Flagellum-triggered sensory system contributing to *Bordetella pertussis* infection

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Abstract

The Gram-negative bacterium *Bordetella pertussis* causes a highly contagious respiratory disease called pertussis (whooping cough) that is characterized by severe and uncontrollable coughing. The mechanisms regulating the expression of virulence factors of *B. pertussis* have been studied using the organism grown *in vitro*, but not within the hosts. In the present study, we found that *B. pertussis* senses gangliosides on the host cell membrane through flagella during host infection and up-regulates the expression of a small RNA designated Bpr4 and subsequent filamentous hemagglutinin (FHA), a major adhesin of the bacteria, leading bacteria to enhance colonization. Our findings indicate that a flagellum-triggered sensory system contributes to *B. pertussis* infection.

Background & Results

Pertussis has recently seen a resurgence despite high vaccination coverage, hypothetically because of the rapid waning of immunity induced by recent acellular pertussis vaccines and adaptation of the bacterium to escape vaccine-induced immunity. Macrolides are the first-line antibiotics to treat pertussis; however, macrolide-resistant *B. pertussis* has emerged worldwide, which prompts the development of novel antibiotic-free therapies to control pertussis.

We focused on the small RNA of *B. pertussis* as a virulence regulator during bacterial infection and identified four small RNAs designated Bpr4, 5, 8, and 9 that were highly expressed upon tracheal colonization in mice (Fig. 1A). Among these small RNAs, we found that a small RNA, Bpr4, which is up-regulated up to 120-fold upon tracheal colonization of *B. pertussis*, binds to the 5'-untranslated region of *fhaB* mRNA encoding FHA and protects it from RNaseE-mediated degradation, resulting in the posttranscriptional up-regulation of FHA, which facilitates bacterial colonization (Fig. 1B). Our results also demonstrated that Bpr4 up-regulation is triggered by the interference of flagellar rotation after the interaction of flagellin and gangliosides on the host cells, which causes the disengagement of MotAB comprising a flagellar stator, from the flagellar complex. The liberated MotA interacted with and activated a diguanylate cyclase designated DgcB to generate cyclic di-GMP, which plays a role in Bpr4 up-regulation through the RisK/RisA two-component system. Our findings indicate that a flagellum-triggered sensory system leads to small RNA upregulation contributing to *B. pertussis* infection.

Significance of the research and Future perspective

Similar to many pathogenic bacteria, *B. pertussis* is also a problem due to the spread of antibiotic-resistant strains. The circumstance prompts the development of therapeutic approaches without antibiotics to control pertussis. The results of this study revealed the flagellum-triggered sensory system contributing to *B. pertussis* infection. Our findings may lead to the development of therapeutic approaches to control pertussis by targeting host (gangliosides)-sensing by flagella. In addition, given that many pathogenic bacteria produce flagella, our idea has the potential to be useful in combating various bacterial infections.

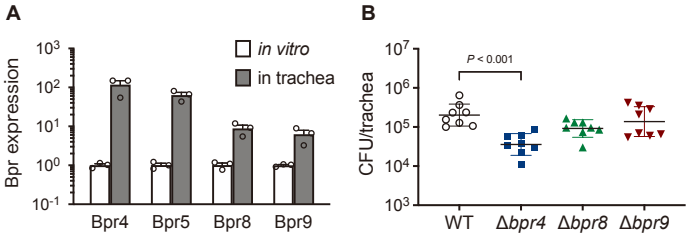

Fig 1. Highly-expressed small RNAs in *B. pertussis* colonizing mouse tracheas.
(A) *In vitro* and *in vivo* (in trachea) expression of *B. pertussis* small RNAs (Bpr).
(B) Colonization of *B. pertussis* WT and mutant strains in mouse tracheas.

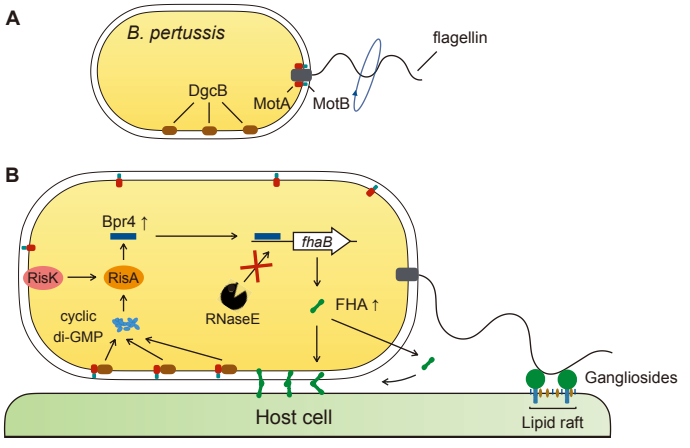

Fig 2. The mechanism of sequential up-regulation of Bpr4 and FHA upon bacterial adherence to the host cell.
(A) MotAB is incorporated into the flagellar basal body in motile *B. pertussis*.
(B) Bpr4 is up-regulated upon cell adherence of *B. pertussis* through a flagellumtriggered sensory system.

Patent

Treatise Hiramatsu, Yukihiro; Nishida Takashi; Nugraha, Krisna, Dendi et al. Interference of flagellar rotation up-regulates the expression of small RNA contributing to Bordetella pertussis infection. Science Advances. 2022, 8(51), eade8971. doi: 10.1126/sciadv.ade8971

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