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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