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BioID screen for bacterial virulence proteins: new tools for infectious disease research

The discovery of penicillin in the 1920s revolutionized our ability to treat bacterial infection. However despite the introduction of antibiotics, infectious bacterial pathogens remain an immense challenge for the healthcare community. The incidence of infection by antibiotic-resistant and multidrug-resistant bacteria has escalated dramatically in recent decades, to the extent that the World Health Organization (WHO) has cited antibiotic resistance as one of the greatest global threats to human health. This has coincided with a progressive decrease in the number of approved new clinical antimicrobials, resulting in an urgent need for drug discovery. To address this call to attention, microbiologists are developing creative alternative strategies to target pathogens therapeutically. Examples include chemically modifying existing antibiotics to make them evade resistance, and combination therapies to reverse antibiotic resistance. One growing area of interest is to target the bacteria's ability to establish infection in the host. As research provides new insight into the molecular mechanisms of virulence factors in the host, this will inspire research into new candidate drug targets.

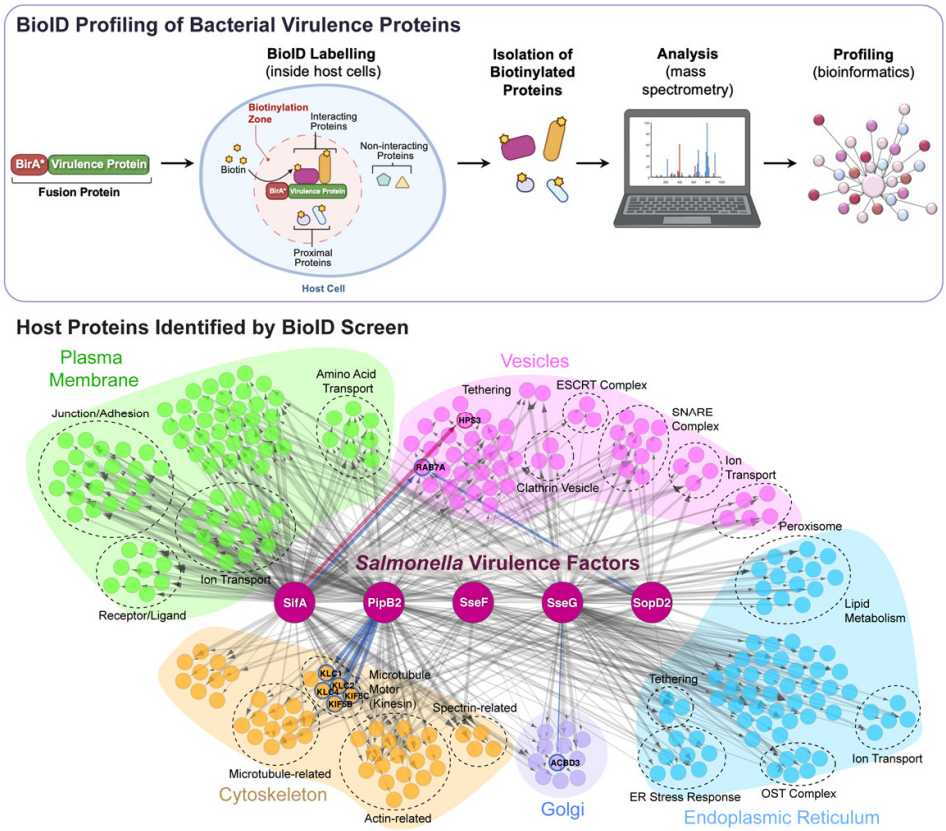

Fig. 1. BioID Identifies Candidate Host Interactors for *Salmonella* Virulence Proteins.
Top: Schematic of BioID Approach. The bacterial effector is fused to a modified biotin ligase (BirA*) to label interacting and nearby proteins with biotin. The biotinylated proteins are then isolated and identified. Bottom: BioID Screen of *Salmonella* Virulence Proteins. Each circle connected to the effectors represents a host protein identified, and line thickness represents peptide counts. Blue outlines are known interactors, and the new host target identified (BLOC-2 subunit HPS3) is outlined in pink.

Virulence proteins called effectors are secreted by pathogenic bacteria into host cells to mediate bacterial uptake, survival, and replication in the host. These virulence factors act by interacting with host target proteins, often on host membranes, and manipulating their function to hijack host cell processes. Identifying the host targets of virulence proteins is essential to understanding their mechanism of action. However in the laboratory, these bacteria-host protein-protein interactions can either be transient and/or weak, resulting in interactions that do not remain intact for long periods of time, or can occur on insoluble host membranes. As such, most traditional methods struggle to identify these host targets due to the inability to capture short-term interactions and incompatibility with insoluble proteins.

Proximity-dependent biotin identification (BioID) has emerged as an important technique for identifying protein-protein interactions. BioID relies on an enzyme called biotin ligase that mediates the irreversible labelling of interacting and nearby proteins with biotin. A fusion protein is created with the enzyme and protein of interest, and is expressed in cells to allow the enzyme to labels proximal proteins. The biotin-labelled proteins are then isolated and identified. BioID is a robust technique that overcomes some of the challenges faced by traditional methods with its ability to capture weak or transient interactions, membrane-targeted proteins, and its compatibility with *in vivo* studies.

In a recent study, the utility of this technique was investigated in the context of host-pathogen interactions, using *Salmonella* virulence proteins as a model system. *Salmonella* is one of the leading causes of foodborne illness globally, responsible for millions of cases each year, and is potentially fatal in young, elderly, and immunocompromised individuals. *Salmonella* secretes over 30 effector proteins to invade host cells and create an intracellular replicative niche within compartments called *Salmonella*-containing vacuoles (SCVs). In this study, a comparative analysis of five established virulence factors was conducted between BioID and a traditional method (Fig. 1). BioID identified eight known host-pathogen protein-protein interactions that could not be detected by the traditional method, suggesting its importance as a complementary tool to study host–pathogen interactions. The technique also identified hundreds of new candidate host interactors, including protein or protein complexes previously reported to have important roles during *Salmonella* infection but whose interaction with a bacterial protein remained unknown.

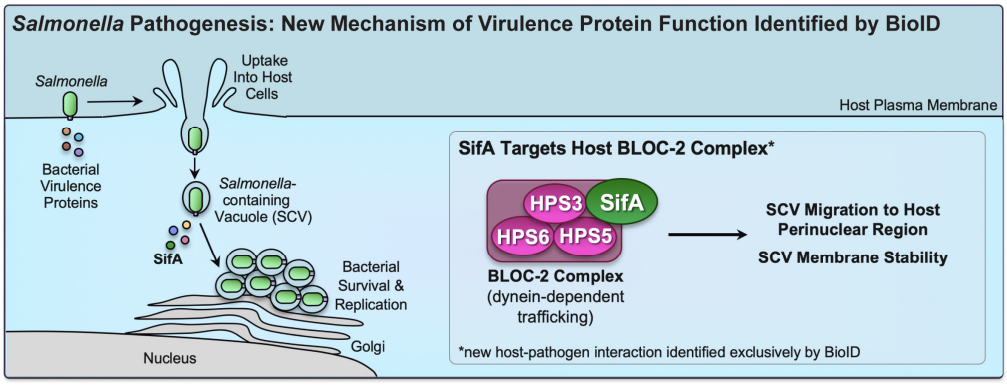

Fig. 2. BioID Identifies a New Host Target for the Virulence Factor SifA. The *Salmonella* effector SifA is important for virulence and bacterial replication in the host. BioID identified a new host target for SifA, the BLOC-2 complex. This host-pathogen interaction was shown to contribute to the *Salmonella*'s ability to direct SCV migration to the perinuclear region inside host cells, and for stability of the SCV membrane to maintain its replicative niche.

Of the new host proteins detected by BioID, six were validated to interact with the effectors experimentally, highlighting the ability of this technique to identify novel interactors of bacterial virulence proteins. The study also revealed that BioID could identify novel host targets that are important for infection. A deeper look into the *Salmonella* virulence protein SifA revealed that its interaction with the host protein complex BLOC-2 contributes to the bacteria's ability to control the positioning and stability of the SCVs inside host cells (Fig. 2).

This research provides novel insight into our understanding *Salmonella* pathogenesis, and highlights a valuable new tool for infectious disease research. This technique could also provide new opportunities for the study of poorly understood pathogens, and novel candidate therapeutic targets for drug discovery.

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BioID screen of Salmonella type 3 secreted effectors reveals host factors involved in vacuole positioning and stability during infection
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