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The Dynamics of Cellular Stress Responses in *Deinococcus radiodurans*

DOE Microbial Cell Program/Genomes to Life Program

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Final Report for Project Period 1997-2004 (7 papers, see below)

Institutional Endorsement: **Henry M. Jackson Foundation for the Advancement of Military Medicine (HMJFAMM),** Rockville, MD 20852

Products:

Work funded by the existing grant contributed to 7 papers in the period 2001-2005 on radiation resistance mechanisms of *D. radiodurans*. Summary of Publications: Microbial Cell Program/Genomes to Life Grant DE-FG02-01ER63220.

2001-2005

1. M. V. Omelchenko, Y. I. Wolf, E. K. Gaidamakova, V. Y. Matrosova, A. Vasilenko, **MICHAEL J. DALY**, E. V. Koonin and K. S. Makarova (2005) Comparative genomics of *Thermus thermophilus* HB27 and *Deinococcus radiodurans* R1: Divergent paths of adaptation to thermophily and radiation resistance. *BMC Evolutionary Biology*, **5**, 57-79.

2. D. Ghosal, M. V. Omelchenko, E. K. Gaidamakova, V. Y. Matrosova, A. Vasilenko, A. Venkateswaran, H. M. Kostandarithes, H. Brim, K. S. Makarova, L. P. Wackett, J. K. Fredrickson and **MICHAEL J. DALY** (2005) How radiation kills cells: Survival of *Deinococcus radiodurans* and *Shewanella oneidensis* under oxidative stress. *FEMS Microbiology Reviews* **29**, 361-375.

3. **MICHAEL J. DALY**, E. K. Gaidamakova, V. Y. Matrosova, A. Vasilenko, M. Zhai, A. Venkateswaran, M. Hess, M. V. Omelchenko, H. M. Kostandarithes, K. S. Makarova, L. P. Wackett, J. K. Fredrickson and D. Ghosal (2004) Accumulation of Mn(II) in *Deinococcus radiodurans* facilitates gamma-radiation resistance. *Science* **306**, 925-1084.

4. M. Sandigursky, S. Sandigursky, P. Sonati, **MICHAEL J. DALY** and W. A. Franklin (2004) Multiple uracil-DNA glycosylase activities in *D. radiodurans. DNA Repair*, **3**, 163-169.

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

6. M. S. Lipton, L. Pasa-Tolic, G. A. Anderson, D. J. Anderson, D. Auberry, J. R. Battista, **MICHAEL J. DALY**, J. K. Fredrickson, K. K. Hixson, H. Kostandarithes, T. Conrads, C. Masselon, M. Markille, R. J. Moore, M. F. Romine, Y. Shen, N. Tolic, H. R. Udseth, T. D.Veenstra, A. Venkateswaran , K. K Wong, R. Zhao and R. D. Smith (2002) Global analysis of the *Deinococcus radiodurans* R1 proteome using accurate mass tags. *Proc. Natl. Acad. Sci. USA*. **99**, 11049-11054.

7. 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.

Final Technical Report:

Introduction: Bacteria belonging to the family *Deinococcaceae* are some of the most ionizing radiation (IR) resistant organisms yet discovered. *Deinococcus radiodurans* is obligate aerobic, capable of growth under chronic IR (60 Gy/hour) and relatively resistant to many DNA damaging conditions including exposure to desiccation, ultraviolet radiation and hydrogen peroxide. The genes and cellular pathways underlying the survival strategies of *D. radiodurans* have been under investigation for fifty years. In the last decade, *D. radiodurans* was subjected to whole-genome sequencing, annotation and comparative analysis, whole-transcriptome and whole-proteome analyses, and numerous DNA repair studies. Collectively, published reports support that the key to survival of *D. radiodurans* resides in its ability to repair DNA, but the mechanisms responsible remain poorly defined. Unexpectedly, many novel genes implicated in recovery from IR by transcriptome and proteome profiling have had little effect on survival when disrupted, and there is reason to ask if something is missing from classical models of radiation resistance. The prevailing dogma of radiation toxicity has been that the cytotoxic and mutagenic effects of radiation are principally the result of DNA damage that occurs during IR. However, in light of available whole genome sequences, one broad observation that is difficult to reconcile with this view is that many organisms that encode a compliment of DNA repair and protection functions are killed at radiation doses that cause little DNA damage. This indicates that there are cellular targets involved in recovery that are more vulnerable to IR damage than DNA. It has been reported that *D. radiodurans* and other resistant organisms accumulate very high intracellular concentrations of Mn(II), and restricting the amount of Mn(II) during recovery from IR substantially reduced survival of *D. radiodurans*. At high intracellular concentrations, Mn(II) is known to act as a true catalyst of the dismutase of superoxde ($O_2^{\bullet-}$), with Mn cycling between the divalent and trivalent states. Superoxide is generated during water radiolysis, particularly in the presence of iron redox-cycling processes, and has been implicated in damaging [4Fe-4S] cluster-containing proteins, with the release of bound Fe(II). Thus, it is possible that Mn(II) accumulation acts as keystone among antioxidant defenses which limits protein damage during both irradiation and recovery, with the result that DNA repair and other enzymic systems involved in recovery function with greater efficiency in *D. radiodurans* than other organisms.

Results:

Transcriptome Dynamics of *D. radiodurans* Recovering from Ionizing Radiation (*Proc. Natl. Acad. Sci. USA*, 100, 4191-4196)

We have reported the dynamic changes in *D. radiodurans* global gene expression profiles using microarrays covering ~94% of its predicted genes. Cells representing early-, mid-, and late-phases of its recovery were examined following high-dose IR (15 kGy) and incubation in undefined rich medium for 0, 0.5, 1.5, 3, 5, 9, 12, 16, and 24 hours. In total, 832 genes (28% of genome) were induced 2-fold or more at least at one time point during

recovery. 25 of 71 (35%) genes predicted for DNA repair, recombination and replication were overexpressed during the early-mid phase of recovery (0-12 h after IR). We have used gene expression patterns during recovery from high-dose IR to help identify open reading frames (ORFs) contributing to radiation resistance in *D. radiodurans*. To determine the function of genes and their possible role in the resistance phenotype, we generated knockout mutants. From 40 (expression-prioritized) ORF disruptions, 3 predicted genes were identified that substantially reduced survival of *D. radiodurans* following IR. The most sensitive mutant obtained was a disruption of ORF DR0070. The phenotype of disrupted DR0070 was examined in detail by stress-sensitivity assays (*e.g.*, gamma-radiation, UV-radiation, and desiccation). Two other resistance genes confirmed among the group of 40 disrupted ORFs are DR2069, which encodes a predicted NAD-ligase and renders cells more sensitive to acute radiation, and DR2339, which encodes a predicted 2'-5' RNA ligase (co-localized with *recA*) and sensitizes cells to chronic (50 Gy/hour) radiation. The *D. radiodurans* genes we have identified by transcriptional profiling following IR, and which have been disrupted and analyzed, are listed online at: http://www.usuhs.mil/pat/deinococcus/index_20.htm.

Proteome of *D. radiodurans* (*Proc. Natl. Acad. Sci. USA*. 99, 11049-11054)

Understanding biological systems and the roles of their constituents is 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 a high-throughput methodology to characterize an organism's dynamic proteome based on the combination of global enzymatic digestion, high-resolution liquid chromatographic separations, and analysis by Fourier transform ion cyclotron resonance (FTICR) mass spectrometry. The peptides produced serve as accurate mass tags for the proteins and have been used to identify with high confidence >61% of the predicted proteome for the ionizing radiation-resistant bacterium *D. radiodurans*. This fraction represents the broadest proteome coverage for any organism to date and includes 715 proteins previously annotated as either hypothetical or conserved hypothetical.

The definition of the *D. radiodurans* proteome involved building a database of accurate mass tags (AMTs) generated for the tryptic peptides from global cellular protein digestions. AMTs are defined as unique peptides that are identified and validated by the combination of sequence-related information from tandem MS and the high mass measurement accuracy of FTICR. Specifically, cells were cultured under a variety of conditions, lysed, and the proteins extracted and proteolytically digested. The global tryptic digest approach thus bypasses the more conventional 2-D polyacrylamide gel electrophoresis (PAGE) separations, and when combined with the enhanced sensitivity and dynamic range of the FTICR instrumentation, expands the identification of proteins while eliminating the inherent complications and more limited coverage associated with 2-D PAGE.

Expansion of MCP/GtL Project to include *Deinococcus geothermalis*

The bacterium *Deinococcus geothermalis* is remarkable not only for its extreme resistance to IR, but also for its ability to grow at temperatures as high as 55^{o}C and in the presence of chronic irradiation (50 Gy/hour). The organism was isolated in 1997 from thermal springs at Agnano, Naples, Italy, and we reported the development of genetic transformation systems for this bacterium in 2003. *D. geothermalis* belongs to the bacterial family *Deinococcaceae*, currently comprised of seven distinct nonpathogenic radiation resistant species, of which *D. radiodurans* strain R1 is the most characterized. Draft genome sequencing of *D. geothermalis* together with our recently reported advances in the genetic management of this bacterium are being used to test hypotheses on mechanisms of radiation resistance. For example, two novel *D. radiodurans* genes (DR0070 and DR2339) that have been shown to be involved in radiation resistance in *D. radiodurans* are also present in *D. geothermalis*. In this context, it should be noted that many uncharacterized genes in *D. radiodiodrans* are absent in *D. geothermalis*, adding significance to the presence of DR0070 and DR2339 in *D. geothermalis*.

In collaboration with DOE's Joint Genome Institute (JGI), we supported whole genome sequencing of *D. geothermalis*; the genome has been fully assembled Preliminary comparative analyses using the *D. geothermalis* draft sequence and other whole or partially sequenced radiation resistant bacteria support the following conclusions: At least five radiation resistant bacteria have been subjected to whole/draft genome sequencing, and available sequences for comparison support that their DNA repair systems depend on a variety of common genes including RecADEG, UvrABC, and RuvABC, *etc*. So far, no shared group of predicted, uncharacterized genes has been identified in those organisms that might comprise an expanded gene core involved in recovery from radiation. Viewed in this context, two competing views are supported, (i) radiation resistant organisms use a relatively limited, conventional set of DNA repair functions, but with greater efficiency than other organisms, or (ii) beyond the core of conventional repair genes, resistance pathways are highly diverse and uniquely adapted to the lifestyle of the host. We have examined by ICP-MS analysis the total Mn and Fe concentrations of the sequenced radiation resistant organisms and find that their intracellular Mn/Fe ratios correlate with their survival capabilities.

Role of manganese in *D. radiodurans*' radiation resistance (*Science* 306, 925-1084)

D. radiodurans is extremely resistant to IR. How this bacterium can grow under chronic gamma radiation [50 grays (Gy) per hour] or recover from acute doses greater than 10 kGy is unknown. We have shown that *D. radiodurans* accumulates very high intracellular manganese and low iron levels compared with radiation-sensitive bacteria and that resistance exhibits a concentration-dependent response to manganous chloride [Mn(II)]. Among the most radiation-resistant bacterial groups reported, *Deinococcus*, *Enterococcus*, *Lactobacillus*, and cyanobacteria accumulate Mn(II). In contrast, *Shewanella oneidensis* and *Pseudomonas putida* have high iron but low intracellular manganese concentrations and are very sensitive. We have proposed that Mn(II) accumulation facilitates recovery from radiation injury.

General Insights and Conclusions from Current Project Period

Current emphasis on DNA damage inflicted during the course of irradiation as the overriding cause of death of irradiated bacterial cells might be overstated, particularly for Fe-accumulating organisms such *S. oneidensis* that encode a comprehensive set of known DNA repair functions but are killed by very low doses of radiation (Ghosal *et al*., 2005). In experiments on *Escherichia coli* where the synthesis of superoxide dismutease (SOD) was externally controlled, it has been shown that the cells calibrate their defences so that they barely withstand the toxic action of endogenous superoxide. Therefore, recovery of the radiation sensitive *S. oneidensis*, *P. putida*, and *E. coli*, which encode similar SOD, catalase and peroxidase functions but have relatively low intracellular Mn/Fe ratios (Daly *et al*., 2004) might be compromised by their inability to recalibrate enzymatic defence systems in time to counter sudden increases in endogenous oxidative stress during irradiation. In contrast, accumulated Mn(II) ions would be functionally poised to act against increases in superoxide, but not affected by radiation. Mn(II) assimilation strategies that support the recovery of highly resistant organisms might include expression of high catalase activities, suppression of hydroxyl radical-production (extremely toxic) by limiting cellular Fe requirements, and suppression of superoxide levels by metabolic regulation during recovery. Similar arguments can be made to explain how radiation resistant vegetative cells or bacterial spores which accumulate Mn(II) but not Fe can also withstand desiccation and exposure to H_2O_2. The desiccation resistance profiles of the seven representative bacteria we have examined mirror both the trend in their Mn/Fe ratios and their resistance to ionizing radiation.