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Nuclear Energy in the Post-Genomic Era

Custer C. Deocaris and Alejandro Q. Nato, Jr.

Philippine Nuclear Research Institute
Commonwealth Avenue, Diliman 1101, Quezon City, Philippines

Abstraction

'Genomics,' the term first proposed in 1986, is the discipline of understanding genomes powered by an unprecedented accumulation of information on genetic sequences of a wide archive of life forms, foremost of which is the genome of man. After more than two decades of international effort in decoding life's alphabet and culminating with the first working draft of the human DNA this year, the 'post-genomic' era aims to annotate the existing genetic data, elucidate gene functions and find revolutionary applications in almost all branches of life and physical sciences, leaving no bough untouched, not even nuclear science.

1. From Nuclear Energy to Nuclear Genome

Nuclear power is not just a form of energy that has been and will continue to be exploited for electricity generation but it is also a fuel to fire the expansion of the human mind and imagination, in understanding all related fields of science, and in trail-blazing to new frontiers that would shape society and improve life on the planet.

The roots of this massive biology project can be traced back to the dynamic offices of the US Department of Energy (DOE), in the same conference rooms that previously conceived their first expensive baby named, Manhattan, somewhere in the hinterlands of New Mexico in the mid-40s. While in a pre-Christmas get-together of geneticists in Little Cottonwoon Canyon, near Salt Lake City, Alta in 1984, DOE contemplated on a single question: Can the current gene technologies determine the extent of damage to the DNA of people exposed to radiation, in particular, the survivors of Hiroshima and Nagasaki and their descendants? While the workshop evaluated the status of current DNA technologies at the time, the pace at which individual laboratories performed gene cloning and sequencing was so slow and inefficient. Finding fine-structured mutations with high sensitivity with minimum effort was then a dream, and unless laboratories unite their talents and resources in a single large, complex and expensive program, the art of human mutation screening would remain a nightmare. A subsequent report on *Technologies for Detecting Heritable Mutations in Human Beings* sparked the idea for a dedicated human genome project by the DOE.

In the months that followed after heated debates and deliberation, in an atmosphere of rare intellectual ferment in 1986, the DOE took a bold step in sowing the seeds for the most expensive scientific endeavor by humanity that would make such detection possible in the future---the human genome project.

The major objective was to generate refined physical maps for human genome research, complete the sequence of the human genome and a variety of other model

organisms, and expand communication networks and computational and database capacities. In 1988, the US Office of Technology Assessment and National Research Council, and the US National Institutes of Health (NIH) set up the National Center for Human Genome Research (NCHGR) while the DOE channeled its genome activities mainly through the Lawrence Berkeley National Laboratory, the Los Alamos National Laboratory and the Lawrence Livermore National Laboratory---all of which had a common denominator: these were former factories of nuclear weapons at wartime. The US Congress officially gave approval to a 15-year US human genome project commencing in 1991 initially worth US$3 billion for the first year. Other human genome programs were established in Denmark, France, Germany, Italy, the Netherlands, the UK, the former USSR, the Nordic regions, Australia, Canada, Japan, Korea and New Zealand.

The impact of the project is now largely felt in medicine and health care. It has also stimulated significant investments by large companies in the hope to capitalize on its profound implications. The careers of scientists in the likes of Craig Ventner, Frank Collins, among others emerged like a steadfast Tower of Babel in the annals of science. The DOE saw far more to the genome project than as a tool to assess mutations in humans, it would yield to more efficient production of energy, to novel strategies in environmental remediation and waste reduction from power plants, and to more cost-effective industrial processing technologies. As a result of the initiative, DOE has completed sequencing several microbial genomes of special relevance to energy issues (**See Box 1**) while post-genomic protein structural studies and genetic manipulation are underway. One special interest in the Microbial Genome Program was the elucidation of the genomic sequence, proteosome and metabolosome of *Deinococcus radiodurans*.

2. D. radiodurans: Radio-tolerance demystified

Deinococcus radiodurans is a gram-positive, red-pigmented, non-motile bacterium accidentally discovered as a contaminant in irradiated-canned meat at the Oregon Agricultural Experiment Station (Anderson *et al*., 1956). All species in the genus *Deinococcus* are extremely resistant to DNA damage from ionizing radiation, ultraviolet rays (UV), desiccation and hydrogen peroxide. In fact, *D. radiodurans* is described to be the most radiation-resistant organisms to date, capable of thriving in exponential growth phase despite being bombarded with 1,750,000 rads. For years, scientists had no idea on how this bacterium evolved its super radiation-resistance and theories had been forwarded that it could only be so if *D. radiodurans* originated from outerspace, perhaps as a touristy microbe on-board a frozen meteorite. It was only until the revelation of its genome sequence that its radiation-tolerance was explained logically and appreciated.

Using the random whole-genome shotgun method, White and his colleagues (1999) published the complete genetic sequence of *D. radiodurans*. The genome is composed of two chromosomes (2,648,638 and 412,348 bp), a megaplasmid (177,466 bp) and a small plasmid (45,704) yielding a total genome of 3,284,156 bp (**See Box 2**).

D. radiodurans' claim to fame in its ability to survive vicious levels of ionizing and non-ionizing radiation, desiccation and chemical treatment can be summarized as the result of three mechanisms: prevention, tolerance and repair.

Scavenging oxygen radicals is first in the agenda of the bacterium. Several such free radical-neutralizing genes are present in the *D. radiodurans*' genome, including two catalases, multiple superoxide dismutases and a homolog of DPS protein in *E. coli*. The red coloration of *D. radiodurans* is due to the presence of high quantities of carotenoid pigments that also act as free radical-scavengers. Finally, it has a cell wall forming three or more layers with complex outer membrane lipids and thick peptidoglycan layer with high amount of omithine, a radioprotective amino acid.

Although prevention and tolerance contribute to the survival tools of *D. radiodurans*, equally tantalizing to account for are the radioresistance-coding genes that provide a highly efficient DNA repair system. Bombarding the bacterium of 1.75 Mrads of ionizing radiation would leave its genes unscathed since DNA repair gets done within 24 hours. Analysis of the genome sequence of *D. radiodurans* reveals a battery of DNA repair enzymes, including nucleotide excision repair, base excision repair, mismatch excision repair and various recombinatorial repair enzymes. There are functional homologs of these enzymes in other prokaryotes, however, no other species has been shown to contain as many DNA repair mediators.

The polyploid nature of *D. radiodurans* at 4-10 genome equivalents during its logarithmic phase is likely important during the homologous recombinatorial repair. Its genes are stacked resembling a 'Lifesaver' perhaps to speed up the repair by connecting/splicing undamaged chromosomes to a damaged one using a highly efficient RecA protein. Another crucial component is the presence of DNA repeat elements scattered throughout the genome. Being intergenic and ubiquitous in the chromosomes, they serve as binding/recognition sites for an inhibitory protein, Irrf, to prevent exhaustive chromosomal degradation after radiation exposure.

Another mechanism unique to *D. radiodurans*' resistance to DNA damage insults is the clean-up system to rid of damaged DNA strands by exporting these genetic glitches out of the cell thus preventing the reincorporation in the genome. The presence of two homologs of UvrA may explain this extraordinary talent. A UvrA homolog performs this task by recognizing and attaching itself to damaged DNA facilitating transport across the cell membrane, not just in *D. radiodurans*, but in other bacteria as well, like *E. coli*.

Phylogenetic analysis of highly conserved genes of the *Deinococci* revealed extensive homologies with the *Thermus* genus (**see Box 3**). Since all *Thermus* species are thermo-tolerant and that some *Deinococcus* members are slightly thermophilic, it is suggested that these two genera evolved from a common ancestor and that the ability of *D. radiodurans* to withstand the high flux of radiation evolved from thermotolerance survival systems (Ferreira *et al*., 1997).

3. Contribution to nuclear energy development

One of the biggest challenges in waste cleanup at a number of DOE repository sites is the management of the *ad mixture* of toxic chemicals, heavy metals, halogenated solvents and radionuclide wastes. Organic chemicals used by the US nuclear weapons industry from 1945 to 1986 in the Cold War were frequently mixed with highly radioactive isotopes and buried in underground waste tanks in various DOE radioactive supersites. Some of the older tanks have leaked their deadly contents into the surrounding soil and some are feared to even have

leached into underground waters. In a futile effort of speciation and disposal of individual contaminants, one proposal was to remove the halogenated solvents by bioremediation. However, most bacteria would fail to survive in the levels of radiation present in these mixed wastes.

The fact that compositional differences in the plasmids of *D. radiodurans* indicate the propensity for horizontal gene transfer, the bacterium would be amenable to genetic engineering. In fact, consistent with its gene structure, it appears that *D. radiodurans* is the most transformable species known (Tigari and Moseley, 1980).

Based on the published sequence data revealing its metabolosome profile, the nutritional requirements of maintaining the vegetative phase of *D. radiodurans* were deduced. Since some *de novo* amino acid synthesis pathways are deficient, exogenous amino acids are essential for the restoration of radiation-resistance and must be added in otherwise nutritionally-limiting radioactive environment (Venkateswaran *et al*., 2000)

D. radiodurans can be released to tolerate the atomic blast and initiate scouring for toxic residues of a nuclear holocaust. Insertion of the gene for toluene dioxygenase from *Pseudomonas putida* into the chromosome of *D. radiodurans* conferred the bacterium with the capacity to degrade toluene and other organic compounds in high-radiation environments. Other microorganisms may be exploited as a talent pool to contribute snippets of genes in engineering *D. radiodurans*. These include *D. ethenogenes*, for degrading tetrachloroethene; *Geobacter sulfurreducens*, *Shewanella oneidensis* and *Thiobacillus ferroxidans* for altering metal-oxidative states with applications in biomining; and *Nitrosomas europaea*, for bioremediating nitrates. Bioconversion of toxic forms of mercury, a common heavy metal pollutant in nuclear weapons production sites, into less harmful end-products have also been performed by inserting heavy metal-binding proteins from a strain of *Escherichia coli* into the chromosomes of *D. radiodurans*. In corollary, this principle can be applied to improve recovery of uranium and thorium for biomining (Lange *et al*., 1998).

D. radiodurans beginnings simulate the early Earth and parallel the time when the Earth's environment approximates that existing on Mars as of present time. Dubbed as a polyextremophile, the properties of *D. radiodurans* make it an exceptional model to simulate life on Mars. On the practical aspects in space exploration, instead of bringing truckloads of pharmacy to space, *D. radiodurans* could be genetically-altered to produce various medicines and even food-supplements for astronauts during lengthy space flights and camping at homesteads in the Martian landscape. With such an approach, the issues of shelf-life could be solved and the weight of a spaceship could be much reduced. The ultimate dream of space biologists is the concept of exploiting this bacterium to restructure Mars for human habitation despite the absence of atmospheric shield against cosmic rays. The early life-forms, such as those belonging to the archaea group, were the early settlers on Earth that shaped the carbon dioxide atmosphere and calcium-rich, soupy seas into a hospitable place for more complex creatures (Richmond *et al*., 1999).

Field tests have yet to be done, but scientists are hopeful that these strange, pink radio-resistant berries (English translation for *Deinococcus radiodurans*) could be customized to gobble-up heavy metals, radioactive wastes and other substances that pollute the soil and groundwater at nuclear production sites. Someday too, these mighty mites might end-up as a favorite pet for geoexploration of uranium deposits needed badly to expand the nuclear energy industry, like a goody pig to a truffle.

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Box 1. Organisms of Research by the DOE*

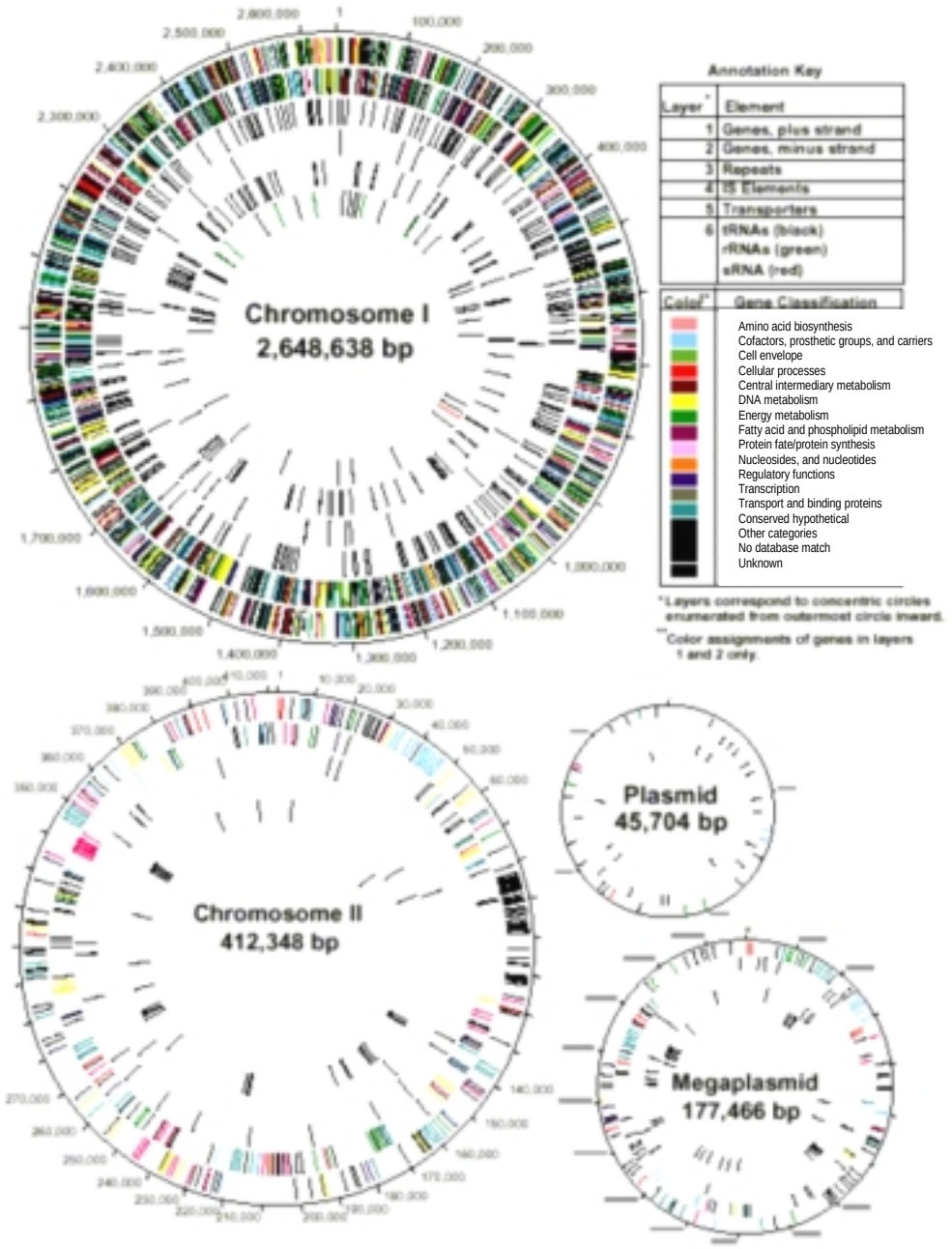
The US Department of Energy has either completed or presently sequencing the entire genomes of the microbes mentioned below which have been found to have significant impact on energy issues. Various applications are being specifically elucidated for each organism as elaborated by the project description and notes indicated in the table.

Organism	Phylogenetic Branch	Genome Size	Project Description	Notes
Aquifex aeolicus	Bacteria Aquifex	1.6 Mb	Genome sequenced, functional genomics	Chemolithoautotrophic thermophile
Archaeoglobus fulgidus	Archaea Methanosarcina	2.2 Mb	Genome sequenced, functional genomics	Sulfur- metabolizing hyperthermophile
Carboxydothermus hydrogenoformans	Bacteria Thermodesulfobacterium	1.8 Mb	Genome sequencing	Hydrogen production
Caulobacter crescentus	Bacteria Purple bacteria	3.8 Mb	Genome sequencing	Used to study reg. of cell cycle events; bioremediation
Chlorobium tepidum	Bacteria Green sulfur bacteria	2.1 Mb	Genome sequenced	Thermophilic photoautotroph, role in global carbon cycling
Clostridium acetobutylicum	Bacteria Gram- positive	4.1 Mb	Genome sequenced	Industrial biotechnology & waste remediation
Dehalococcoides ethenogenes	Bacteria Green nonsulfur bacteria		Genome sequencing	Anaerobic chemoautotroph useful for bioremediation
Deinococcus radiodurans	Bacteria Deinococci	3.3 Mb	Genome sequenced	Chemoorganotroph, highly resistant to desiccation and gemma radiation
Desulfovibrio vulgaris	Bacteria Purple bacteria	1.7 Mb	Genome sequencing	Sulfate- reducing bacteria; bioremediation
Geobacter sulfurreducens	Bacteria Purple bacteria	1.0 Mb	Genome sequencing	Bioremediation
Methanobacterium thermoautotrophicum	Archaea Methanobacterium	1.8 Mb	Comparative & functional genomics	Methane- producing, moderate thermophile
Methanococcus jannaschii	Archaea Methanococcus	1.7 Mb	Comparative & functional genomics	Autotrophic, methane- producing thermophile (also listed under NASA)
Mycoplasma genitalium	Bacteria Gram- positive	0.6 Mb	Sequenced, functional genomics	Free- living organsim with smallest known genome
Nitrosomonas europaea	Bacteria Purple bacteria	1.6 Mb	Genome sequencing	Carbon/ nitrogen management
Nostoc spp.	Bacteria Cyanobacteria	<10.0 Mb	Genome sequencing	Carbon management
Prochlorococcus marinus	Bacteria Cyanobacteria		Genome sequencing	Carbon management
Pseudomonas putida	Bacteria Purple bacteria	5.0 Mb	Genome sequencing	Opportunistic plant and animal pathogen; bioremediation
Pyrobaculum aerophilum	Archaea Thermoproteus	2.2 Mb	Sequenced, functional genomics	Hyperthermophile; model for high temperature growth
Pyrococcus furiosus	Archaea Thermococcus	2.1 Mb	Genome sequenced	Heterotrophic, sulfur- reducing thermophile
Rhodobacter capsulatus	Bacteria Purple bacteria	3.7 Mb	Genome sequencing	Carbon and nitrogen fixation
Rhodopseudomonas palustris	Bacteria Purple bacteria	<5.0 Mb	Genome sequencing	Hydrogen production
Shewanella putrefaciens Strain: MR- 1	Bacteria Purple bacteria	4.5 Mb	Genome sequencing	Model bacterial system for reductive dehalogenation reactions; bioremediation
Thermotoga maritima	Bacteria Thermotogales	1.9 Mb	Genome sequenced, functional genomics	Hyperthermophile; model for molecular mechanisms of protein thermostability
Thiobacillus ferrooxidans	Bacteria Purple bacteria	2.9 Mb	Genome sequencing	Obligate chemolithoautotroph; carbon management, bioremediation

*Source: **Interagency Report on the Federal Investment in Microbial Genomics.** (2000) Report from the Biotechnology Research Working Group, Subcommittee on Biotechnology, Committee on Science, National Science and Technology Council, Executive Office of the Pres. of the United States. 43pp**.**

Box 2. Circular representation of the *D. radiodurans* genome*

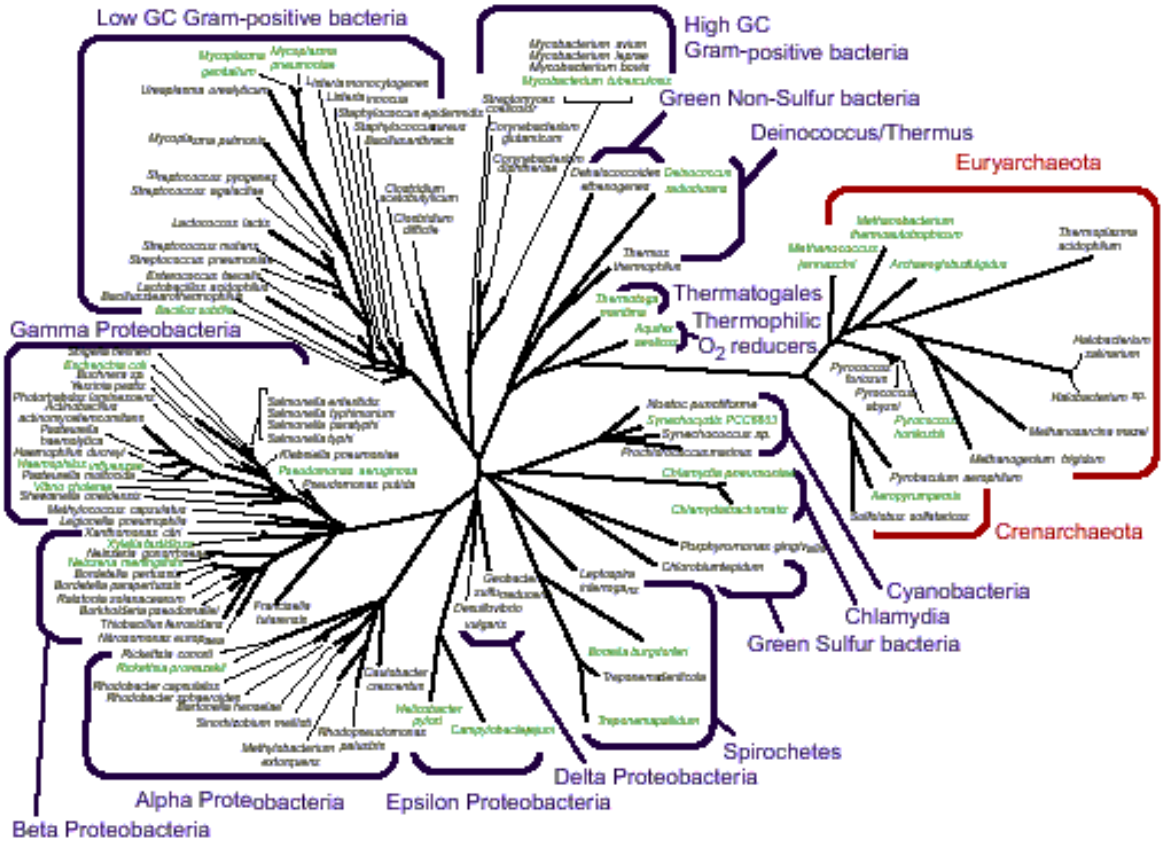
The locations of predicted coding regions are color-coded by biological role, repeats, insertion (IS) elements, rRNA genes, tRNA genes, sRNA genes, and transposons which are indicated on the four circular molecules of *D. radiodurans*.



*Source: White O *et al.* (1999) *Science*. **286**:1571.

Box 3. Unrooted Phylogenetic Tree on 16s rRNA Sequences of Prokaryotic Organisms*

The prokaryotic organisms whose complete genomic sequence has been published (highlighted in green) and other ogranisms for which genome sequencing projects are underway are arranged in the unrooted phylogenetic tree. The major prokaryotic phylogenetic groupings are indicated by the brackets (archaea in red, eubacteria in blue). The phylogenetic tree was derived from sequences from the Ribosomal Database Project (RDP) using Phylip for tree construction. In some instances where the rRNA sequence of the particular strain was not available, the rRNA of a close relative was substituted.



*Source: Nelson KE *et al.* (2000). *Nature Biotech.* **18:**1049.