Insights into the Structural Basis of Cell-Surface Signaling by the Sigma-Regulator PupR in *Pseudomonas capeferrum*.

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Cell-surface signaling (CSS) pathways are highly conserved in Gram-negative bacteria and provide a rapid and robust transcriptional response to extracellular stimuli. CSS pathways usually involve three distinct proteins: 1) an outer membrane (OM) transducer responsible for sensing the stimuli, 2) an inner membrane (IM) sigma-regulator that conveys the signal from the periplasm to the cytoplasm, and 3) a cytosolic extra-cytoplasmic function sigma-factor, which regulates transcription of stimulus response genes. Our studies have focused on the structural and biochemical characterization of PupR, the IM sigma-regulator of the pseudobactin BN7/BN8 Fe$^{3+}$-signaling axis from *Pseudomonas capeferrum*; and its interactions in the cytoplasm and the periplasm. We have demonstrated that PupR has three distinct domains: a cytoplasmic, N-terminal anti-sigma domain (ASD), a single transmembrane helical domain, and a periplasmic, C-terminal cell surface signaling domain (CCSSD). The ASD interacts with the sigma-factor, PupI, while the CCSSD interacts with the N-terminal signaling domain (NTSD) of the OM transducer, PupB.

Our recent structural and biochemical data provide key insights into how CSS signal transduction is regulated by interactions of the PupR CCSSD in the periplasm that prevent CSS activation by regulated intramembrane proteolysis (RIP). In response to ferric pseudobactin RIP is initiated by the degradation of the PupR CCSSD by the site-1 protease, Prc. This is followed by cleavage of PupR by the site-2 protease, RseP, to release the PupR ASD:PupI complex to activate transcription. Our recent 1.56 Å structure of the PupR CCSSD:PupB NTSD complex demonstrates that the CCSSD has two distinct subdomains: the juxtamembrane domain that has a novel protein fold that has not previously been described and a Secretin and TonB short N-terminal domain that is in a novel architectural arrangement at the very C-terminus of the PupR CCSSD. This structure allows us to elucidate the mechanism by which the PupR CCSSD interacts with the PupB NTSD. Our biochemical analyses verify this interaction serves to stabilize the PupR CCSSD. Additionally, recognition of the CCSSD by Prc is blocked by the CCSSD:NTSD interaction although Prc does efficient process the CCSSD in the absence of the NTSD. Together, our data provides evidence for a mechanism that differs from the canonical view of CSS activation.