Investigation of protein-protein interactions involving Deinococcus radiodurans PriA, DnaB and SSB.

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Abstract

Deinococcus Radiodurans is a species of bacteria that has sparked a lot of interest due to its resilience to ionizing radiation. D. radiodurans demonstrates its repair capacity by restarting replication after exposure to radiation, typically lethal. This project examined the mechanism of replication restart in D. radiodurans by investigating the protein PriA interacting with DnaB and SSB. Many different types of gel electrophoresis were employed to investigate potential protein complex formations between D. radiodurans PriA and DnaB. Through agarose gel electrophoresis, an interaction between D. radiodurans PriA and DnaB was identified. Results in this work indicate that although D. radiodurans PriA does not characteristically and functionally appear normal, it could still behave as we would classically expect in replication restart.

DNA Damage

- When cells are exposed to ionizing radiation their genome becomes torn apart and shattered [1]. Depending on the intensity of exposure the DNA sequence of a cell can receive several hundred single stranded breaks, double stranded breaks, and base modifications [1].
- A broken genome will leave a cell incapable of replicating its DNA and typically lead to cell death [1].

• D. radiodurans is a species of bacteria that has shown a rare resistance to ionizing radiation [2].
• This species of bacteria was first discovered while attempting to use radiation to sterilize spoiled meat [3].
• A study found that after spending six years in a desiccator D. radiodurans remained 10% viable [1]. This resistance to desiccation is particularly interesting because it suggests an evolutionary explanation for the radiation resistance displayed in D. radiodurans.
• The bacterium does not avoid the damaging effects of radiation. D. radiodurans therefore demonstrates the ability to both repair and restart replication of all vital genes [1].

Figure 1 – D. radiodurans viability compared with E. coli viability after exposure to ionizing radiation. D. radiodurans (represented by squares) is able to remain viable after sustaining much greater amounts of ionizing radiation than E. coli (represented by diamonds) [1].

• D. radiodurans does not display helicase activity within its genome [1].
• This species of bacteria was first discovered while attempting to use radiation to sterilize spoiled meat [3].
• A study found that after spending six years in a desiccator D. radiodurans remained 10% viable [1]. This resistance to desiccation is particularly interesting because it suggests an evolutionary explanation for the radiation resistance displayed in D. radiodurans.
• The bacterium does not avoid the damaging effects of radiation. D. radiodurans therefore demonstrates the ability to both repair and restart replication of all vital genes [1].

Figure 2 – A proposed mechanism for the replication restart mechanism of D. radiodurans on a collapsed replication fork. Yellow circles represent PriA. Red circles represent DnaB.

PriA typically acts as a DNA helicase

- PriA in E. coli and many other species of bacteria utilize the energy from ATP to unwind double stranded DNA and load DnaB onto the replication fork.
- PriA in D. radiodurans does not display this classically observed helicase activity.

D. radiodurans does not display helicase activity

Table 1 – PriA Size Comparison Over Several Different Phyla. The PriA found in D. radiodurans is considerably larger than many other species in different phyla.

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Organism</th>
<th>PriA Length (a.a.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deinococcus-Thermus</td>
<td>Deinococcus radiodurans R1</td>
<td>925</td>
</tr>
<tr>
<td>Proteobacteria (alpha)</td>
<td>Ricetella ficus (UTW0134)</td>
<td>648</td>
</tr>
<tr>
<td>Proteobacteria (gamma)</td>
<td>Heliobacter pylori</td>
<td>619</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>Clostridium acetobutylicum ATCC 824</td>
<td>733</td>
</tr>
<tr>
<td>Chloroflexi</td>
<td>Chlorobium phaeoviridum TW-183</td>
<td>769</td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td>Thermosynechococcus elongatus BP-2</td>
<td>850</td>
</tr>
<tr>
<td>Planctomycetes</td>
<td>Thermotoga maritima ICT 4243</td>
<td>865</td>
</tr>
<tr>
<td>Chloroflexi</td>
<td>Thermotoga maritima ICT 4243</td>
<td>865</td>
</tr>
</tbody>
</table>

Figure 3 – Sequence logo comparing the PriA Walker A motif amino acid sequence of 10 different organisms. D. radiodurans clearly shows a great number of differences in Walker A Motif. This motif is believed to be the binding site of ATP on PriA in other organisms.

Replication Restart

This project focused on the mechanism of replication restart in D. radiodurans. The mechanism shown below is a hypothesized model of replication restart base on what is known from other species of bacteria.

Research Goal

- Is PriA involved in DNA replication restart in D. radiodurans?
- If PriA does function as a replication restart protein in D. radiodurans then PriA must be able to physically interact with DnaB. This project was focused on identifying whether or not D. radiodurans PriA and DnaB are capable of forming a protein complex with each other.

Experimental Methods

Protein Preparation

- D. radiodurans PriA and DnaB were grown in E. coli using the pET expression system and purified through various different chromatographic techniques such as size exclusion chromatography and nickel affinity chromatography.

Protein Mixing Analysis

- Native agarose electrophoresis – Agarose gel electrophoresis was the final analytical technique used in this project. The gels were all within 0.6%–1.0% agarose.
- The most successful gels were 0.7% agarose and run at 50 V for several hours.

Conclusions

- PriA and DnaB show a clear capacity to interact and form protein complexes with each other.
- PriA and SSB also indicate that they may be able to interact. However, the evidence generated in this project was not conclusive enough to claim that a complete formation occurs when these two are in close proximity.
- The results of this project indicate that despite lacking classically observed helicase activity, PriA can physically interact with DnaB and therefore could still be involved in the replication restart process in D. radiodurans.

References