October 15, 2003

Re: Final Report for Grant no. DE-FG02-98ER62583 "Functional Analysis of the Genome Sequence of *Deinococcus radiodurans*."

Expired: 3/14/2003 (after a one-year no-cost extension).

Dear Sir/Madam:

The resources provided by grant DE-FG02-98ER62583 were used to study the bacterium *Deinococcus radiodurans*. This organism is historically most famous for its ability to survive hundreds of DNA double-stranded breaks following acute exposures to high-dose ionizing radiation. The genome sequence has been key to defining the repertoire of genes and pathways responsible for this remarkable phenotype and has laid the foundation on which the mystery of extreme radiation resistance will be solved. We are very grateful for the longstanding interest and support provided to us by the U.S. Department of Energy.

Sincerely,

Michael J. Daly, Ph.D.
Associate Professor
Uniformed Services University of the Health Sciences
Pathology, Room B3153
4301 Jones Bridge Road
Bethesda, MD 20814-4799
USA
Tel: 301-295-3750
Fax: 301-295-1640
Email: mdaly@usuhs.mil

Summary of Products:

Comparative Genomics (5 papers): Our group published a comprehensive genomic analysis of *D. radiodurans* in March 2001 that included cataloging ~1,000 deinococcal genes of unknown function from a total of 3,195 detected ORFs (<ftp://ncbi.nlm.nih.gov/pub/koonin/Deinococcus/>). We recognize that there are still many important informatic areas that remain unexplored and have investigated operon organization and its repertoire of regulatory signals using whole genome expression arrays and proteomic approaches.

1. K. S. Makarova, L. Aravind, Y. I. Wolf, R. L. Tatusov, K. Minton, E. V. Koonin, and MICHAEL J. DALY (2001) The genome of the extremely radiation resistant bacterium *D.*

- radiodurans* viewed from the perspective of comparative genomics. *Microbiology and Molecular Biology Reviews* **65**, 44-79.
2. K. S. Makarova, L. Aravind, MICHAEL J. DALY, and E. V. Koonin (2000) Specific expansion of protein families in the radioresistant bacterium *Deinococcus radiodurans*. *Genetica* **108**, 25-34.
 3. O. White, J. A. Eisen, J. F. Heidelberg, E. K. Hickey, J.D. Peterson, R. J. Dodson, C. Zalewski, K. S. Makarova, L. Aravind, MICHAEL J. DALY, K. W. Minton, R. D. Fleischmann, K. A. Ketchum, K. E. Nelson, S. Salzberg, H. O. Smith, J. C. Venter, C. M. Fraser *et al.*, (1999) Sequencing and functional analysis of the *Deinococcus radiodurans* genome. *Science* **286**, 1571-1577.
 4. J. Lin, R. Qi, C. Aston, J. Jing, T. S. Anantharaman, B. Mishra, O. White, K. W. Minton, MICHAEL J. DALY, J. C. Venter, and D. C. Schwartz (1999) Whole genome shotgun optical mapping of *Deinococcus radiodurans* using genomic DNA molecules. *Science* **285**, 1558-1561.
 5. K. S. Makarova, Y. I. Wolf, K. W. Minton, O. White, and MICHAEL J. DALY (1999) Short repeats and insertional elements in *Deinococcus radiodurans* and comparison to other bacterial species. *Res. Microbiol.* **150**, 711-724.

Microarray Results (1 paper): To define the repertoire of *D. radiodurans* genes responding to acute irradiation (15 kGy), transcriptome dynamics were examined in cells representing early, middle, and late phases of recovery using DNA microarrays covering 94% of its predicted genes. At least at one time point during *D. radiodurans* recovery, 832 genes (28% of the genome) were induced and 451 genes (15%) were repressed two-fold or greater. The expression patterns of the majority of the induced genes resemble the previously characterized expression profile of *recA* following irradiation. *D. radiodurans recA*, which is central to genomic restoration following irradiation, is substantially up-regulated upon DNA damage (early phase) and down-regulated before the onset of exponential growth (late phase). Many other genes were expressed later in recovery, displaying a growth-related pattern of induction. Genes induced in the early phase of recovery included those involved in DNA replication, repair, recombination, cell wall metabolism, cellular transport, and many encoding uncharacterized proteins. Collectively, the microarray data suggest that *D. radiodurans* cells efficiently coordinate their recovery by a complex network, within which both DNA repair and metabolic functions play critical roles. Components of this network include a predicted distinct ATP-dependent DNA ligase, and metabolic pathway switching that could prevent additional genomic damage elicited by metabolism-induced free radicals.

1. Y. Liu, J. Zhou, A. Beliaev, J. Stair, L. Wu, D.K. Thompson, D. Xu, A. Venkateswaran, M. Omelehenko, M. Zhai, E. K. Gaidamakova, K. S. Makarova, E. Koonin, and MICHAEL J. DALY (2003) Transcriptome dynamics of *D. radiodurans* recovering from ionizing radiation. *Proc. Natl. Acad. Sci. USA* **100**, 4191-4196.

Proteomic Results (1 paper): The ability to understand biological systems and their constituents would be greatly facilitated by the ability to make quantitative, sensitive, and comprehensive measurements of how their proteome changes *e.g.*, in response to environmental perturbations.

To this end we have developed new instrumentation and a high throughput methodology to characterize an organism's dynamic proteome based upon the combination of global enzymatic digestion, high-resolution liquid chromatographic separations and analysis by Fourier transform ion cyclotron resonance mass spectrometry. Using accurate mass tags, 61% of the predicted proteome of the ionizing radiation resistant bacterium *D. radiodurans* was characterized with high confidence. This represents the broadest proteome coverage for any organism to date, and includes 715 proteins previously annotated as either hypothetical or conserved hypothetical. This work was published in the Proceedings of the National Academy of Sciences (PNAS) in August 2002.

1. M. S. Lipton, L. Pasa-Tolic, G. A. Anderson, D. J. Anderson, D. Auberry, J. R. Battista, MICHAEL J. DALY, J. K. Fredrickson, A. Venkateswaran, K. K. Wong, R. Zhao, and R. D. Smith *et al.* (2002) Global analysis of the *D. radiodurans* R1 proteome using accurate mass tags. *Proc. Natl. Acad. Sci. USA*. **99**, 11049-11054.

Purification of DNA repair proteins (2 papers)

The RecA protein of *D. radiodurans* (RecA(Dr)) is essential for the extreme radiation resistance of this organism. The RecA(Dr) protein has been cloned and expressed in *Escherichia coli* and purified from this host. In some respects, the RecA(Dr) protein and the *E. coli* RecA (RecA(Ec)) proteins are close functional homologues. RecA(Dr) forms filaments on single-stranded DNA (ssDNA) that are similar to those formed by the RecA(Ec). The RecA(Dr) protein hydrolyzes ATP and dATP and promotes DNA strand exchange reactions. DNA strand exchange is greatly facilitated by the *E. coli* SSB protein. As is the case with the *E. coli* RecA protein, the use of dATP as a cofactor permits more facile displacement of bound SSB protein from ssDNA. However, there are important differences as well. The RecA(Dr) protein promotes ATP- and dATP-dependent reactions with distinctly different pH profiles. Although dATP is hydrolyzed at approximately the same rate at pHs 7.5 and 8.1, dATP supports an efficient DNA strand exchange only at pH 8.1. At both pHs, ATP supports efficient DNA strand exchange through heterologous insertions but dATP does not. Thus, dATP enhances the binding of RecA(Dr) protein to ssDNA and the displacement of ssDNA binding protein, but the hydrolysis of dATP is poorly coupled to DNA strand exchange. The RecA(Dr) protein thus may offer new insights into the role of ATP hydrolysis in the DNA strand exchange reactions promoted by the bacterial RecA proteins. In addition, the RecA(Dr) protein binds much better to duplex DNA than the RecA(Ec) protein, binding preferentially to double-stranded DNA (dsDNA) even when ssDNA is present in the solutions. This may be of significance in the pathways for dsDNA break repair in *Deinococcus*.

1. J-I Kim, A. K. Sharma, S. N. Abbot, E. A. Wood, D. Dwyer, A. Jambura, K. W. Minton, R. B. Inman, MICHAEL J. DALY, and M. M. Cox (2002) RecA protein from the extremely radioresistant bacterium *Deinococcus radiodurans*: Expression, purification, and characterization. *J. Bacteriol.* **184**, 1649-60.
2. M. Sandigursky, S. Sandigursky, P. Sonati, MICHAEL J. DALY, and W. A. Franklin

(2003) Multiple uracil-DNA glycosylase activities in *Deinococcus radiodurans*. *DNA Repair*, Submitted.

Relationship between Metabolism, oxidative stress and radiation resistance in *Deinococcus* (1 paper)

We have previously shown that *D. radiodurans* can grow in the presence of continuous radiation (60 Gy/hour) without lethality, on rich medium. Now we have developed a chemically defined minimal medium to analyze growth in the presence and absence of continuous radiation; whereas cell growth was unaffected in the absence of radiation, cells did not grow, and were killed, under continuous radiation. In nutrient-limiting conditions, DNA repair was found to be limited by this organism's metabolic capabilities and not by any nutritionally induced defect in genetic repair. Our growth studies and analysis of the complete *D. radiodurans* genomic sequence support the existence of several defects in *D. radiodurans*' global metabolic regulation that limit carbon, nitrogen and DNA metabolism. We have identified key nutritional constituents that restore growth of *D. radiodurans* in nutritionally limiting radioactive environments.

1. A.Venkateswaran, S. C. McFarlan, D. Ghosal, K. W. Minton, A. Vasilenko, K. Makarova, L. P. Wackett, and MICHAEL J. DALY (2000) Physiologic determinants of radiation resistance in *D. radiodurans*. *Appl. Environ. Microbiol.* **66**, 2620-2626.

Role of DNA protection in the extraordinary survival of *D. radiodurans* (1 paper)

With experimental comparisons made to *Escherichia coli* and *Shewanella oneidensis*, we have tested a new hypothesis that DNA damage may not be key in determining survival following acute irradiation. Instead we have found evidence supporting that Fe-dependent protein systems involved with energy production and protection against oxygen radicals (*e.g.*, cytochromes and Fe-SOD) are highly vulnerable to free radicals generated during radiation and during early recovery. For example, we believe that the very high intracellular Mn(II) concentration detected in *D. radiodurans* mediates protection against high levels of superoxide generated by irradiation-damaged electron transport during recovery. Evidence is consistent with the conclusion that *Deinococcus* cells emerge from irradiation with a functional non-enzymatic Mn(II)-based protection system ready for immediate defence against metabolism-generated reactive oxygen species (ROS). In contrast, irradiated *E. coli* and *S. oneidensis*, which have elevated free Fe(II) levels and lack high Mn concentrations, are vulnerable to Fenton-type chemistry. Without the benefit of a broad-based, radiation-tolerant system for ROS removal, cells with high levels of accumulated DNA damage appear to be committing suicide as they increase metabolic rates to levels needed for cellular repair.

1. A. Vasilenko, E. K. Gaidamakova, V. Matrosova, D. Ghosal, M. Zhai, A. Venkateswaran, H. Brim, M. Hess, M. Omelchenko, H. M. Kostandarithes, K. S. Makarova, J. K. Fredrickson, and MICHAEL J. DALY (2003) Role of Manganese (II) Protection in the Extraordinary Survival of *Deinococcus radiodurans*. To be Submitted.