Radiation Induced Chromatid-type Aberrations after Irradiation of Late-S/G2 Cells: Roles of Homologous Recombination and Non-Homologous End Joining

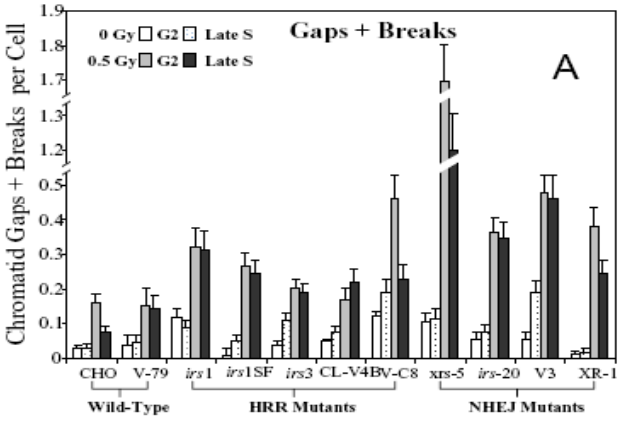
Hatsumi Nagasawa[a], John R. Brogan[a], Yuanlin Peng[a], John B. Little[b], and Joel S. Bedford[a]
[a]*Department of Environmental and Radiological Health Sciences*
Colorado State University, Fort Collins, Colorado 80523, U.S.A.
[b]*Department of Genetics and Complex Diseases*
Harvard School of Public Health, Bldg I, Room 505, Boston, MA, 02115 U.S.A.

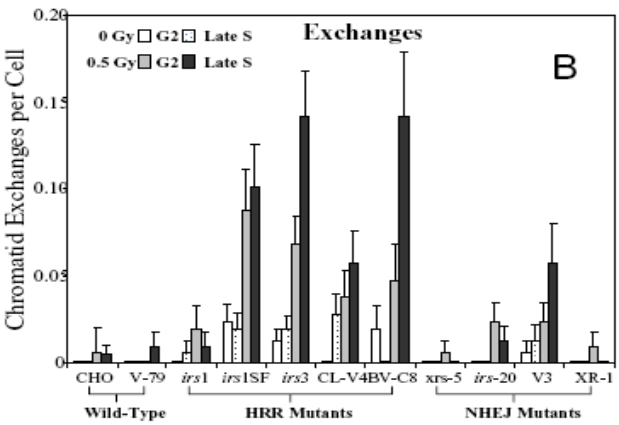
There has been considerable discussion, with some data reported, addressing the question of the relative contributions of non-homologous end joining (NHEJ) and homologous recombinational repair (HRR) on the repair or rejoining of radiation-induced DNA double strand breaks, especially in relation to their operation during the cell cycle. Reports have included studies on chromosomal aberration induction in G1/G0 cells defective in NHEJ or A-T cells, for example, but relatively little on S/G2 cells and especially on mutants defective in HRR. The broad biological importance of homologous recombination is not an issue here. The question is how much does HRR contribute to the processing of radiation induced DNA dsb that can affect the yield of chromosomal aberrations in mammalian cells?

We have recently undertaken a study to compare, specifically, the contributions of these two processes in protecting cells from induction of chromatid-type aberrations after gamma-irradiation of late S/G2 cells, where presumably HRR should provide its greatest contribution. This study involved measuring the yields of chromatid aberrations in wild-type Chinese hamster cell strains and a variety of mutants defective in components of either the NHEJ or HRR systems.

The normal wild-type cells used were CHO K1 (1), CHO AA8 (2), V79B (3), or V79-4 (4). Various mutant sub-lines were used that were defective in pathways involving either HRR [irs-1(*Xrcc2*) (5), irs1SF(*Xrcc3*) (6), irs3(*Rad51C*) (5), CL-V4B(Rad51C) (7), V-8C (*Brca2*) (7)] or NHEJ [Xrs-5(*Ku70/80*) (8), V3(*DNA-PKcs*) (9), irs-20 (*DNA-PKcs*) (10,11), and XR-1 (12)]. Briefly, log phase cultures of these cell lines were irradiated (0.0, 0.25, 0.5, or 1.0Gy) and then Colcemid was added 0.5 hours later for the first collection interval (0.5 to 2.5h) which we called the G2 sample, or starting 2.5 hours later for a second collection interval (2.5 – 4.5h) which we celled the late S sample. After collecting these mitotic cells, slides were prepared for chromatid aberration analysis.

The results for the yield of chromatid gaps plus breaks are plotted below in the left panel and the yield for chromatid exchanges in the right panel. The ratio of gaps to breaks was about 1:1 for the G2 samples but less for the late S samples. The background yields for no radiation are shown as open bars (G2 irradiation) and dotted bars (late S irradiation. The yields for 0.5 Gy are shown as grey bars for G2 irradiation, and black bars for late S irradiation. Uncertainties are standard deviations, and between 100 and 300 cells were scored for each yield estimate. A total of about 11,000 mitotic cells were scored for these experiments. Regarding gaps and breaks, the induced yields for both G2 and late S irradiations were appreciably higher for all the NHEJ mutants (presumably with intact HRR). This was especially striking for the xrs-5 (Ku70/80) mutant,





indicating a large contribution to repair by the NHEJ system. (Without it the cells have more gaps and breaks).

There also appeared to be some increase in levels of induced gaps plus breaks for the HRR-deficient cells with (presumably) an intact NHEJ system. The right panel shows the result for chromatid exchanges. One particularly interesting feature was the relatively low level of increase in the exchanges for the NHEJ mutants, especially the xrs-5 (Ku70/80$^{-/-}$) and XR-1 (*XRCC4/LIGASE4*) mutants. Deficiency in dsb rejoining might be expected to result in a reduction in exchanges with a corresponding increase in unrejoined breaks and this is indeed what we observed. The exchange frequency was higher in V3 cells (DNA-PKcs null) than its wild-type parent, and it may be pertinent that cells which have drastically low levels of DNA-PKcs are also deficient in ATM protein (13). For the HRR deficient cells, we also saw a marked increase in exchanges indicating that the HRR system was also effective in repairing some breaks that interact to form exchanges. It should be noted, however, that the scales for the right (gaps + breaks) and left (exchanges) panels differ by a factor of about 5, so on average the yield of gaps and breaks was approximately five-fold higher than the yield of exchanges.

In summary, our results agree with the conclusion that, along with NHEJ, HRR does contribute to repair of DNA damage leading to chromatid aberrations after irradiation of late S or G2 cells, and this is especially pertinent regarding the relative contributions from the two processes to exchange formation. HRR seems to function more effectively to repair damage in late S and G2 that would lead to exchange formation, with NHEJ less effective in this regard, but NHEJ seems much more effective in promoting the restitution of breaks that might otherwise lead to deletions.

Reference List

1. T. T. Puck, S. J. Ciecuira, and A. Robinson, Genetics of somatic mammalian cells. III. Long-term cultivation of euploid cells from human and animal subjects. *J. Exp. Med.* 108, 945-955 (1958).
2. L. H. Thompson, S. Fong, and K. Brookman, Validation of conditions for efficient detection of HPRT and APRT mutations in suspension-cultured Chinese hamster ovary cells. *Mutat. Res.* 74, 21-36 (1980).
3. D. K. FORD and G. Yerganian, Observations on the chromosomes of Chinese hamster cells in tissue culture. *J. Natl. Cancer Inst.* 21, 393-425 (1958).
4. E. H. Y. Chu, P. Brimer, and K. B. Jakobson, Mammalian genetics. 1. Selection and characterization of mutations auxotrophic for l-glutamine or resistance to 8-azaguanine in Chinese hamster cells in vitro. *Genet.* 62, 359-377 (1969).
5. N. J. Jones, R. Cox, and J. Thacker, Isolation and cross sensitivity of X-ray-sensitive mutants of V79-4 hamster cells. Mutat. Res. 183, 279-286 (1987).
6. L. F. Fuller and R. B. Painter, A Chinese hamster ovary cell line hypersensitive to ionizing radiation and deficient in repair replication. *Mutat. Res.* 193, 109-121 (1988).
7. M. Z. Zdzienicka and J. W. I. M. Simons, Mutagen sensitive cell lines are obtained with a high frequency in V79 Chinese hamster cells. *Mutat. Res.* 178, 235-244 (1987).
8. P. A. Jeggo, L. M. Kemp, and R. Holliday, The application of the microbial "tooth-pick" technique to somatic cell genetics, and its se in the isolation of x-ray sensitive mutants of Chinese hamster ovary cells. *Biochimie* 64, 713-715 (1982).
9. Q. Ding, Y. V. Reddy, W. Wang, T. Woods, P. Douglas, D. A. Ramsden, S. P. Lees-Miller, and K. Meek, Autophosphorylation of the catalytic subunit of the DNA-dependent protein kinase is required for efficient end processing during DNA double-strand break repair. *Mol. Cell Biol.* 23, 5836-5848 (2003).
10. M. A. Stackhouse and J. S. Bedford, An ionizing radiation-sensitive mutant of CHO cells: irs-20 Isolation and initial characterization. *Radiat. Res.* 136, 241-249 (1993).
11. A. Priestley, H. J. Beamish, D. Gell, A. G. Amatucci, M. C. Muhlmann-Diaz, B. K. Singleton, Smith G.C., T. Blunt, L. C. Schalkwyk and others, Molecular and biochemical characterization of DNA-dependent protein kinase-defective rodent mutant irs-20. *Nucl. Acids Res.* 26, 1965-1973 (1998).
12. T. D. Stamato, R. Weinstein, A. Giaccia, and L. Mackenzie, Isolation of a cell cycle-dependent gamma-ray sensitive Chinese hamster ovary cell. *Somat. Cell Mol. Genet.* 9, 165-173 (1983).
13. Y. Peng, R. G. Woods, H. Beamish, R. Ye, S. P. Lees-Miller, M. F. Lavin, and J. S. Bedford, Deficiency in the catalytic subunit of DNA-dependent protein kinase causes down-regulation of ATM. *Cancer Res.* 65, 1670-1677 (2005).

This research was supported by the Low Dose Radiation Research Program, Office of Science (BER), U.S. Department of Energy, Grant No. DE-FG-02-07ER64350