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Mechanisms of Radiation Resistance in *Deinococcus radiodurans* R1 Revealed by the Reconstruction of Gene Regulatory Network Using Bayesian Network Approach

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Abstract

The mechanisms of radiation resistance in the extreme anti-radiation bacterium *Deinococcus radiodurans* R1 have fascinated researchers for more than sixty years. In this study, with the DNA microarray data, we first constructed the gene regulatory network in *D. radiodurans* R1 using Bayesian network approach. The results of our analysis for the gene regulatory network reveal twelve significant mechanisms of radiation resistance in *D. radiodurans* R1, of which two mechanisms (DNA repair and reactive oxygen species) are consistent with the well-known knowledge; and two mechanisms (metal ion homeostasis and oxygen transport process) support Daly's manganese-based radiation protection hypothesis. We also demonstrate that different mechanisms act in concert under the radiation stress in *D. radiodurans* R1.

Keywords: Mechanisms of radiation resistance; *Deinococcus radiodurans* R1; Gene Regulatory network; Bayesian network

Introduction

The Gram-positive bacterium *Deinococcus radiodurans* R1 is the most radiation-resistant organism described to date [1]. The radiation resistance of *D. radiodurans* R1 makes it an ideal candidate of many applications in fields ranging from environmental biotechnology to health care.

Although *D. radiodurans* R1 was discovered in 1956 [2], little is known about the mechanisms of radiation resistance in the bacterium till now. To uncover the mechanisms of the radiation resistance in *D. radiodurans* R1, White et al. [3] sequenced the whole genome of *D. radiodurans* R1 in 1999. The genome is composed of two chromosomes (2.65 and 0.412 Mb), a megaplasmid (0.177 Mb), and a small plasmid (0.046 Mb). It was expected that the complete genome sequence would shed light on the extraordinary DNA repair capabilities of *D. radiodurans* R1. However, analysis of the genome sequence did not provide insight into the genetic basis of the DNA repair capabilities [3,4]. In 2007, Griffiths and Gupta [5] identified 399 unique proteins to *D. radiodurans* R1, but did not illustrate the biological functions of these proteins. The emergence of DNA microarray technology [6] enables researchers to measure all the gene expression levels of an organism simultaneously. In 2003, Liu et al. [7] described the transcriptional dynamics in *D. radiodurans* R1 cultures following exposure to 15 kGy ionizing irradiation. The irradiated culture was then transferred to fresh media to recover. During the recovery, the concentration of 2750 *D. radiodurans* R1 transcripts relative to the unirradiated control at nine different time points over a 24 hour period were analyzed. The results indicated that 832 genes were induced and 451 genes were repressed at one or more of the time points during the recovery period. Liu et al. [7] analyzed the DNA microarray results using hierarchical clustering. The clustering algorithm is useful in discovering genes that have similar expression patterns over a set of experiments, but difficult to reveal the process of the transcriptional regulation [8]. Due to the large number of loci responding during recovery following irradiation, it is difficult to interpret the significance of these findings.

Currently, two mechanisms explain the radiation resistance in *D. radiodurans* R1 [1,9]. However, DNA repair system in *D. radiodurans* R1 seems having no difference with other organisms. The radiation-resistant mechanisms in *D. radiodurans* R1 are far from complete understood. Bayesin network approach [10] is a promising tool for DNA microarray data analysis [11,12] and biological network reconstruction [13-15]. To explore the mechanisms of radiation resistance in *D. radiodurans* R1, in this study we for the first time reconstructed the *D. radiodurans* R1 gene regulatory network using Bayesin network approach. Our results revealed more mechanisms involved in radiation-resistant processes in *D. radiodurans* R1.

Data and Methods

D. radiodurans R1 DNA microarray data

The *D. radiodurans* R1 DNA microarray data were retrieved from reference [7]. The data include 2750 genes. Each gene contains gene expression values at nine time points.

Reconstruction of *D. radiodurans* R1 gene regulatory network using Bayesian network approach

Bayesian network is a probabilistic graphical model that represents a set of random variables and their conditional dependencies via a directed acyclic graph (DAG). It consists of two components: the first

component is a directed acyclic graph; and the second component is a set of parameters that quantify the network [12]. A Bayesian network is defined as:

$$P_B = (X_1,...,X_n) = \prod_{i=1}^{n} P_B(X_i \mid pa(X_i))$$

Where:

X_i denotes each variable in DAG, $pa(X_i)$ denotes all parent nodes of X_i.

In this study, we used the R package bnlearn (http://cran.r-project.org/) to learn the Bayesian network structure.
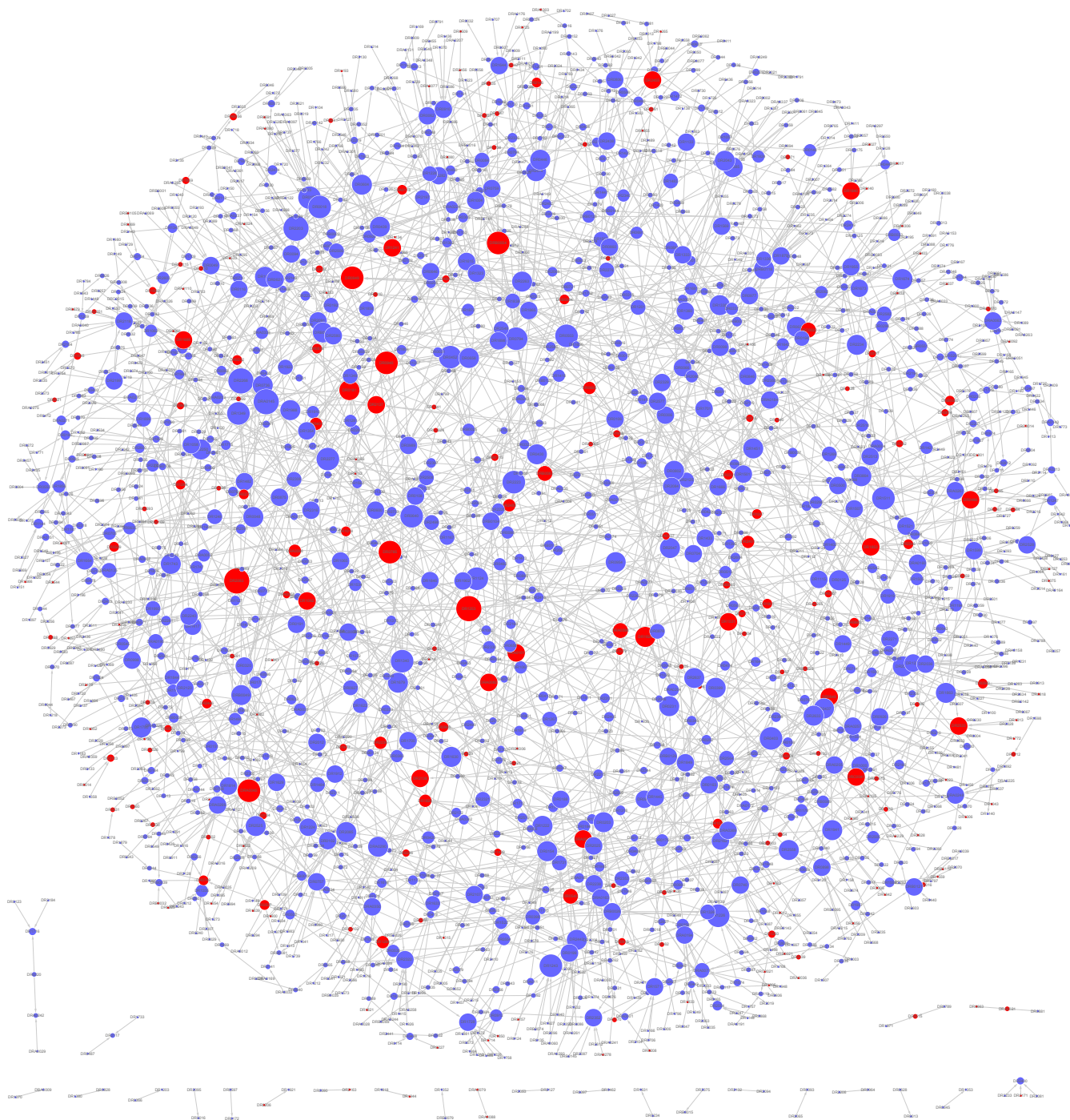

Figure 1: The gene regulatory network in *D. Radiodurans* R1. The gene regulatory network includes 1938 nodes and 2635 edges. The red nodes represent unique genes in *D. Radiodurans* R1. The node size is proportion to the degree of the node.

Bootstrap test for the Bayesian network

Bootstrap test for the Bayesian network was performed according to the method proposed by Friedman et al. [16]. In this study, we performed the bootstrap test for 150 times. Pair nodes with edge bootstrap values equal to or larger than 0.85 will be used to reconstruct the gene regulatory network.

Visualization of the gene regulatory network

Visualization of the gene regulatory network was performed using the software Cytoscape 2.8.3 (http://www.cytoscape.org/) [17].

GO term enrichment analysis

To find the significant biological processes in the gene regulatory network, we first annotated the GO terms for each gene via InterProScan tool [18]. Then, we conducted the GO term enrichment analysis using R package topGO (http://www.bioconductor.org/packages/2.12/bioc/html/topGO.html). The significant level for the enrichment is p-value < 0.05.

GO term annotations for the unknown genes based on Markov blanket

In a Bayesian network, the Markov blanket of node includes its parents, children and the other parents of all of its children. To annotate the function of an unknown gene, we first extracted the Markov blanket of the gene from the gene regulatory network, and then conducted the GO term enrichment analysis for the Markov blanket. Finally, we assigned the enriched GO term(s) to the unknown gene as its function(s).

Results

Gene regulatory network of *D. radiodurans* R1

We first reconstructed *D. radiodurans* R1 gene regulatory network using Bayesian network approach. The gene regulatory network includes 1938 nodes and 2635 edges (Figure 1). Each edge between two nodes has a bootstrap value greater than 0.85. The distribution of node degrees showed that only a minority of nodes have high degree of connectivity (Figure 2), and followed the power law (Figure S1). The result suggested that *D. radiodurans* R1 gene regulatory network is a scale-free network. The scale-free networks are remarkably resistant to accidental failures [19]. Since the hub nodes (i.e. nodes having high degree of connectivity) in a scale-free network dominate the overall connectivity of the network, they are of great importance [20]. Therefore, we next focused our analysis on the hub nodes in *D. radiodurans* R1 gene regulatory network, and expected to find out the key mechanisms of radiation resistance in *D. radiodurans* R1.

Hub node sub-network reveals a variety of radiation-resistant mechanisms in *D. radiodurans* R1

In this study, we defined the nodes with degree equal to or greater than 8 as hub nodes. We collected all of 74 hub nodes and 511 nodes directly connected with the hub nodes (Table S1). Figure 2 is the sub-network which hub nodes involved in. Next, we performed the GO term enrichment analysis for the hub node sub-network, and found that twenty-two biological processes were enriched (Table 1, Figure S2). The enriched biological processes could be classified into eight categories: (1) Arginine metabolic process; (2) GTP metabolic process; (3) metal ion homeostasis; (4) oxygen transport; (5) dicarboxylic acid transport; (6) protein transport; (7) protein oligomerization and (8)
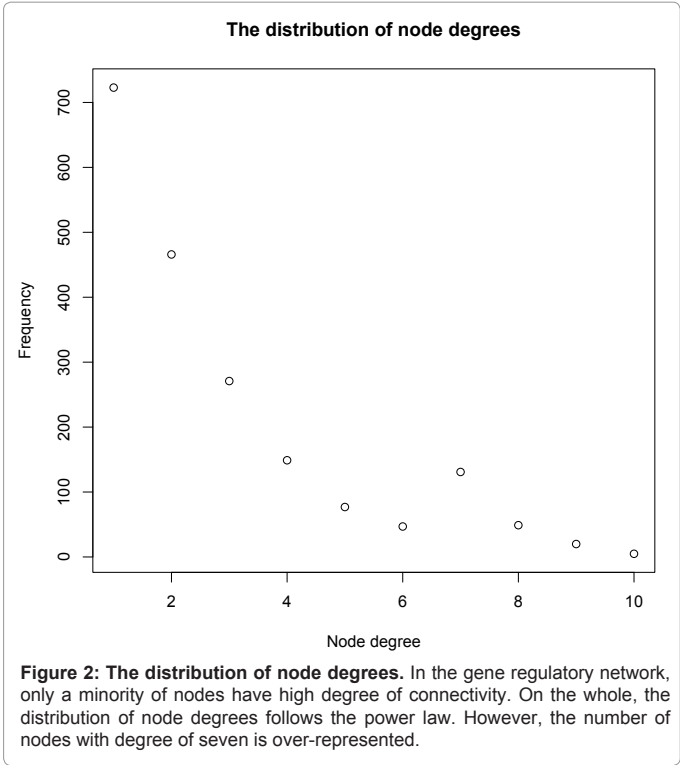

Figure 2: The distribution of node degrees. In the gene regulatory network, only a minority of nodes have high degree of connectivity. On the whole, the distribution of node degrees follows the power law. However, the number of nodes with degree of seven is over-represented.

Category	GO.ID	Term	p-value
1	GO:0006520	cellular amino acid metabolic process	0.034
	GO:0006525	arginine metabolic process	0.043
2	GO:0046039	GTP metabolic process	0.036
3	GO:0048878	chemical homeostasis	0.031
	GO:0006873	cellular ion homeostasis	0.031
	GO:0006875	cellular metal ion homeostasis	0.031
	GO:0006879	cellular iron ion homeostasis	0.031
	GO:0030003	cellular cation homeostasis	0.031
	GO:0050801	ion homeostasis	0.031
	GO:0055065	metal ion homeostasis	0.031
	GO:0055072	iron ion homeostasis	0.031
	GO:0055080	cation homeostasis	0.031
4	GO:0015669	gas transport	0.031
	GO:0015671	oxygen transport	0.031
5	GO:0006835	dicarboxylic acid transport	0.031
6	GO:0033036	macromolecule localization	0.023
	GO:0008104	protein localization	0.023
	GO:0045184	establishment of protein localization	0.016
	GO:0015031	protein transport	0.016
7	GO:0051259	protein oligomerization	0.031
8	GO:0055082	cellular chemical homeostasis	0.031
	GO:0071555	cell wall organization	0.031

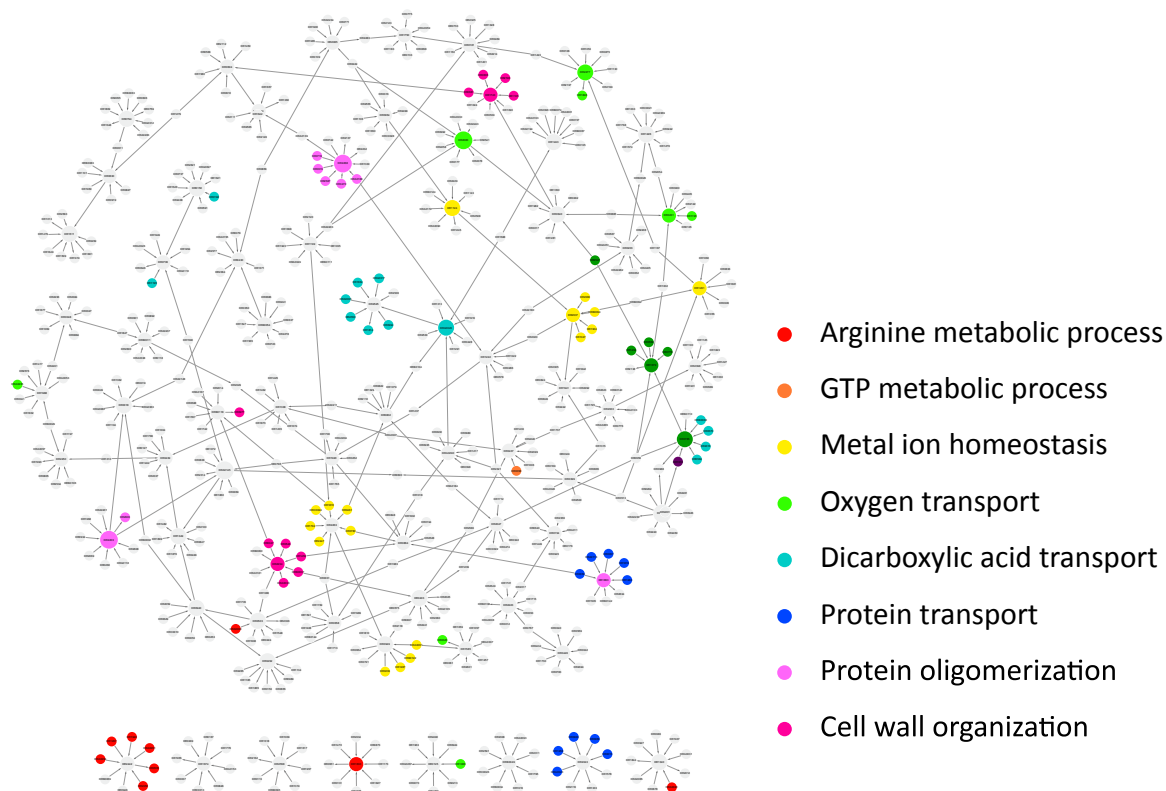**Table 1: S**ignificant biological processes in the hub node sub-network.

Figure 3: The sub-network of hub node in *D. Radiodurans* R1. The sub-network includes 74 hub nodes with degree of 8~10 and 563 nodes directly connected with the hub nodes. The node sized is proportion to the degree of the node. The sub-network reveals eight mechanisms of radiation-resistance in *D. radiodurans* R1 (shown in different colors).

cell wall organization (Table 1). We found that some nodes involved in different categories simultaneously. For example, nodes DR0569, DR0778, DR0785, DR1454, DR1679 and DR2547 involved in the oxygen transport and the dicarboxylic acid transport at the same time. The node DR1910 participated the dicarboxylic acid transport as well as the cell wall organization (Table S1).

Nodes with degree of seven are over-represented in *D. radiodurans* R1 gene regulatory network

We observed that 131 nodes with degree of seven appeared in *D. radiodurans* R1 gene regulatory network (Figure 3). Theoretically, the number should be less than that of the node with degree of six (47 nodes). To understand the biological meaning behind the over-representation, we extracted the sub-network of the 131 nodes with the degree of seven from the whole gene regulatory network, and performed the GO term enrichment analysis for the sub-network. We found that thirteen biological processes were enriched (Table 2). The enriched biological processes could be classified into 4 categories: (1) DNA repair; (2) reactive oxygen species (ROS) metabolic process and superoxide metabolic process; (3) glycine catabolic process and (4) tRNA aminoacylation (Table 2).

Discussion

In this study, we reconstructed the first gene regulatory network for the extreme radiation-resistant bacteria *D. radiodurans* R1 using Bayesian network approach. Further analysis for the gene regulatory network revealed that twelve mechanisms involved in the radiation-

Category	GO.ID	Term	p-value
1	GO:0006950	response to stress	0.048
	GO:0033554	cellular response to stress	0.027
	GO:0006974	response to DNA damage stimulus	0.041
	GO:0006281	DNA repair	0.041
2	GO:0072593	reactive oxygen species metabolic process	0.015
	GO:0006801	superoxide metabolic process	0.015
3	GO:0009071	serine family amino acid catabolic process	0.043
	GO:0006546	glycine catabolic process	0.043
4	GO:0019538	protein metabolic process	0.011
	GO:0044267	cellular protein metabolic process	0.042
	GO:0043038	amino acid activation	0.041
	GO:0043039	tRNA aminoacylation	0.041
	GO:0006418	tRNA aminoacylation for protein translation	0.041

Table 2: Significant biological processes in the sub-network of 131 nodes with degree of 7.

resistant processes in *D. radiodurans* R1.

We find eight mechanisms of radiation-resistance in *D. radiodurans* R1: (1) Arginine metabolic process; (2) GTP metabolic process; (3) Dicarboxylic acid transport; (4) Protein transport; (5)

Protein oligomerization; (6) Cell wall organization; (7) Glycine catabolic process and (8) tRNA aminoacylation. Some mechanism has been proved to be radiation-resistant in other organisms. For example, arginine could protect hematopoietic progenitor cells against ionizing radiation in mammalian [21]. The existences of nodes involve different mechanisms (Table S1) suggest that different mechanisms may act in concert under the radiation stress.

Previous studies have found that DNA repair and reactive oxygen species (ROS) mechanism respond to radiation in all biological systems including mammalian [4]. In this study, we also found that DNA repair process and reactive oxygen species metabolic process are two significant radiation-resistant mechanisms in *D. radiodurans* R1 (Table 2). Therefore, our results are consistent with the known knowledge. Our finding of the over-representation of node with degree 7 in *D. radiodurans* R1 gene regulatory network suggests that *D. radiodurans* R1 may has a more complex DNA repair and reactive oxygen species (ROS) mechanism than other organisms.

In 2009, Daly proposed the manganese-based radiation protection hypothesis [9]. The hypothesis involves metal ion homeostasis and oxygen transport process. In this study, we also find that the two mechanisms are related to radiation resistance (Table 1). Therefore, our results support Daly's manganese-based radiation protection hypothesis.

One limitation of our study is that Bayesian network is a directed acyclic graph (DAG). So our gene regulatory network did not include the relationship of negative feedback regulation. Nevertheless, our results are not only validated by the well-known mechanisms, and also support the recent hypothesis of radiation resistance. Thus, our findings uncover more radiation-resistant mechanisms in *D. radiodurans* R1.

In summary, using Bayesian network approach, we reconstruct the first gene regulatory network of the extreme radiation-resistant bacterium *D. radiodurans* R1. We find more radiation-resistant mechanisms in *D. radiodurans* R1. Our results are not only consistent with the known knowledge, but also support the current radiation-resistant hypothesis.

Acknowledgement

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