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Inactivation of Cell Division Protein FtsZ by SulA Makes Lon Indispensable for the Viability of a ppGpp^0 Strain of *Escherichia coli*

Aanisa Nazir,[a,b] Rajendran Harinarayanan[a]

Laboratory of Bacterial Genetics, Center for DNA Fingerprinting and Diagnostics, Hyderabad, India[a]; Graduate Studies, Manipal University, Manipal, India[b]

ABSTRACT

The modified nucleotides (p)ppGpp play an important role in bacterial physiology. While the accumulation of the nucleotides is vital for adaptation to various kinds of stress, changes in the basal level modulates growth rate and vice versa. Studying the phenotypes unique to the strain lacking (p)ppGpp (ppGpp^0) under overtly unstressed growth conditions may be useful to understand functions regulated by basal levels of (p)ppGpp and its physiological significance. In this study, we show that the ppGpp^0 strain, unlike the wild type, requires the Lon protease for cell division and viability in LB. Our results indicate the decrease in FtsZ concentration in the ppGpp^0 strain makes cell division vulnerable to SulA inhibition. We did not find evidence for SOS induction contributing to the cell division defect in the ppGpp^0 Δ*lon* strain. Based on the results, we propose that basal levels of (p)ppGpp are required to sustain normal cell division in *Escherichia coli* during growth in rich medium and that the basal SulA level set by Lon protease is important for insulating cell division against a decrease in FtsZ concentration and conditions that can increase the susceptibility of FtsZ to SulA.

IMPORTANCE

The physiology of the stringent response has been the subject of investigation for more than 4 decades, with the majority of the work carried out using the bacterial model organism *Escherichia coli*. These studies have revealed that the accumulation of (p)ppGpp, the effector of the stringent response, is associated with growth retardation and changes in gene expression that vary with the intracellular concentration of (p)ppGpp. By studying a synthetic lethal phenotype, we have uncovered a function modulated by the basal levels of (p)ppGpp and studied its physiological significance. Our results show that (p)ppGpp and Lon protease contribute to the robustness of the cell division machinery in *E. coli* during growth in rich medium.

Adaptation in response to stress is a universal trait among living organisms. In eubacteria and plants, two modified nucleotides, namely, guanosine tetraphosphate (ppGpp) and guanosine pentaphosphate (pppGpp), collectively referred to as (p)ppGpp, have been implicated in adaptation to various nutritional and environmental stresses (1–5). By far the best-studied stress response mediated by (p)ppGpp is the stringent response in the bacterial model organism *Escherichia coli*, and it is characterized by the repression of rRNA transcription and the activation of amino acid biosynthetic genes. This adaptation requires the RelA-mediated synthesis of (p)ppGpp in response to amino acid starvation and results in the extensive reprogramming of gene expression globally (6, 7). More commonly, the stringent response also refers to responses caused by elevating (p)ppGpp by other means. In *E. coli*, *relA* and *spoT* genes are involved in (p)ppGpp metabolism and are part of the RSH (Rel-Spo homolog) superfamily of genes conserved in eubacteria and plants (3, 8). (p)ppGpp is synthesized in response to amino acid starvation when uncharged tRNA binds to the ribosomal A site and activates the ribosome-bound RelA protein (9, 10). RelA synthesizes pppGpp/ppGpp using GTP and GDP through the transfer of pyrophosphate from ATP. The amount of (p)ppGpp made following amino acid starvation is much higher than the amount of ribosome-bound RelA protein in the cell, indicating multiple rounds of catalysis by each RelA molecule. Based on *in vitro* and *in vivo* studies, some mechanistic models have been proposed for how this could be achieved (10–13). SpoT-dependent (p)ppGpp synthesis has been documented in response to deprivations such as iron, phosphorus, fatty acid, and carbon sources (14–19). SpoT is a bifunctional protein with (p)ppGpp synthetase and pyrophosphohydrolase activities; the latter activity generates GTP and GDP from (p)ppGpp, predominates during normal growth conditions, and is reported to be an essential function (20). Based on biochemical and structural studies, models have been proposed for the regulation of the opposing catalytic activities within the protein (21–24).

Consistent with the profound alteration in the gene expression profile induced by (p)ppGpp, RNA polymerase (RNAP) is a primary target of (p)ppGpp (25–28). The regulation of transcription has been shown to occur at the level of initiation, elongation, or competition of sigma factors for the RNA polymerase core (2, 3, 29, 30). Effects of (p)ppGpp on transcription are potentiated by DksA, especially that of the negative and positive regulation of rRNA and amino acid biosynthetic genes, respectively (31–33). DksA is a structural homolog of transcription elongation factors GreA/GreB and regulates transcription through the secondary channel of RNAP (34, 35). However, DksA also functions inde-

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Address correspondence to Rajendran Harinarayanan, hari@cdfd.org.in.

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