Toward the Cell Cycle Synchronization of *Rhodobacter sphaeroides*

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INTRODUCTION

**Bacterial Cell Cycle:** In bacteria, the cell cycle consists of B period (G_phase), C period (S phase), and D period (G_phase and M phase). Finally, septum formation completes cell division, as shown in Figure 1. Rhodobacter sphaeroides, a member of the Proteobacteria, members of which are metabolically versatile. Unlike most bacteria, it possesses a complex genome comprised of two circular chromosomes [2]. Thus, its evolutionary features may have significant differences from bacteria whose genomes consist of a primary circular chromosome. Furthermore, it has a longer generation time than fast-growing bacteria with overlapping cell cycles. The long generation time may possibly make DNA replication happen once in a round of cell cycle only, which is better for studying bacterial cell cycle. Since the genome of R. sphaeroides has been completely sequenced, assembled, and fully annotated; and genetic and biochemical approaches for its genome analysis are available, R. sphaeroides becomes an ideal organism for the study of cell cycle regulation in prokaryotes with a complex genome structure.

**Cell Cycle Synchronization:** Synchronization of cell cycle is an important and also limiting step in studying DNA replication or other cell cycle events. For bacteria, several approaches are used to synchronize the cell cycle as shown in Table 1 with different aspects (1). Recently, a new method has been proposed and proven to be working effectively in *Escherichia coli*. An amino acid analog, DL-serine (SHX), was used to induce the stringent response to arrest cell cycle at the stage of initiation of DNA replication [5]. SHX can lead to serine-RNA synthetase competitively prevent the serine-RNA from being charged. This results in the amino acid starvation during which the chargeable RNA decreases leading to a stop in protein synthesis. In amino acid starvation induced stringent response, the uncharged tRNAs trigger a signal to RII, a ribosomal-bound enzyme, to produce the guanosine pentaphosphate (ppppGpp) and guanosine tetraphosphate (ppppGpp). pppGpp can binds to RNA polymerases leading to an activation or repression of many genes. Thus the cell can be globally reprogrammed and adapt to the new environment. Most significantly for arresting cells, it down regulates the expression of genes encoding for ribosomal proteins, cell division and DNA replication, and also reduces the amount of stable tRNAs [6-8]. In *Bacillus subtilis* (ppppGpp has been shown to inhibit replication directly by inhibiting primase in DNA replication) [9].

**OBJECTIVES**

In *E. coli*, SHX-induced synchronization method has been shown to be an effective approach. Compared with the traditional approaches, it has great advantages on getting higher amount of synchronization cells and also being similar to normal status of wild type cells. Also, it is a rapid and reversible replication arrest that helps maintain the genomic stability. However, it has been shown that SHX also induces a significant reduction but not complete block in septum formation and cell division, resulting in some cells to be arrested in D period (R-unde [5]. Therefore, the objective of this study is to evaluate this method or its effectiveness in *R. sphaeroides*, together with the other methods, to develop an effective approach to synchronize the cell cycle in this organism.

**MATERIALS AND METHODS**

**Cell Culture and Treatment:** *R. sphaeroides* was growing in Sistons’s minimal medium at 30°C. After reaching the early mid-log phase (OD~0.2~0.3), cultures were treated with different concentrations of serine hydroxamate (Sigma-Aldrich, S4503; 1mg/ml, 0.5mg/ml and 0.1mg/ml, respectively). For DNA content analysis, cells from different sets of treatment were collected by fixing them using 70% ethanol after 2 hours, 4 hours and 6 hours treatment. On the other hand, cells were also washed of SHX and introduced to drug-free medium after different durations of treatment. Cells were collected at different time points after release.

**Flow Cytometric Analysis:** Fixed cells were suspended in 1x Tris-EDTA (TE) pH 8.0. Propidium, a DNA-specific fluorescence dye, was used for staining DNA; then cells were incubated under dark for 0.5~6 hours at room temperature. Poicogen flow cytometry for DNA content was done using Becton Dickinson FACScan (M. D. Anderson Cancer Center, Houston, TX). Data were analyzed using Flowjo software (Tree Star, Inc.)

**RESULTS**

**SHX treatment affects the bacterial doubling time:**

As shown in Figure 4, it was indicated that there is a dose-dependent effect between SHX concentration and DNA content. With high dose treatment (1mg/ml), the change of DNA content in C period (between B and D period) is significant; while using lower concentration (0.5 and 0.1mg/ml), the effect of drug is not significant.

**SHX arrests cells in both B and D periods:**

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DISCUSSION

In flow cytometric analysis, we consistently observed only 1N and 2N peaks, meaning that it is most likely that DNA replication in *R. sphaeroides* occurs once per cell cycle. As results indicated, cells treated with higher concentration of SHX had a more significant effect on both cell growth and DNA content that indicates the distribution of cells in different cell cycle stages. This suggested that SHX may work as a dose-dependent manner. This is consistent with the previous research, in which they showed that the replication inhibition by (pppGpp) was in a dose-dependent manner [8].

However, our results showed no significant synchronization of cell cycles. DNA synthesis is significantly inhibited by SHX. This is due to the stringent response induced by amino acid starvation that results in the accumulation of (pppGpp). Therefore, the initiation of DNA replication is blocked while the ongoing process will continue as shown in *E. coli*. The proportion of cells in B period (before initiation of DNA replication) increased during treatment; however, still many cells were arrested in D period (before cell division), which suggested that stringent response triggered by SHX may also have a very significant reduction in septum formation and cell division; while in *E. coli*, it was shown that there was a preference for stringent arrest in B period [4-5]. Significant arrest in both B and D period resulted in no significant and successful cell cycle synchronization.

Interestingly, after release from SHX, the amount of D period cells starts decreasing when the proportion of B period cells keeps increasing. This may be due to the more rapid release of cells from D period: cells start dividing faster compared with the DNA replication.

FUTURE WORK

- Evaluate the SHX-triggered synchronization method further by modifying protocol and conditions and use different approaches including flow cytometric and fluorescence microscopic analysis to examine the effect.
- Evaluate the other synchronization methods in *R. sphaeroides*.
- Identify a best approach for cell cycle synchronization in *R. sphaeroides* for further cell cycle studies.

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REFERENCES