Studies on the Regulation of (p) ppGpp Metabolism and Its Perturbation Through the Over-Expression of Nudix Hydrolases in Escherichia coli

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Regulation by small molecules is a conserved feature across all life forms. ppGpp and pppGpp are modified nucleotides (collectively referred to as (p)ppGpp) found in eubacteria and plants. The metabolism of these nucleotides are regulated by the proteins RelA and SpoT, the former functions in the synthesis of the molecules while the latter is capable of both synthesis and degradation of the molecule. The synthesis of (p)ppGpp by RelA normally requires the interaction of this protein through its C-terminal domain with ribosomes having an un-acylated tRNA in the A-site, which arises usually following amino acid starvation. In this study, using reduced activity relA alleles, we provide experimental evidence for SpoT-mediated negative regulation of the amplification of RelA-dependent stringent response. We investigated the kinetics of ppGpp degradation in cells recovering from stringent response in the complete absence of SpoT function. Although greatly diminished, there was slow ppGpp degradation and growth resumption after a lag period, concomitant with decrease in ppGpp pool. From a genetic screen, the nudix hydrolases MutT and NudG were identified as over-expression suppressors of the growth defect of ΔspoT and ΔspoT ΔgppA strains. The effect of over-expression of these hydrolases on the stringent response to amino acid starvation and basal (p)ppGpp pool was studied. Over-expression of each hydrolase reduced the strength of the stringent response to amino acid starvation, and additionally, perturbed the ratio of ppGpp to pppGpp in strains with reduced SpoT hydrolase activity. In these strains that do not accumulate pppGpp during amino acid starvation, the expression of NudG or MutT supported pppGpp accumulation. This lends support to the idea that a reduction in the SpoT hydrolase activity is sufficient to cause the loss of pppGpp accumulation and therefore the phenomenon is independent of hydrolases that target pppGpp, such as GppA.

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