rate is of major importance to a number of cellular functions that determine the survival of irradiated cells, the response of proton-irradiated oidia exposed at a rate of 1.67 krads/min was compared to oidia irradiated at 0.17 krads/min (see Fig. 2). Once radiation starts to have an effect, the survival percentage declines exponentially with slope D_o (the increased dose required to produce a 37% decrease in survival). The amount of radiation required (D_o) was drastically increased and the quasi-threshold dose (D_q, the place where the exponential crosses 100%) was reduced for the lower dose rate. This sur-

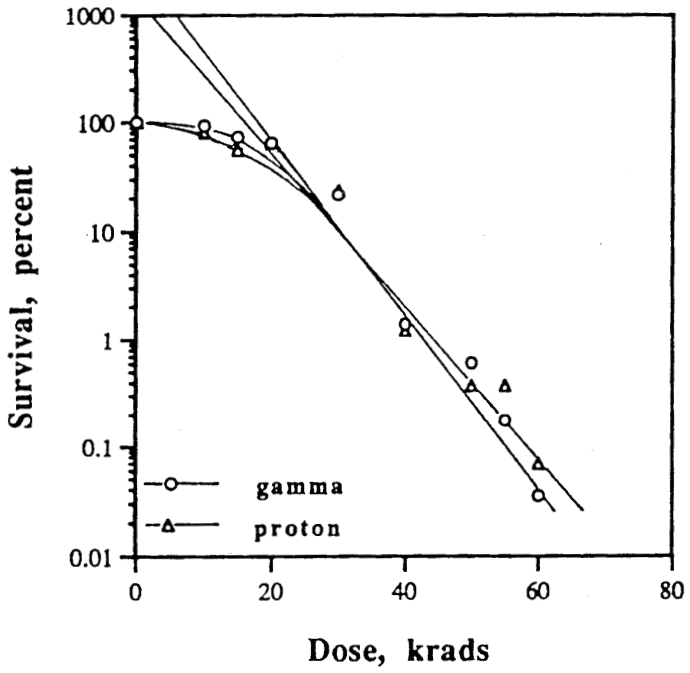

Figure 1. Dose response curves for gamma- and proton-irradiated *C. cinereus.* Duplicate samples of oidia were consecutively irradiated under identical conditions by either gamma-rays or protons at a dose rate of 1.67 krads/min. The linear portions of the curves on this semi-log plot have been extended to emphasize the differences in D_o and D_q.

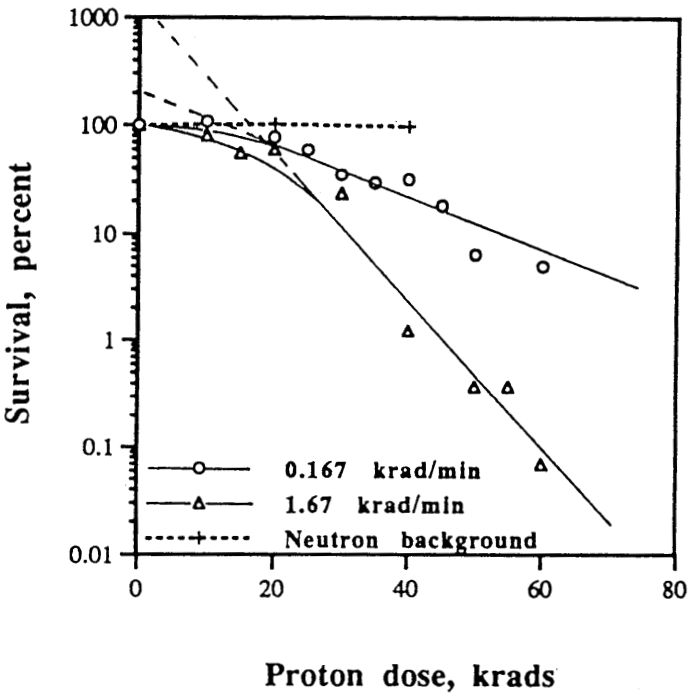

Figure 2. Dose rate dependence exhibited in wild type *C. cinereus.* Identical samples of oidia were proton irradiated at 1.67 and 0.167 krads/min. The linear portion of the curves has been extended to emphasize differences. Background neutron radiation effects were evaluated at the higher dose rate.

vival dependence upon proton delivery rate has also been demonstrated in a number of mammalian systems. The influence of the relatively high neutron background dose was also examined in this experiment by placing samples in proximity to the proton beam, but distant from its direct influence. The experiment indicated no detectable difference between neutron-irradiated and control samples. Future work will examine the relationship between gamma- and proton-radiation dose rate as well as the effect on mutant damage repair.

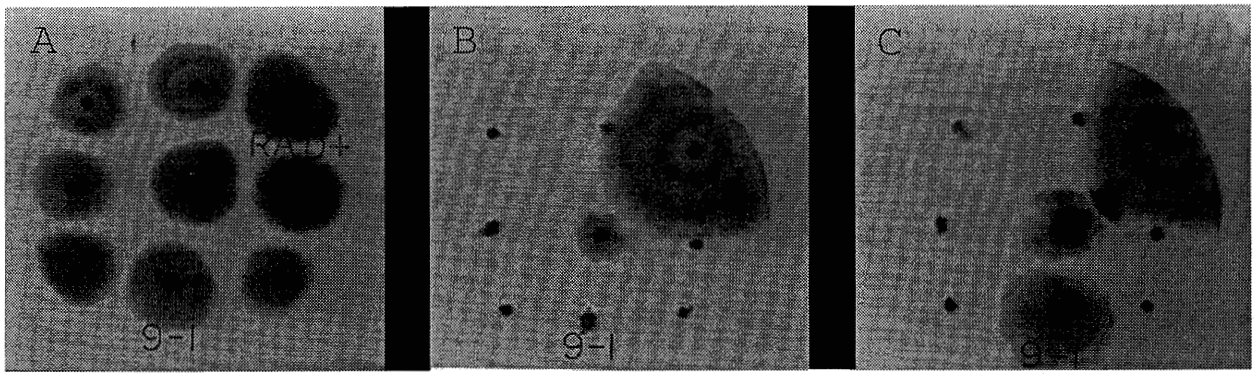

Figure 3. Screen for mutants differentially sensitive to gamma and proton radiation. Small chunks of mutant haphae were explanted and irradiated with low dose rate, high LET protons or gamma radiation from a ^{60}Co source. The un-irradiated mutants (A) grew at the same rate as the congenic wild type, Rad+, strain (photographed at +3 days). Mutants exposed to 40 krads of either gamma (B) or proton (C) radiation grew considerably slower than the unirradiated samples (photographed at +5 days). The Rad+ control is located in the upper right corner of each photograph .

Of the 20,000 mutangenized cultures screened for gamma radiation sensitivity, 37 retained sensitivity through at least one outcrossing to a wild type strain.[1,2] Of these, 16 mutants were backcrossed to the same wild type strain five additional times. Backcrossing reduced the extraneous gene mutations, increasing the probability that a single mutated gene was responsible for gamma radiation sensitivity. These 16 mutants were individually cultured; a small square "chunk" was excised, and they were transplanted to fresh growth medium. The chunks were irradiated at 20, 40, and 60 krads with either gamma radiation or protons. Three mutants indicated differential sensitivity to the two types of radiation. This difference in response indicated specific mutations that contributed to gamma-radiation sensitivity but less so to proton sensitivity. This implied that the genes responsible for the mutations were more important for gamma-radiation repair. The most dramatic difference was demonstrated by the mutant rad9-1 (Fig. 3). The rad9 gene has been isolated and sequenced, and found to encode a new metabolism protein whose function is likely conserved in evolution.[3] Because the initial screen was performed for gamma radiation sensitivity, genes specific for proton radiation repair would not be disclosed in these preliminary experiments. Primary screens for proton sensitivity are planned for the future.

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2. M.E. Zolan, C.J. Tremel, P.J. Pukkila, Genetics **120**, 379 (1988).
3. L. Seitz, K. Tang, W.J. Cummings, M.E. Zolan, in preparation.