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Title

<u>The SOS Response In Escherichia Coli: Single Cell Analysis Using Fluorescence Microscopy</u>

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Abstract

During the course of DNA replication, replication forks often stall or collapse as they proceed from oriC to the terminus due to housekeeping types of DNA damage or proteins bound to DNA. If the DNA is not repaired or if the replication forks do not restart, viability of the cell then becomes compromised. In Escherichia coli, if DNA damage is detected, approximately 40 genes are expressed to repair the offending DNA lesion. This is known the SOS response. Two proteins RecA and LexA regulate the SOS response, where RecA (when bound to ssDNA), serves as the sensor for DNA damage and LexA serves the repressor for SOS expression. When the RecA nucleoprotein filament forms, this complex will accelerate autocleavage of LexA inducing the response. Recently it has been observed that in a population of cells approximately 15% of the population had RecA bound to DNA, however at any given time approximately 0.3% of the population is induced for SOS expression suggesting that the cell can decide whether induce the SOS response or not. The aim of this work is to understand how the cell decides whether or not to express the SOS response at housekeeping types of DNA damage. Regulation is important because the cell would not want to express the SOS response every time replication forks encounter housekeeping types of DNA damage. The first component of this work looks at SOS expression in populations of cells during log phase growth using the fluorecense microscopy and the transcriptional fusion sulA-gfp. Results show that a SOS expression is stochastic and occurs in a small population of wild type cells. The second component of my work focuses on how the cell decides when to express the SOS response by using recA constitutive mutants that are defective in this regulation. Results show that the concentration and conformation of the RecA nucleoprotein filament is crucial for this to occur. Lastly novel recA mutants were created and examined for their role in suppressing constitutive SOS expression. It is observed that suppression of constitutive SOS expression could be seen when these mutations were supplied in cis and in trans, suggesting multiple levels of SOS regulation.

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