2017

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The Localization and Function of Novel *Tetrahymena thermophila* Cytoskeletal Genes BBC29 and BBC39

Emily Moore, Nicole Zanolli, and Douglas Chalker, PhD

**Introduction**

Ciliary function is known to play an important role in many human conditions, including chronic sinus infections, pulmonary diseases, and problems with infertility. Cilia are cytoskeletal structures that protrude from the cell body and facilitate movement. Basal bodies are complex protein structures located at the base of each cilium. They anchor the structure to the cell body and help coordinate ciliary beating. Kinetochemical fibers form from segmented coils of protein and serve to both stabilize and organize cytoskeletal elements, including basal bodies. A clear understanding of structural proteins and their interactions with one another will not only increase our knowledge of the cilium but also better understand the diseases that arise when they do not function correctly. Here, we examine two novel cytoskeletal proteins in *Tetrahymena thermophila*, BBC29 and BBC39. *T. thermophila* serves as a useful model for the study of cytoskeletal genes, due to their robust and longitudinally organized cilia. BBC29 and BBC39 localized to locations consistent with kinetosomes and basal body structures, respectively, indicating a potential role for these proteins in cilial organization. We also examined the interaction between the two proteins using co-immunoprecipitation to determine if the two proteins interact closely with one another when expressed in the cell. We believe that the classification of proteins involved in cilial will offer key insights into the organization of this structure essential for eukaryotic life.

**Methods**

**DNA Preparation for Localization**

**Co-immunoprecipitation**

**Figure 3:** Both BBC29 and BBC39 were amplified with PCR using gene specific primers. TOPO cloning allowed for direct insertion of the gene of interest (GOI) into the pENTR vector. LR recombination interchanged the GOI with the Gateway cassette of the pCFV vector. The pCFV-GDI vector was transformed into T. thermophila through electroporation. The pCFV-GDI vector added a yellow fluorescent protein (YFP) to the GOI allowing for visualization with fluorescence microscopy. Successful transformants were selected for using an ampicillin resistance gene in the pCFV vector. The GOI was expressed via an MTT mammalian inducer promoter.

**Figure 4:** Both BBC29 and BBC39 were recombined into the pTRIC2_ICA_gtw vector. The pTRIC2_ICA_gtw vector contains a HA tag. The pTRIC2_ICA_gtw vector was digested using SacI and PstI restriction enzymes to create linear DNA fragments. Linear DNA was loaded onto DNAHM gel containing gels and fixed into T. thermophila containing a plasmid with the second gene tagged with YFP. The linear HA tagged DNA integrated directly into the T. thermophila genome. The presence of both genes in T. thermophila with different tags allowed for co-immunoprecipitation.

**Figure 5:** BBC29 (therm_0068560) is a 753 nucleotide gene that codes for a 252 amino acid protein in *T. thermophila*. There are no introns in this gene. Within the protein product, the shaded area indicates a conserved domain from the family of SF-assemblin proteins.

**Figure 6:** BBC39 (therm_0088340) has a 753 nucleotide coding region producing a 252 amino acid protein in *T. thermophila*. There are four introns in this gene. Within the protein product, the shaded area indicates a conserved domain from the family of SF-assemblin proteins.

**Figure 7:** Model of coiled coil protein structure.

**Figure 8:** A schematic representation of a cilium showing cross sectional area of the projection and basol body.

**Figure 9:** (A) White light image of *T. thermophila* in the growing stage. Cilia can be seen on the surface of plasma membrane extending into extracellular space. (B) *T. thermophila* under UV light. BBC29-YFP tagged protein appears in dashed lines indicating its localization to kinetodesmal fibers. This suggests BBC29 plays a role in cilial structure.

**Figure 10:** (A) White light image of *T. thermophila* in the growing stage. Basal bodies can be seen longitudinally along the surface of plasma membrane. (B) *T. thermophila* under UV light. BBC39-YFP tagged protein localized to dot-like structures indicative of basal bodies. This suggests a role in cilial structure. A large, non functional, protein aggregate can be seen in the cytosol.

**Figure 11:** Schematic diagram of co-IP protocol. Antibodies are used to isolate specific proteins which can be separated using SDS-PAGE gel electrophoresis and visualized with fluorescence microscopy.

**Figure 12:** Co-immunoprecipitation of BBC29-HA with BBC39-YFP, done with HA stain (red) and YFP stain (green). Lane 1: Cell lysate, Lane 2: ladder, Lane 3: wild type strain 429, Lane 4: HA antibody isolation, Lane 5: YFP antibody isolation.

**Figure 13:** Comparison of BBC29 and BBC39 Localization

**BBC29: Kinetodesmal Fibers**

**Figure 14:** Lane 5, with only green (YFP) bands. Isolation using HA antibodies did not pull down proteins containing a YFP tag, seen in lane 4. Both a green band around size 56 kDa (the expected size for BBC39-YFP) and a red band around size 30 kDa (the expected size for BBC29-HA) are visible.

**Figure 15:** Isolation using YFP antibodies did not pull down proteins containing an HA tag, seen in lane 5, with only green (YFP) bands.

**Conclusion**

Our results demonstrate the complex interactions that occur between cilial proteins, such as those in the basal bodies and kinetodesmal fibers of cilia, and illuminate the need to further understand the relationship among the many proteins that allow for proper cilial function.

- BBC29 is a kinetodesmal fiber that localizes in dashed lines along the plasma membrane. This indicates a stabilizing role for this protein in the cilium. Kinetodesmal fibers also associate with basal bodies and help with their organization.
- BBC39 is a basal body protein that localizes to the plasma membrane in longitudinal rows. Basal bodies anchor cilia and facilitate proper ciliary motility.
- BBC29 and BBC39 appear to directly interact with one another. During co-immunoprecipitation, HA antibodies isolated not only HA tagged proteins but also ones tagged with YFP. This suggests that BBC29 has a dynamic interaction with BBC39 in vivo in *T. thermophila*. Because cilial proteins are often highly conserved between species, results gleaned through study with *T. thermophila* may be extended to elucidate the biochemical basis of human ciliary diseases.

**Acknowledgments**

We would like to thank our teaching assistants Lisa McLellan and Justin Miller for their help on this project. Funding for this project was made possible through the Howard Hughes Medical Institute and a research grant from the National Science Foundation to Douglas Chalker.