STRUCTURAL BASIS OF CAPSULE POLYMERASES IN PATHOGEN BACTERIA

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Bacterial capsules are long-chain carbohydrate polymers that form a protective layer around the cell surface. They have critical roles in host-pathogen interactions and provide a protective envelope against host recognition, leading to immune evasion and bacterial survival. Capsule biosynthesis enzymes are potential drug targets and valuable biotechnological tools for generating vaccine antigens [1]. In this work we define the capsule biosynthesis pathway of *Haemophilus influenzae* serotype b (Hib), a Gram-negative bacterium that causes severe infections in infants and children [2]. Reconstitution of this pathway enabled the fermentation-free production of Hib vaccine antigens starting from widely available precursors and detailed characterization of the enzymatic machinery. The X-ray crystal structure of the capsule polymerase Bcs3 reveals a multi-enzyme machine adopting a basket-like shape that creates a protected environment for the synthesis of the complex Hib polymer. This architecture is commonly exploited for surface glycan synthesis by both Gram-negative and Gram-positive pathogens. Supported by biochemical studies and comprehensive 2D nuclear magnetic resonance, our data explain how the ribofuranosyltransferase CriT, the phosphatase CrpP, the ribitol-phosphate transferase CroT and a polymer-binding domain function as a unique multi-enzyme assembly (Figure 1).

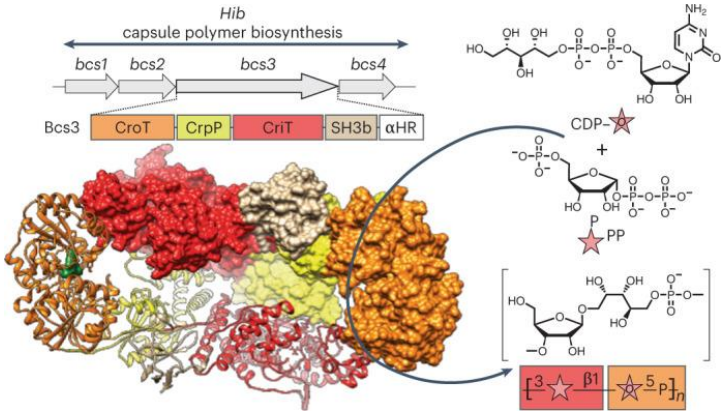

Figure 1. Overall structure of the Bcs3 dimer in complex with CMP, with both protomers shown in surface representation (left) and one protomer shown in cartoon representation (right) to visualize the secondary structural organization and the CMP (green). Each protomer of the homodimer is composed of (1) the ribofuranosyltransferase CriT (red), the phosphatase CrpP (yellow), (3) the ribitol-phosphate transferase CroT (orange) and (4) an SH3b domain (tan).

References:
1. T. Hallström and K.Riesbeck, Trends Microbiol, 2010, 18, 258-265
2. L. M. Willis and C. Whitfield, Carbohydr Res, 2013, 378, 35-44