RADIATION BIOLOGY

PROTON IRRADIATION OF RADIATION-SENSITIVE MUTANTS OF *COPRINUS CINEREUS*

S.B. Klein and C. Bloch
Indiana University Cyclotron Facility, Bloomington, Indiana, 47408
M.E. Zolan
Biology Department, Indiana University, Bloomington, Indiana, 47405

Proton radiation has important biological consequences in two specific cases: radiation therapy and high energy particle (particularly solar) radiation encountered by biological systems traveling outside of the Earth's magnetosphere. Thus there is a strong impetus to understand the basic biology that has produced the empirically derived consequences of exposure to proton radiation. These consequences include cell death, neurological malfunction, accelerated aging, cataracts, genomic instability, mutagenesis, carcinogenesis, bone-mass loss, and shortened life span. Unfortunately, the classical methods of irradiating mammalian cells in culture have limited application to the *in vivo* situation. We have selected the basidiomycete fungus (mushroom), *Coprinus cinereus* for a model system because it is a genetically tractable, haploid organism that presents a synchronous meiotic (sexual) cycle. With this organism, it is possible to examine the nondifferentiated (stem) cells, differentiated cells (organs), meiotic (germ) cells, and haploid genetics (damage to single genes) not accessible in a mammalian tissue culture system. *C. cinereus* will be used to identify the genes involved in proton radiation sensitivity, the mechanisms responsible for cell death and genomic instability; the pathway overlaps between DNA repair, radiation sensitivity, and meiotic function; and the unique consequences of particle radiation compared to photon radiation.

The proton radiation survival characteristics of vegetative spores (oidia) have been characterized for the wild-type fungus and four gamma radiation-sensitive mutants (see Fig. 1).[1] These mutants each express different defective genes[2] that are involved in the same radiation-sensitivity pathway as well as in meiosis.[3] Small differences in radiation quality (protons vs. ^{137}Cs radiation) and dose rate effects were noted. The data were fit by minimization of χ^2 with the linear or quadratic equations traditionally used to describe *in vitro* radiation survival effects. The radiobiological effectiveness (RBE) of protons compared to ^{137}Cs gamma radiation for cell killing was found to be between 1.27 and 1.39. This is comparable to the values obtained for mammalian cell systems. The biphasic character of the survival curves for wild type *C. cinereus* was not maintained in the survival curves for the sets *rad12, rad11*, and *rad3*; the survival followed a simple linear relationship. The standard interpretation of this phenomenon is that the mutant has lost the ability to repair specific types of DNA damage. Interestingly, the survival

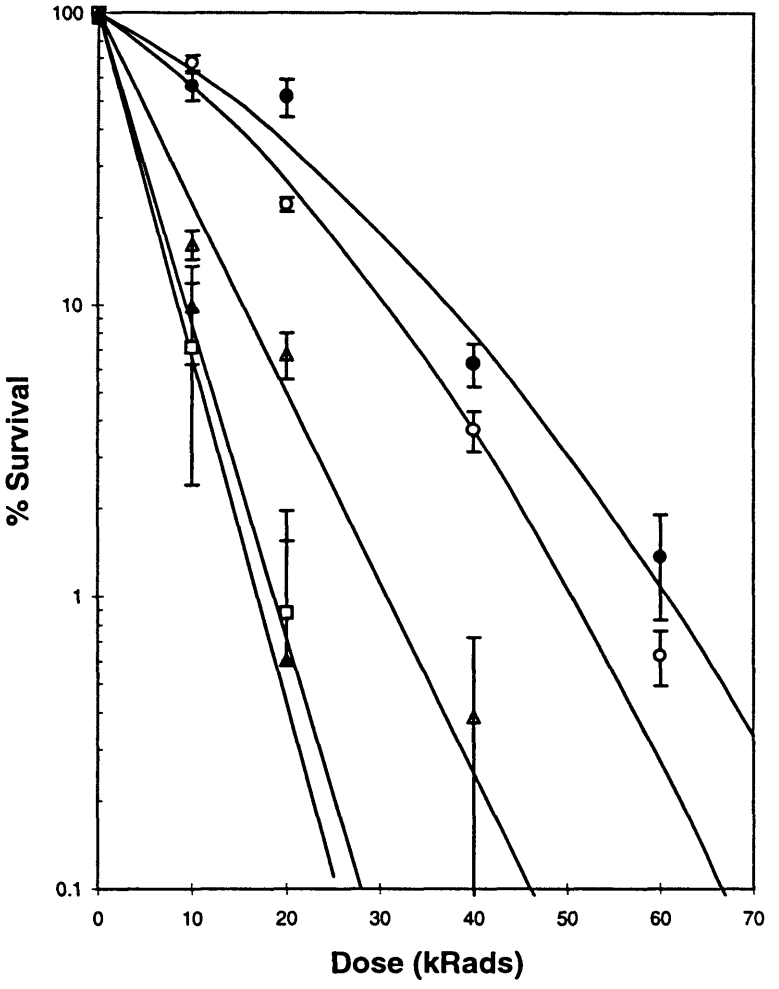

Figure 1. Survival of proton-irradiated vegetative spores. The oidia from wild type *C. cinereus* (filled circle), and four mutants: *rad3-2* (hollow triangle), *rad9-1* (hollow circle), *rad11-1* (filled triangle), and (hollow square), were proton irradiated at increasing doses. The oidia were plated at clonal densities, and the number of colonies surviving following irradiation were scored. The data were fit by the equation, $S = Ae^{-(\alpha D+\beta DD)}$, where S = survival, D = dose and α and β are free parameters.

curves for *rad9* maintained the biphasic character, did not indicate profound loss of repair function compared to wild type, and only exhibited a slightly increased sensitivity to proton radiation.

Small explants of vegetative hyphae were also irradiated by ^{137}Cs gamma radiation and protons. Unlike the oidia, which are held at the G2 cell cycle check point, the vegetative cells are cycling through mitosis, and are asynchronous. The explants were exposed to 5, 10, 20, and 40 krads of ^{137}Cs gamma radiation, or protons, and the sensitivity was assessed by the colony diameter at 2 days (see Fig. 2). Two mutants, *rad11* and *rad12*, were extremely sensitive to both types of radiation, as had been indicated by the survival curves for irradiated oidia. In addition, the sets *rad3* and *rad9* exhibited sensitivity to both types of radiation, but unlike the oidia survival curves *rad9* was drastically growth inhibited at 40 krads. Because the metabolism of the two vegetative cells differed, it was necessary to determine whether the difference between the survival-curve data and explant data was biological or artifactual. The clonal growth of irradiated oidia was reanalysed for growth rate (the number of colonies appearing each day). Figure 3 shows that although the mutant, *rad9*, eventually produced as many colonies as the wild type, the initiation of growth was delayed. The molecular character of the *rad9* gene has been described[4] and the functions of the *rad9* protein in meiosis and radiation damage repair are currently under investigation.

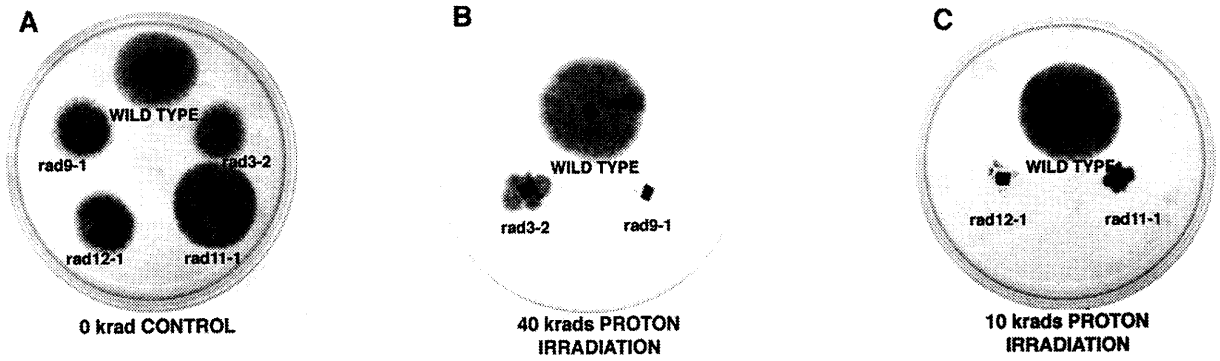

Figure 2. Irradiation of mitotically-cycling vegetative explants. Control (A) panel shows the relative growth rates of unirradiated wild type and four mutant hyphae explants two days after transplantation. Proton irradiation at 40 krads (B) or at 10 krads (C) demonstrated the increased radiation sensitivity of the four meiotic mutant strains at four days post-irradiation.

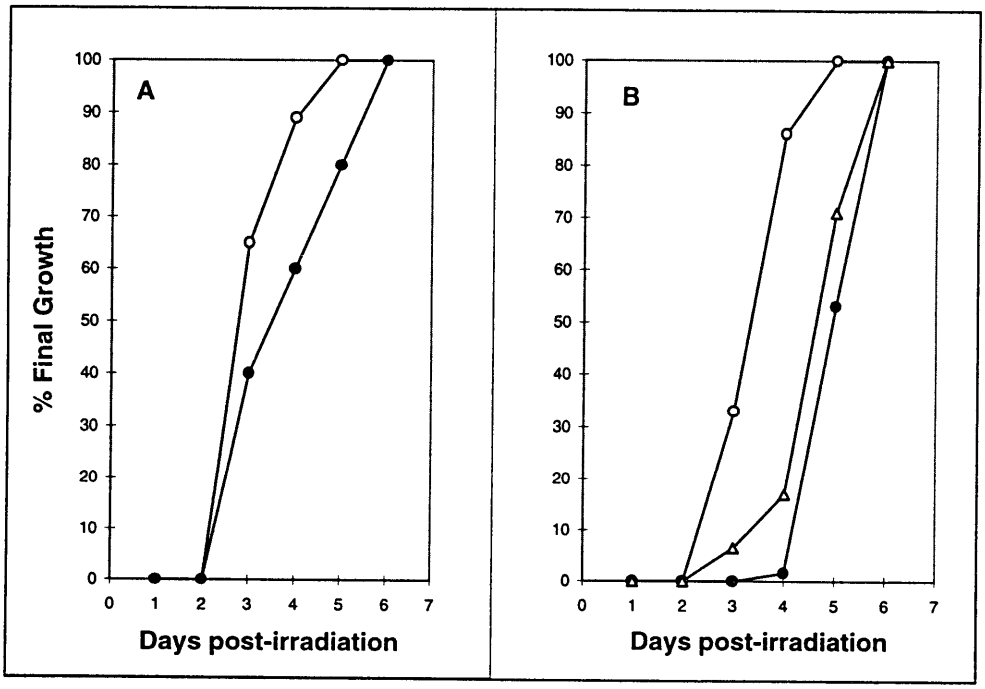

Figure 3. Growth-rate analysis of vegetative spores. Clonal survival was analyzed for growth rate by scoring the appearance of colonies each 24 hours. The results are presented as a percent of total colonies. The growth rate of the wild type (A), unirradiated (hollow circle) vs. irradiated at 60 krads (filled circle), and the growth rate of *rad9-1* (B), unirradiated (hollow circle), and irradiated at 40 (hollow triangle) and 60 (filled circle) krads are compared.

1. G. Valentine, Y.J. Wallace, F.R. Turner and M.E. Zolan, Molec. Gen. Genet. **247**, 169 (1995).
2. M.E. Zolan, J.R. Crittenden, N.K. Heyler and L.C. Seitz, Nucleic Acids Res. **20**, 3993 (1992).
3. M.E. Zolan, N.Y. Stassen, M.A. Ramesh, B.C. Lu and G. Valentine, Can. J. Bot. **73**, S226 (1995).
4. L.C. Seitz, K. Tang, W.J. Cummings and M.E. Zolan, Genetics **142**, 1105 (1996).