Structural Insight into Ependymal Cilia and Oviduct Cilia

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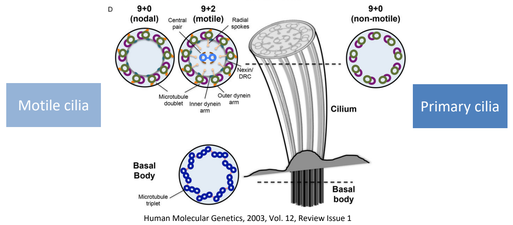
Abstract

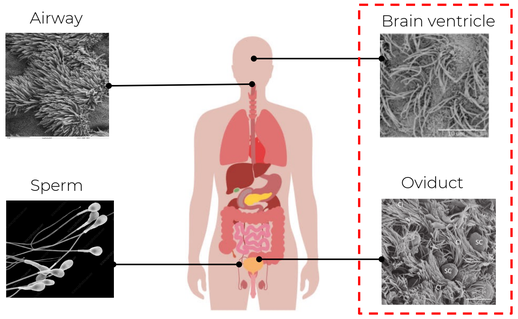
Motile cilia in the female oviduct propel to generate a fluid flow to transport oocytes to the uterus. The coordinated beating of ependymal cilia is required for facilitating the absorption of cerebrospinal fluid. The excess accumulation of cerebrospinal fluid in brain ventricles is the primary characteristic of hydrocephalus. However, little is known about the ultrastructure and the molecular composition of the ependymal cilia and oviduct cilia. In this work, we present the 48 nm and 96 nm atomic models of the axoneme doublet microtubule isolated from the porcine brain ventricles and the bovine fallopian tube. We have identified a tissue-specific Ca^{2+} binding protein in the ependymal cilia. It promotes glioma cell proliferation, making it a potential therapeutic target. The 96 nm doublet microtubule structure reveals unprecedented details of the molecular composition of the inner dynamin arms. These data provide new insights into the function and tissue specificity of mammalian cilia and inform the potential therapeutic targets for ciliopathies.

Introduction

❖ Cilia



❖ **Motile cilia** are present in the organs in the body including oviduct, lungs, brain and airway and act to propel fluid or mucus



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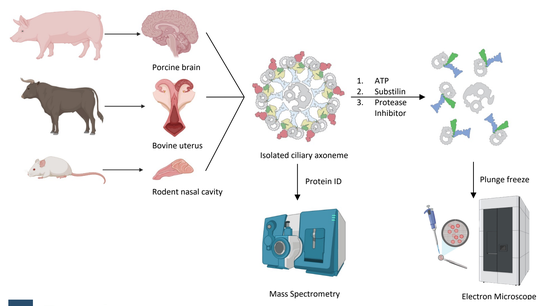
❖ **Oviduct cilia** help transport fertilized eggs

❖ **Ependymal cilia** are essential for the circulation of cerebrospinal fluid

❖ **Olfactory sensory cilia** play a crucial role in the olfactory signaling cascade by housing receptors for odorant molecules and transducing their binding into electrical signals, which are then relayed to the brain for the perception and interpretation of different smells

Methodology

In this study, we isolated the ependymal cilia from pig brain ventricles and the oviduct cilia from bovine fallopian tube with mechanical force and Calcium shock. Purified cilia were demembraned and splayed into doublet microtubules. Single particle cryo-EM analysis were performed all samples. All samples were sent to mass spec to identify all protein components in the sample. Below is the workflow.



Results

Olfactory sensory cilia have longer tip than motile cilia

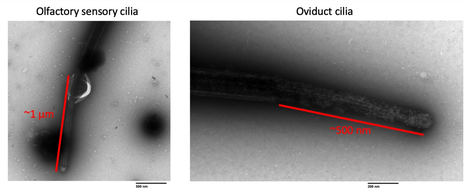


Figure 1. Comparison of negative stain morphology between olfactory sensory cilia (OSC) and oviduct cilia tip region. The average length of OSC measures approximately 1 µm, while oviduct and ependymal cilia are approximately 500 nm in length. Notably, the tips of oviduct and ependymal cilia exhibit prominent decoration by globular proteins, whereas OSC doesn't have.

Microtubule transformation at the tip region

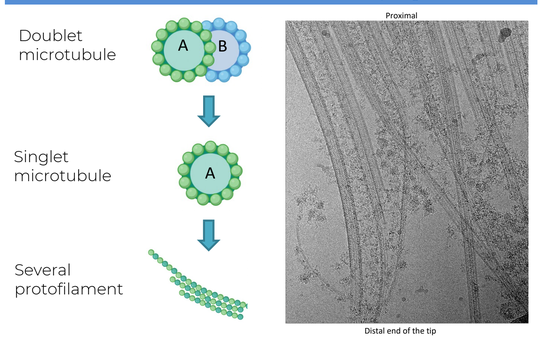


Figure 2. Doublet microtubule transform into singlet microtubule from proximal to the distal end of the cilia and eventually become one or a few protofilaments at the end of the tip.

Results

High resolution structure of 48 nm repeat doublet microtubule of three types of cilia

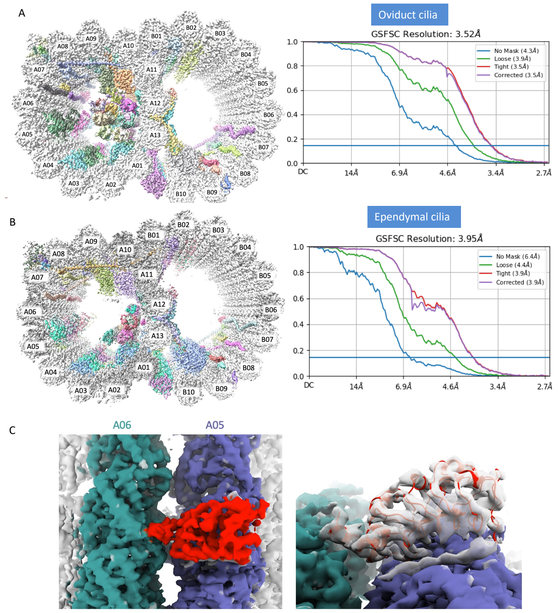


Figure 3. The 48 nm repeat doublet microtubule map of oviduct cilia (A) and ependymal cilia (B) at 3.5, and 3.9 Å resolution respectively. The structure we obtained from the OSC dataset is highly similar to that of the respiratory cilia. Further 3D classification is needed to separate the two types of cilia in that dataset. (C) A new protein identified at the outer junction of the A05 and A06 protofilament that is a leucine-rich repeat containing protein mainly function as scaffold.

The monomeric actin of inner dynein arm (IDA) a and b have ATP density in the nucleotide cleft

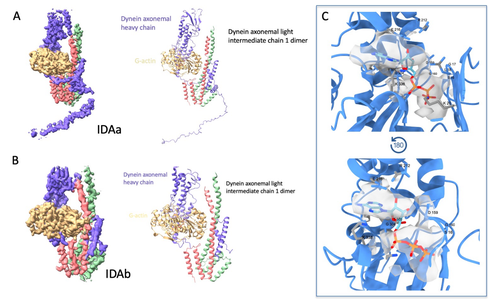


Figure 4. Both IDAa and IDAb are composed of a monomeric actin, a dimer of dynein light intermediate chain 1 and a dynein axonemal heavy chain. There is ATP density in the nucleotide cleft of monomeric actin indicating

Results

Localized protein synthesis within cilia validated by high resolution ciliary ribosome structure

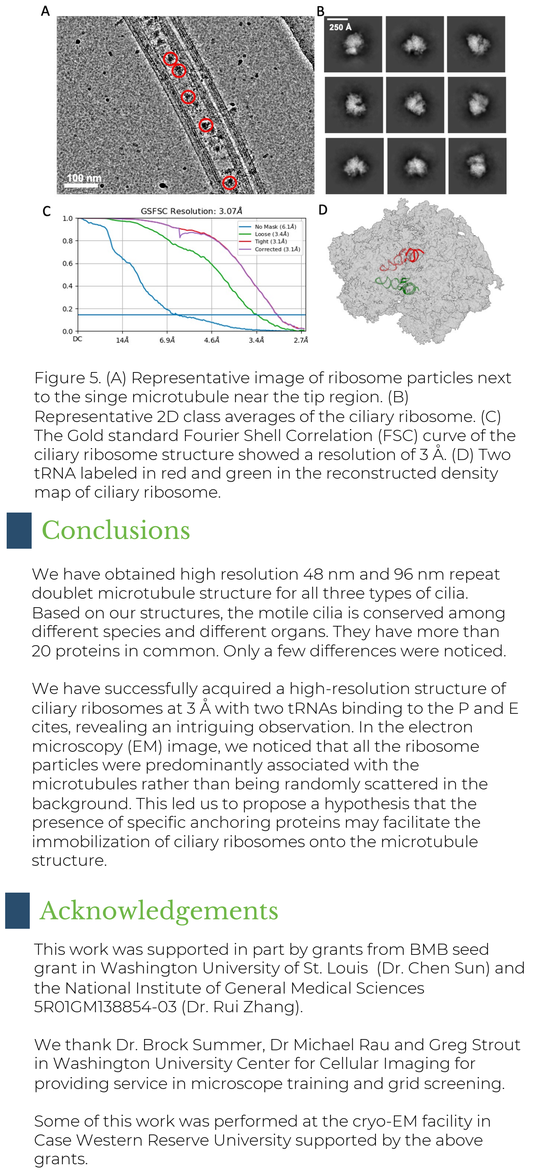


Figure 5. (A) Representative image of ribosome particles next to the singe microtubule near the tip region. (B) Representative 2D class averages of the ciliary ribosome. (C) The Gold standard Fourier Shell Correlation (FSC) curve of the ciliary ribosome structure showed a resolution of 3 Å. (D) Two tRNA labeled in red and green in the reconstructed density map of ciliary ribosome.

Conclusions

We have obtained high resolution 48 nm and 96 nm repeat doublet microtubule structure for all three types of cilia. Based on our structures, the motile cilia is conserved among different species and different organs. They have more than 20 proteins in common. Only a few differences were noticed.

We have successfully acquired a high-resolution structure of ciliary ribosomes at 3 Å with two tRNAs binding to the P and E cites, revealing an intriguing observation. In the electron microscopy (EM) image, we noticed that all the ribosome particles were predominantly associated with the microtubules rather than being randomly scattered in the background. This led us to propose a hypothesis that the presence of specific anchoring proteins may facilitate the immobilization of ciliary ribosomes onto the microtubule structure.

Acknowledgements

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