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Collateral effects of deletion of nlpD on rpoS and rpoS-dependent genes

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Letter to the Editor **Infectious disease**

To the Editor: A seminal article, titled “Active bacterial modification of the host environment through RNA polymerase II inhibition,” was published in the JCI in February 2021 (1). The article depicts a novel bacterial phenomenon mediated by the NlpD protein, which was demonstrated using an nlpD deletion mutant, a recombinant NlpD protein (rNlpD), and an nlpD deletion mutant complemented with the nlpD-rpoS operon. The idea in this article is impressive and has a potential impact on bacteriology, especially for studies on NlpD. However, since nlpD is complicated, as marginally referred to in Supplemental Figure 5 in the article by Ambite et al., we here include detailed information about nlpD. nlpD is positioned upstream of rpoS; rpoS encodes the RNA polymerase sigma factor σ38 (RpoS) that regulates many genes, as shown in a recent study that identified differential expression of 1044 genes between the wild-type and rpoS mutant (2). Importantly, nlpD includes rpoS promoters, including the P2 promoter, which is critical for rpoS expression (2–4). The nlpD deletion mutant lacks the rpoS promoter, resulting in no expression of both rpoS and nlpD. Whether the phenotypes observed in the nlpD deletion mutant depend on the NlpD functions should be confirmed using nlpD and not the nlpD-rpoS operon, and we have reviewed the article by Ambite et al. with this view in mind. […]

Collateral effects of deletion of *nlpD* on *rpoS* and *rpoS*-dependent genes

To the Editor: A seminal article, titled "Active bacterial modification of the host environment through RNA polymerase II inhibition," was published in the JCI in February 2021 (1). The article depicts a novel bacterial phenomenon mediated by the NlpD protein, which was demonstrated using an *nlpD* deletion mutant, a recombinant NlpD protein (rNlpD), and an *nlpD* deletion mutant complemented with the *nlpD-rpoS* operon. The idea in this article is impressive and has a potential impact on bacteriology, especially for studies on NlpD. However, since *nlpD* is complicated, as marginally referred to in Supplemental Figure 5 in the article by Ambite et al., we here include detailed information about *nlpD*. *nlpD* is positioned upstream of *rpoS*; *rpoS* encodes the RNA polymerase sigma factor σ^{38} (RpoS) that regulates many genes, as shown in a recent study that identified differential expression of 1044 genes between the wild-type and *rpoS* mutant (2). Importantly, *nlpD* includes *rpoS* promoters, including the P2 promoter, which is critical for *rpoS* expression (2–4). The *nlpD* deletion mutant lacks the *rpoS* promoter, resulting in no expression of both *rpoS* and *nlpD*. Whether the phenotypes observed in the *nlpD* deletion mutant depend on the NlpD functions should be confirmed using *nlpD* and not the *nlpD-rpoS* operon, and we have reviewed the article by Ambite et al. with this view in mind. An *nlpD* SNP observed in SN25 was mapped to the critical *rpoS* promoter P2 "TATAAT" (5). SN25 showed low or no expression of RpoS (Supplemental Figure 5B in the article by Ambite et al.), appearing to be a mutant with substantial RpoS deficiency. No *nlpD* deletion mutant complemented solely with *nlpD* was tested; however, the mutant complemented with the *nlpD-rpoS* operon that expressed RpoS in addition to NlpD was studied. Furthermore, since no experiment using rNlpD SNP to confirm the phenotypes of SN25 was performed, whether these phenotypes depend on a loss of function of NlpD remains to be determined. These approaches raise the possibility that the phenotypes of SN25 observed can be attributed to the effects of *rpoS*/*rpoS*-dependent genes. We believe that this information will be useful for future studies on host-microbe interactions, especially those focusing on *nlpD*.

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