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Investigating cysteine-mediated interactions between *Chlamydia trachomatis* Chaperones Scc1 and Scc4

Background: *Chlamydia trachomatis* is an obligate intracellular pathogen responsible for widespread human diseases, including the most common bacterial sexually transmitted infection. To establish infection, it utilizes a type III secretion (T3S) system to translocate virulence effectors into the host cell. SccI and Scc4 are two critical T3S chaperones that function together to stabilize and facilitate the secretion of CopN, a crucial effector and "plug" protein. Beyond secretion, Scc1 interacts with bacterial RNA polymerase to regulate transcription.

Objectives: This study investigates whether the two cysteine residues in Scc1 (C28 and C99), which potentially form covalent disulfide linkages, are essential for its interaction with Scc4.

Methods: A combined strategy of site-directed mutagenesis and a transcription activation-based bacterial two-hybrid assay was utilized. Mutated SccI fragments (C28A, C99A, and the C28A/C99A double mutant) were cloned into pBR vectors to express fusions with the N-terminal domain of the *E. coli* RNA polymerase α-subunit (α-NTD) under an IPTG-inducible promoter. These constructs were co-transformed with a plasmid encoding a λCI DNA-binding domain-Scc4 fusion into the FW102 OL2-62 reporter strain. Protein-protein interactions were quantified via β-galactosidase assays measuring *lacZ* expression.

Results and Conclusions: The β-galactosidase assays revealed that the single C28A and C99A mutations caused no significant change in *lacZ* activity compared to the wild-type control. However, the double mutant exhibited a significant decrease in reporter activity under IPTG-induced conditions. Additionally, all mutant variants demonstrated significantly higher *lacZ* activity when induced compared to their uninduced states. These findings suggest that while individual cysteines are dispensable, the presence of at least one functional cysteine residue is critical for the Scc1–Scc4 chaperone interaction.