Analysis of the initiator proteins DnaA and TrfA in *Escherichia coli* cells during amino acid starvation

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Bacteria can rapidly adapt to changing environmental conditions through precise regulation of cellular processes, including DNA replication. The molecular basis of chromosomal DNA replication initiation is well understood, but much less is known about how this process is inhibited under stress. Even less information is available on the regulation of plasmid replication during stress, despite its key role in the dissemination of antibiotic resistance genes. The aim of this study was to analyze the initiator proteins, DnaA and TrfA, in *Escherichia coli* cells during stress conditions. DnaA is responsible for initiating chromosomal replication, whereas TrfA serves as the initiator of RK2 plasmid replication. Under optimal conditions, DnaA binds to the origin of chromosomal replication (*oriC*), leading to the initiation of DNA replication. I demonstrated that during amino acid starvation, DnaA molecules do not bind *oriC*, and this phenomenon correlates with an increase of DnaA acetylation level. Moreover, during stress the intracellular level of DnaA gradually decreases, although it never drops below the detection limit. ChIP-seq analysis revealed that DnaA molecules present in the cell during stress exhibit an altered DNA interaction profile. During stress, DNA regions bound under optimal conditions are no longer occupied, while new interactions appear in other sites. The intracellular localization of DnaA also changes, as under stress the protein does not form the distinct foci characteristic for cells growing under optimal conditions. Taken together, the results show that the level, localization, DNA interaction profile, and acetylation of DnaA change under stress, which ultimately prevents initiation of chromosomal DNA replication. In *E. coli*, DnaA cooperates with the plasmid initiator TrfA during the initiation of RK2 plasmid replication. I showed that during amino acid starvation, initiation of RK2 plasmid replication is inhibited due to the absence of DnaA and TrfA binding to plasmid replication *origin* (*oriV*). The reduction in initiator binding to *oriV* correlates with a decrease of the levels of TrfA monomers and dimers as well as DnaA. Interestingly, the plasmid also does not replicate in cells expressing a hyperactive monomeric TrfA variant, suggesting that replication blockade involves mechanisms other than the formation of handcuff complexes.

The results indicate that complete inhibition of DNA replication initiation under stress is a consequence of several parallel mechanisms. Changes occurring during stress are reversible, which allows for resumption of both chromosomal and plasmid replication once stress is relieved. This work expands our understanding of DNA replication regulation under stress and provides new insights into the mechanisms that enable bacterial survival in unfavorable conditions, which may form the basis for developing novel antimicrobial therapies as well as strategies that support antibiotic treatment and limit plasmid-mediated antibiotic resistance.