



Using DNA microarray data to understand the ionizing radiation resistance of *Deinococcus radiodurans*

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In a recent paper, Liu *et al.* documented the changes in gene expression as stationary phase *Deinococcus radiodurans* cultures recover from acute exposure to gamma radiation. Given that the biochemical details of the response of *D. radiodurans* to ionizing radiation are poorly understood, this work represents an important first step towards achieving an understanding of the ionizing radiation resistance in this species.

Deinococcus radiodurans, one of the most radiation-resistant organisms known, can survive extensive DNA damage by UV and ionizing radiation, toxic chemicals and desiccation. When the decision to sequence this unique microorganism was made, it was optimistically predicted that the complete genome sequence would provide insight into the extraordinary DNA repair capabilities of *D. radiodurans*. However, analysis of the genome sequence (published in 1999 [1]) did not elucidate the genetic basis of the DNA repair capabilities. Basically, all of the typical prokaryotic DNA repair genes were found in *D. radiodurans*. The lack of clearly identifiable unique DNA repair genes or pathways has led to two competing hypotheses: either *D. radiodurans* simply uses the same DNA repair pathways as other prokaryotes, but it is much more efficient; or there are unidentified unique genes that define a novel repair system in *D. radiodurans*.

The sequencing of the *D. radiodurans* genome clearly defines a starting point for research with a primary goal of understanding the genes and mechanisms behind DNA repair in *D. radiodurans*. Several current research efforts are ongoing to characterize the *in vivo* functions of the DNA repair proteins, determine from where the improved efficiencies arise, and analyzing genes with unknown function to identify novel DNA repair pathways. From these experimental approaches, improved insights into the DNA repair mechanisms might be found. The results that follow from an understanding of *D. radiodurans* DNA repair will find many applications in fields ranging from environmental biotechnology to health care.

Transcriptional dynamics

Liu *et al.* have recently published a paper that might ultimately help to identify the genes endowing *D. radiodurans* with an extremely efficient DNA repair system [2]. They described the transcriptional dynamics in *D. radiodurans* cultures following exposure to an acute dose of

ionizing irradiation. In this study, stationary phase cultures of *D. radiodurans* were irradiated to 15 kGy. The irradiated culture was then transferred to fresh media to recover. During the recovery, the concentration of nearly all the *D. radiodurans* transcripts relative to the unirradiated control at nine different time points over a 24 hour period were analyzed [2]. The group reported that 832 genes were induced and 451 genes were repressed at one or more of the time points during the recovery period [2].

The large number of loci responding during recovery following irradiation makes it difficult to interpret the significance of these findings. Although an increase in gene expression suggests that the resultant protein has some crucial function in the cell's response to ionizing radiation, this need not always be true. There is not a perfect correlation between genes that are essential under a given condition and the genes that are induced under the same condition [3,4]. In this study the situation is even more complex because it was necessary for the irradiated stationary phase cultures to be diluted in fresh media to observe recovery. Cultures were, therefore, recovering from ionizing radiation-induced intracellular damage and exiting stationary phase simultaneously. The cell's need to deal with both conditions could help to explain the large number of differentially expressed transcripts. Despite this, Liu *et al.* elegantly tried to sort through the long list of genes responding in these experiments and identify genes truly involved in DNA repair by applying bioinformatics. For example, it is well known that *recA* is essential for DNA repair in *D. radiodurans* and that it is upregulated on irradiation [5–8]. Using hierarchical clustering, Liu *et al.* identified all genes in the dynamic response that behave similarly to *recA*. Additionally, also using hierarchical clustering, they showed that genes primarily involved in growth are not induced until after 9 hours, and hence they conclude that the genes responding early are in response to the irradiation and not the culture conditions. However, a modified experimental protocol that controls for the change in growth states might remove the need for the bioinformatics analysis to filter through the large number of genes. Furthermore, it is likely that the authors have missed many DNA repair genes owing to the bioinformatics methods used because there is not reason to believe that all DNA repair pathways will respond with a pattern like *recA*.

Constructing mutant strains

Ultimately, the goal of Liu *et al.*'s microarray analyses of *D. radiodurans* is to identify the genes involved in repair of

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ionizing radiation-induced intracellular damage. This work represents the first step in that process. Genes that exhibit a relative expression ratio above the threshold limit must be evaluated for their role in radioresistance, and constructing mutant strains, a classical genetic approach for verifying a gene product's involvement in a repair pathway, remains the best method to quickly ascertain the function of individual proteins in a complex biological pathway. Liu *et al.* recognized this and combined their expression analysis with mutagenesis. They constructed a mutant in which DR0070 was disrupted and demonstrated that *D. radiodurans* strains carrying this disruption are more sensitive to irradiation than those that do not. DR0070 encodes a hypothetical protein that is among those genes induced in response to ionizing radiation, and might represent a novel component of this cell's defense against ionizing radiation.

Future work

Many challenges remain for the research community studying *D. radiodurans*. Specifically, using DNA microarrays to identify DNA repair genes will not identify the constitutively expressed genes that are essential for DNA repair. The essential constitutively expressed gene can only be identified by an experiment that directly inactivates that gene. Furthermore, because of noise in the microarray studies, transcripts must experience a relative change greater than twofold to be considered up-regulated or down-regulated with any statistical certainty [2,9]. However, it is likely that many biologically important responses result in a less than a twofold induction or repression. Therefore, improvements in the monitoring technology and the statistical analysis are required to

fulfill the full promise of DNA microarrays in the study of this and other species.

In conclusion, the dynamic analysis of the transcriptome is likely to produce valuable data for uncovering the intricacies in the DNA repair pathways found in *D. radiodurans*, but these preliminary results constitute a collection of predictions that require testing. *D. radiodurans* has not given up its secrets yet. Additional detailed information will be required to obtain a true understanding of the complexities of this organism and its repair pathways.

References

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