

Abstract: Cilia are microtubule-based antenna-like projections from the apical cell surface and perform important biological functions¹⁻⁴. Multiciliated cells (MCCs) possess hundreds of motile cilia that beat in a wave-like motion to generate directional fluid flow (**Fig. 1**). Airway and brain ependymal MCCs play essential roles in mucociliary clearance and cerebrospinal fluid circulation, respectively. Dysfunctional cilia are associated with chronic infection and hydrocephalus⁵⁻⁸. Although they are critical for human health, the molecular players and mechanisms underlying ciliogenesis remain poorly understood.

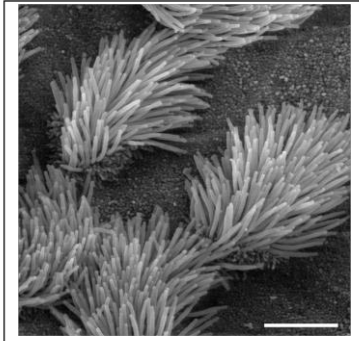


Fig 1. Airway multiciliated cells (MCCs). Adapted from Wikimedia. Scale bar, 5 μ m

We reported that the evolutionarily conserved coiled-coil protein Chibby 1 (Cby1) localizes to the ciliary base and plays a key role in ciliogenesis (**Fig. 2**)⁹⁻¹⁴. Cby1-knockout (Cby1^{-/-}) mice show ciliopathy phenotypes including airway infection and hydrocephalus^{9, 10, 15, 16}. Cby1 forms a complex with lipid-binding Cby1-interacting BAR domain-containing proteins 1 and 2 (ciBAR1 and 2)¹⁷. The Cby1/ciBAR complex localizes to the distal appendages (DAs) of basal bodies as a ring (**Fig. 2**)¹².

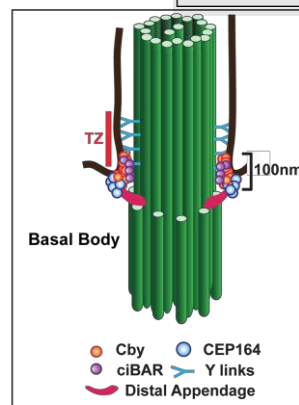


Fig 2. Model for localization of Cby1 and its associated proteins at the base of cilia. TZ, transition zone; DA, distal appendage. Adapted from (13).

During early ciliogenesis, the Cby1/ciBAR complex is recruited to the ciliary base through physical interactions with CEP164^{12, 17, 18}. The Cby1/ciBAR complex is present at the base of mature cilia, suggesting its crucial role in the maintenance of cilia and ciliary membranes, although its precise function remains unclear. In addition, the *in vivo* roles of ciBAR1 and 2 remain to be explored. Cby1, ciBAR1, and CEP164 are mutated in human ciliopathies including Joubert syndrome, Bardet-Biedl syndrome, and polydactyly¹⁹⁻²¹.

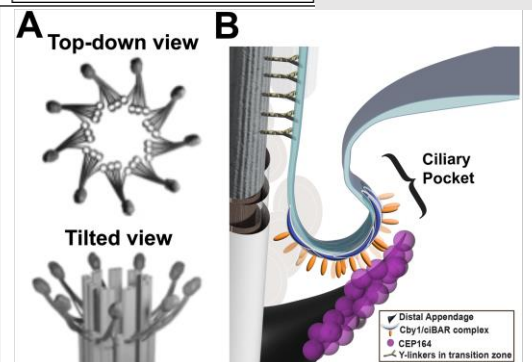


Fig. 3. Distal appendages (A) and working model for the role of the Cby1/ciBAR complex at the ciliary base (B). The Cby1/ciBAR complex binds to and maintains the curved membrane shape of ciliary pockets. The Cby1/ciBAR1 complex is physically linked to the DAs through interactions of Cby1 with CEP164. (A) adapted from (28).

The ciliary membrane is continuous with the cell membrane yet maintains a distinct protein and lipid composition²²⁻²⁴. DAs are nine radial fibrous extensions (150 nm) originating from each of the nine microtubule triplets of the basal body and thought to directly contact the tip of a membrane invagination known as the ciliary pocket (**Fig. 3**)²⁵⁻²⁸. However, the DA components responsible for the membrane attachment are currently unknown. Our hypothesis is that ciBAR1 and 2, in complex with Cby1, bind to lipid membranes at the ciliary pocket region (Fig. 3B) and play essential roles in ciliogenesis and MCC differentiation *in vivo*. To address this hypothesis, we plan to perform *in vitro* lipid-binding assays using reconstituted liposomes and recombinant proteins to examine the lipid-binding properties and specificities of the Cby1/ciBAR complex (Aim 1). Furthermore, we will utilize newly created ciBAR1^{-/-} and ciBAR2^{-/-} mouse models to investigate their functions in the ciliogenesis and differentiation of tracheal MCCs (Aim 2). Collectively, our proposed project will reveal a fundamental concept of how an organelle is physically anchored to the cell membrane and its impact on animal development and health.