Para encoded on chromosome II of Deinococcus radiodurans involves in cell division regulation through nucleoid occlusion mechanism

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Abstract

Deinococcus radiodurans is a multipartite genome containing bacteria known for its extreme tolerance to DNA damaging agents. This bacterium when exposed to such agents it undergoes a cell cycle arrest during which it repairs its genome. The regulation of this arrest with respect to chromosome segregation and cell division is worth investigating. We have been actively involved in understanding the basic process of cell division and chromosome segregation and their role in bacterial response to DNA damage. In this work, we characterized an actin-like genome partitioning protein encoded on secondary chromosome II (named it ParA2) for its role in cognate chromosome maintenance in D. radiodurans. We observed that ParA2 in the absence or with lower stoichiometric ratio of its cognate partner ParB2 play a regulatory role in cell division through a process very similar to nucleoid occlusion. When ParB2 was provided in trans it presumably resumed its normal function and its inhibition of cell division was not observed. These results suggest a conditional regulatory role of ParA2 in cell division depending upon its need in ParB2 mediated genome maintenance in D. radiodurans.

Introduction

An accurate duplication and transmission of genetic information is a fundamental attribute of the life and that determines the successful inheritance of different phenotypes into daughter cells. In bacteria, the genome partitioning occurs mainly by pushing or pulling of duplicated genome toward the cell poles [1]. This involves three core components like cis-element that functions like centromere in bacteria, a centromere binding protein and an actin homologue of bacterial ATPase. Deinococcus radiodurans R1 is a Gram positive bacterium that shows extraordinary tolerance to different DNA damaging agents [2]. This bacterium also has peculiar cytogenetic features. It has multipartite genome system comprise of chromosome I (ChrI), chromosome II (ChrII) and plasmids [3]. Each of these genome elements is present in multiple copies. Genome of this bacterium encodes putative ParA and ParB types proteins located on each of the four genome elements. The cis elements are not known in the genome of this bacterium. Understanding the function of ‘Par’ proteins would be a step forward in knowing the mechanism(s) of chromosome segregation in this bacterium harboring complex genome. Recently, chromosome I partitioning system has been characterized [4] and cell division proteins like FtsZ and FtsA have been characterized in vivo and in vitro [5,6]. The work received this award was on characterization of ParA2 and parB2 roles in chromosome II partitioning during cell division.
ParA2 is characterized as a DNA binding ATPase

Multiple sequence alignment of ParA2 with other ParA-type proteins using CLUSTAL X program showed that ParA2 contains the typical A, A' and B components of Walker motifs that are found in P-loop ATPases. Unusually, it was found that ParA2 is missing one of the largely conserved lysine at the beginning of the Walker A motif, which was also conserved in ParA encoded on chromosome I of D. radiodurans. Phylogenetic analysis indicated that ParA2 is evolutionarily different from other chromosomal type ParAs including ParA1 of D. radiodurans and it was placed between chromosomal type ParAs and other small ATPases involved in regulation of bacterial cell division. Recombinant ParA2 was purified and its identity was further confirmed by mass spectrometry.

The purified ParA2 showed both DNA binding (Fig. 1a) and ATPase activities (Fig.1b). The DNA binding efficiency of ParA2 increased further in the presence of ATP, and its ATPase activity was stimulated with DNA. These results suggested that ParA2 is evolutionarily different from other bacterial ParAs and is a DNA-binding ATPase.

![Fig. 1: ParA2 activity characterization. Purified recombinant ParA2 was checked for DNA binding activity (A) in the presence and absence of ATP. Similarly, its ATPase activity was measured with increasing concentration of protein (B).](image)

ParA2 localizes on nucleoid and causes cell elongation

ParA2-CFP was expressed in E. coli AB1157 on pA2CFP plasmid and cells were examined under fluorescence microscope. Results showed cell elongation and localization of multiple spots of ParA2-CFP on false blue coloured DAPI-stained nucleoid spread throughout the cell (Fig 2). Effect of ParA2 on growth and cell division was monitored in E. coli. Transgenic E. coli expressing ParA2 showed a significant reduction in colony forming units (CFU) as compared to untransformed cells and the cells expressing ParA1on multicycopy plasmid [4]. Nearly no effect in optical density at 600 nm while several-fold decrease in CFU in the cells expressing ParA2 could be attributed to the continued increase in cell volume but inhibition of the cytokinesis.
These results indicated that ParA2 co-localizes with nucleoid in *E. coli* and is most likely affecting cell division in *E. coli*. Since, FtsZ is key player in bacterial cell division, the possibility of ParA2 overexpression making FtsZ inefficient was hypothesized. However, we observed that FtsZ expressing on plasmid did not rescue the ParA2 effect in ParA2 over-expressing *E. coli* cells and recombinant FtsZ activity was not affected by ParA2 in vitro. These results suggested that the increase in cell length and decrease in CFU per mL of cells expressing ParA2 seems to be due to ParA2 effect on cell division in *E. coli* and its direct effect on FtsZ function is less supported.

![LM DAPI CFP MERGED](image)

**Fig.2:** Cellular localization of ParA2 in transgenic *Escherichia coli*. The *E. coli* cells expressing ParA2-CFP fusion was observed under fluorescence microscope using normal mode (LM), excited for DAPI emission (DAPI) and fluorescence protein (CFP). These images were merged (Merged) to find out if green fluorescent ParA2-CFP is localized on DAPI stained nucleoid.

**ParA2 complemented known nucleoid occlusion function in *E. coli***

Binding of ParA2 with nucleoid and its effect on inhibition of cell division are the functions known also for nucleoid occlusion proteins such as SlmA in *E. coli* [7] and ‘Noc’ in Bacillus subtilis [8]. These proteins are known to inhibit cell division by bringing nucleoid occlusion to the vicinity of FtsZ ring formation. Therefore, the possibility of ParA2 inhibiting cell division either by nucleoid occlusion or by affecting DNA replication and genome segregation could be hypothesized. ParA2 was expressed in *E. coli* strain TB85 (ΔslmA) and *E. coli* strain TB86 (ΔminCDEΔslmA) cells, and the effect of ParA2 on growth characteristics of these mutants were examined. The slmA mutant expressing ParA2 grew similar to mutant control, while ParA2 could help slmAΔminCDE double mutant to recover its growth defect in rich medium (Fig. 3) and produce asymmetric cell division generating high frequency of minicells. Double mutants expressing ParA2 produced 19.24±2.12% minicells as against 5.12±1.26% in control without ParA2. Asymmetric cell division has been observed in *E. coli* cells lacking MinCDE system.
This indicated that ParA2 expression could make slmAminCDE double mutants similar to minCDE single mutants, implying the complementation of SlmA loss in the double mutant. These results may, therefore, suggest that ParA2 effect on cell division is most likely by affecting genome compaction, a process integral to nucleoid occlusion mechanisms operated by two distinctly different proteins SlmA in *E. coli* and ‘Noc’ in *B. subtilis.*

![Functional complementation of SlmA](image)

Fig 3: Functional complementation of SlmA (a nucleoid occlusion protein) loss by ParA2 in *E. coli*. ParA2 was expressed in trans into slmA mutant (ΔslmA+ParA2) and slmAminCDE mutant (ΔslmA ΔminCDE +ParA2) and growth characteristic was compared with these mutants harboring vector as control.

*E. coli* expressing ParA2 showed cell elongation which was not observed when either ParA1 was expressed or cells harbor vector as control. This indicated the functional difference of ParA2 from ParA1 and seems to specific effect of ParA2. This was probed further and observed that ParA2 localized on nucleoid and able to interact nonspecifically with DNA *in vitro*. The mechanism underlying cell division inhibition by ParA2 was investigated and observed that ParA2 could rescue the growth defect in minCDEΔslmA double mutant and thereby produced a phenotype that was specific to minCDE system implying that ParA2 could complement double mutant for slmA phenotype but not minCDE phenotype. Thus in conclusion, ParA2 is a DNA binding protein which might play an indirect role in spatial regulation of FtsZ function in *D. radiodurans*.

References


