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The investigation of acetate sensitivity within *shf1 Chlamydomonas* mutants

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ABSTRACT

Cilia and flagella are essential for human health. Defects in the assembly and function of these organelles are associated with a collection of disorders called ciliopathies. Studies have suggested that regulation of ciliary size is associated with external environmental factors. Although TOR signaling pathway has recently been implicated as playing a pivotal role in linking the cellular environment with determination of cell and organelle size, additional biological pathways involved in this process remain largely unknown. Short flagellar (*shf*) mutants of *Chlamydomonas* assemble flagella that are half the length of wild-type cells. Consistent with the observation that ciliary length and cell size are interconnected, *shf1* cell volume is increased compared to wild-type cells. Interestingly, *shf1* mutants are aflagellate when grown in the presence of acetate. To learn more about the acetate sensitivity, we examined the ultrastructure of *shf1* mutants following the addition of acetate. Microscopic analysis revealed notable deformities in the flagellar ultrastructure. Currently, we are using a biochemical and global proteomic approach to learn more about the function of the SHF1 gene product.

INTRODUCTION

Figure 1. Flagellar phenotypes of mutants defective in length control

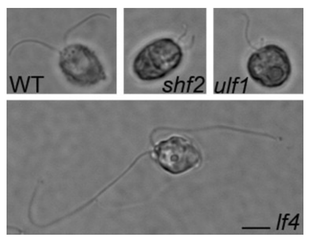


Figure 2. Ciliopathies are pleiotrophic

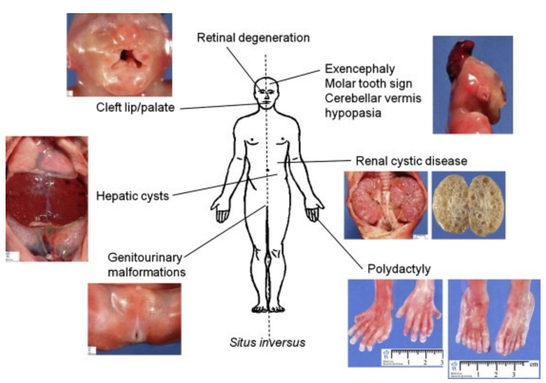


FIGURE 165-4. Emery and Rimoin's Principles and Practice of Medical Genetics (Sixth Edition)

METHODS

Treatment with acetate

Both WT and *shf1* cells were grown in M media with continuous aeration and under light-dark cycle to an equal cell density. 20 mM acetate was added and cells were removed in 30 minute intervals. Cells were then fixed with an equal volume of 1% glutaraldehyde.

Measuring the cell body volume

Cells were examined by phase contrast microscopy and electron microscopy. Cell body area was determined.

Figure 3. Mean cell body areas of wild-type and *shf1* and the histogram distribution.

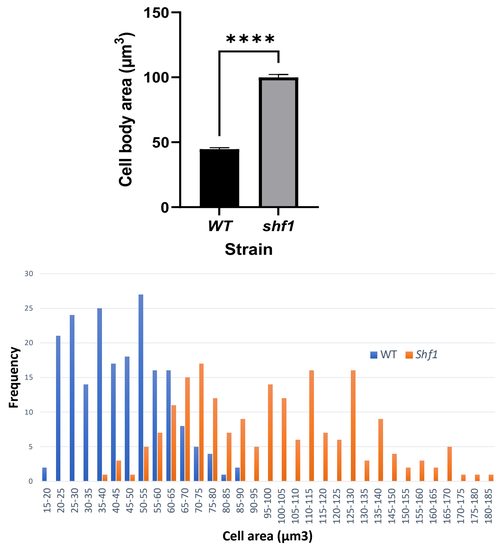
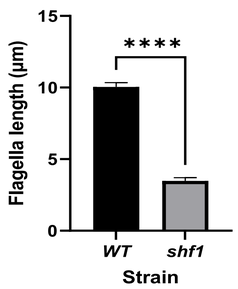


Figure 4. Flagellar length comparison of wild-type and *shf1.*



RESULTS

Figure 5. Abnormal flagellar morphologies of *shf1* flagella with acetate.

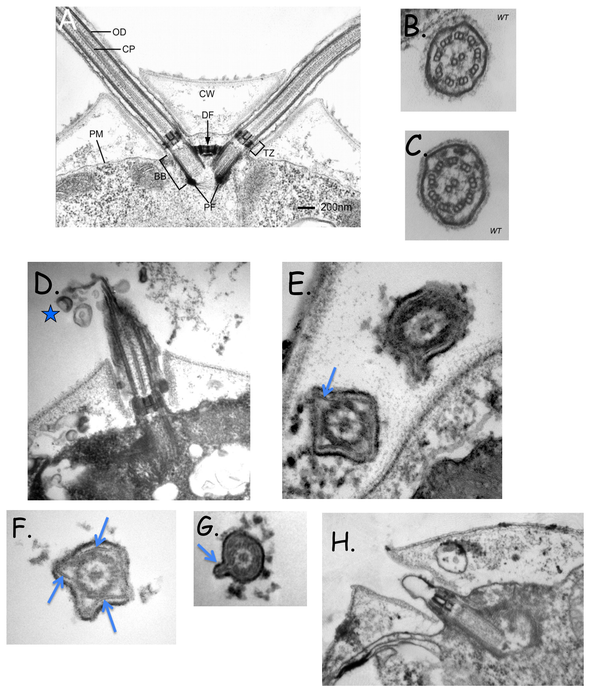


Figure 5. Ultrastructural analysis of wild-type and *shf1* cells with acetate. A.) Image provided by William Dentler, Ph.D.; Univ of Kansas demonstrating normal features of flagella and basal bodies. B and C.) Cross-sections of wild-type flagella showing normal 9+2 microtubular arrangement. Panels D, E, F, and G are images of *shf1* after 60 minutes of acetate. D.) Longitudinal section through basal body and flagella demonstrates membrane blebbing when microtubules are lost. E – G) Cross-sections through *shf1* flagella. Blue arrows indicate "rod-like" particles that distort the normal morphology of the flagellar membrane. H.) *shf1* cells grown for 14 days in acetate-containing media completely lack all microtubules past the transition zone. Abbreviations: OD – outer doublet; CP – central pair; PM – plasma membrane; CW – cell wall; DF – distal striated fiber; PF – proximal striated fiber; TZ – transition zone; BB – basal body

CONCLUSION

Although *shf1* assembles short flagella, their cell bodies are approximately twice the size of wild-type cells. This suggests that regulation of flagellar length and cell body size are coupled together. As originally reported, inclusion of acetate in growth media results in the disassembly of *shf1* flagella. Ultrastructural analysis demonstrates a dramatic change to the morphology of *shf1* flagella upon acetate treatment. It is intriguing to speculate that the "rodlike" structures present between the axonemes and the flagellar membrane are axonemal fragments. Currently, we are examining the composition of these "rods" to determine their biochemical components

FUTURE DIRECTIONS

- Analyzing the effect of acetate on *shf1* mutant flagella and cell volumes for a longer period.
- Analyzing the effect with different concentrations of acetate.
- Quantitative proteomic analysis of *shf1* mutant and wildtype cell bodies and flagella.

REFERENCES

1. "Target-of-rapamycin complex 1 (Torc1) signaling modulates cilia size and function through protein synthesis regulation" Shiaulou Yuan, Jade Li, Dennis R. Diener , Michael A. Choma, Joel L. Rosenbaum, Zhaoxia Sun, Proceedings of the National Academy of Sciences Feb 2012, 109 (6) 2021-2026
2. "Regulation of flagellar length in Chlamydomonas", Nedra F. Wilson, Janaki Kannan Iyer, Julie A. Buchheim, William Meek, Seminars in Cell & Developmental Biology, Volume 19, Issue 6,2008,Pages 494-501,