MOL231: Characterization of ΔUnc13 in the multicellular stage of the choanoflagellate, *Salpingoeca rosetta*

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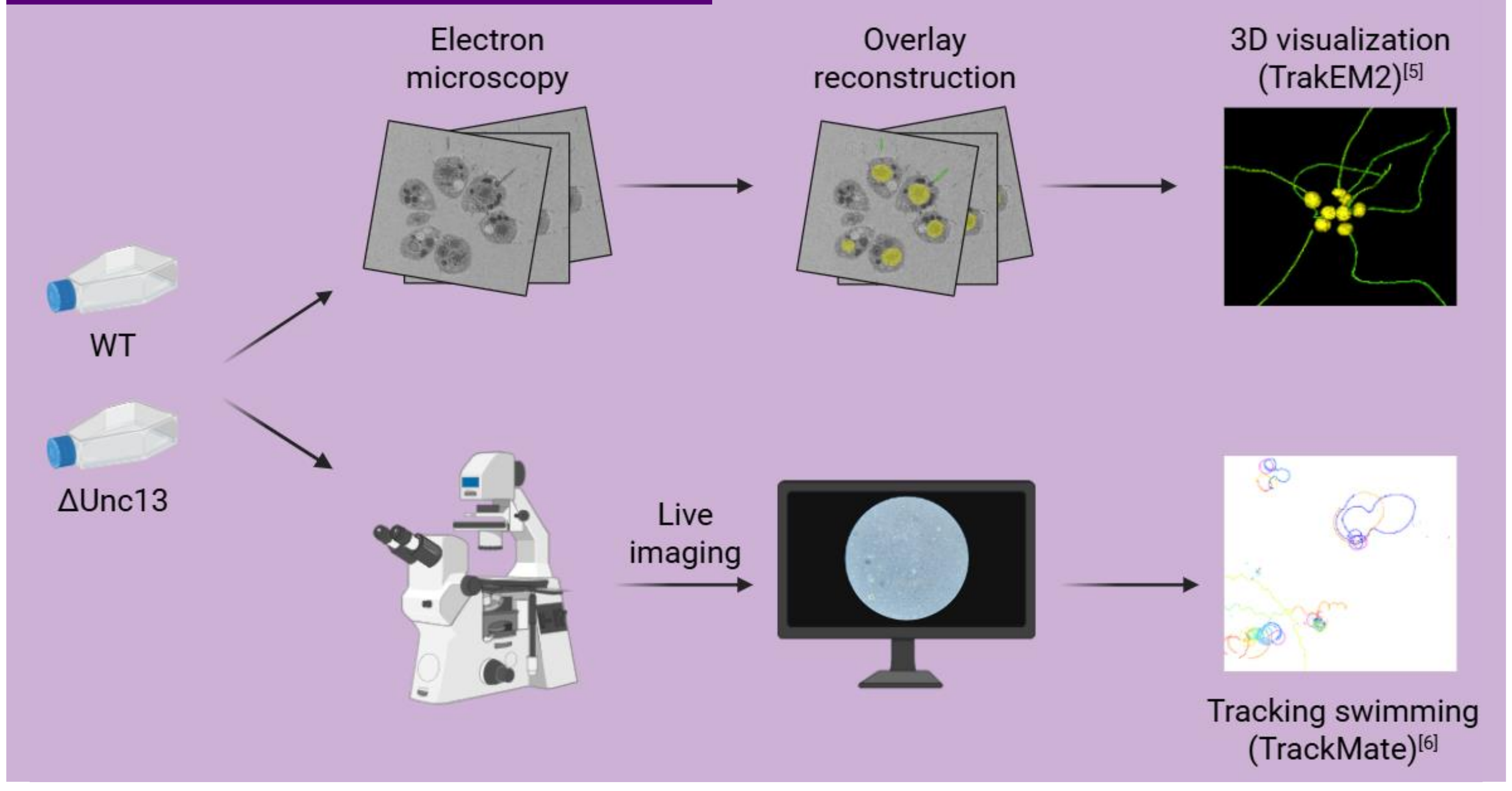
Background

Choanoflagellates are the sister group to animals and possess many genes once thought to be animal specific[1]. One such gene encodes the Unc13 protein, which is a key synaptic protein in animals[2]. The choanoflagellate *Salpingoeca rosetta* exists as single cells which contain an apical feeding structure, composed of a flagellum, surrounded by a microvillar collar [3]. Certain environmental cues can trigger the formation of clonal rosette colonies [4], which are believed to increase feeding efficiency. Knockout of Unc13 in *S.rosetta* results in severe defects in the apical structure[3]. The goal of this study was to elucidate the role of Unc13 in *S. rosetta* colonies.

Workflow



ΔUnc13 results in absence of flagella in some cells and change in the mobility of the colonies

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Colony	Flagella present		Volume flagella [µm³]	
	WT	Unc13	WT	Unc13
1	15/15	5/7	2,21	0,85
2	9/9	11/16	2,25	0,57
3	11/11	4/4	1,41	1,18
4	7/7	3/4	2,30	1,02
5	13/13	6/11	2,29	0,01
6	8/8	5/6	1,90	1,50
7	11/11	7/8	1,96	0,82
			2,05	0,85

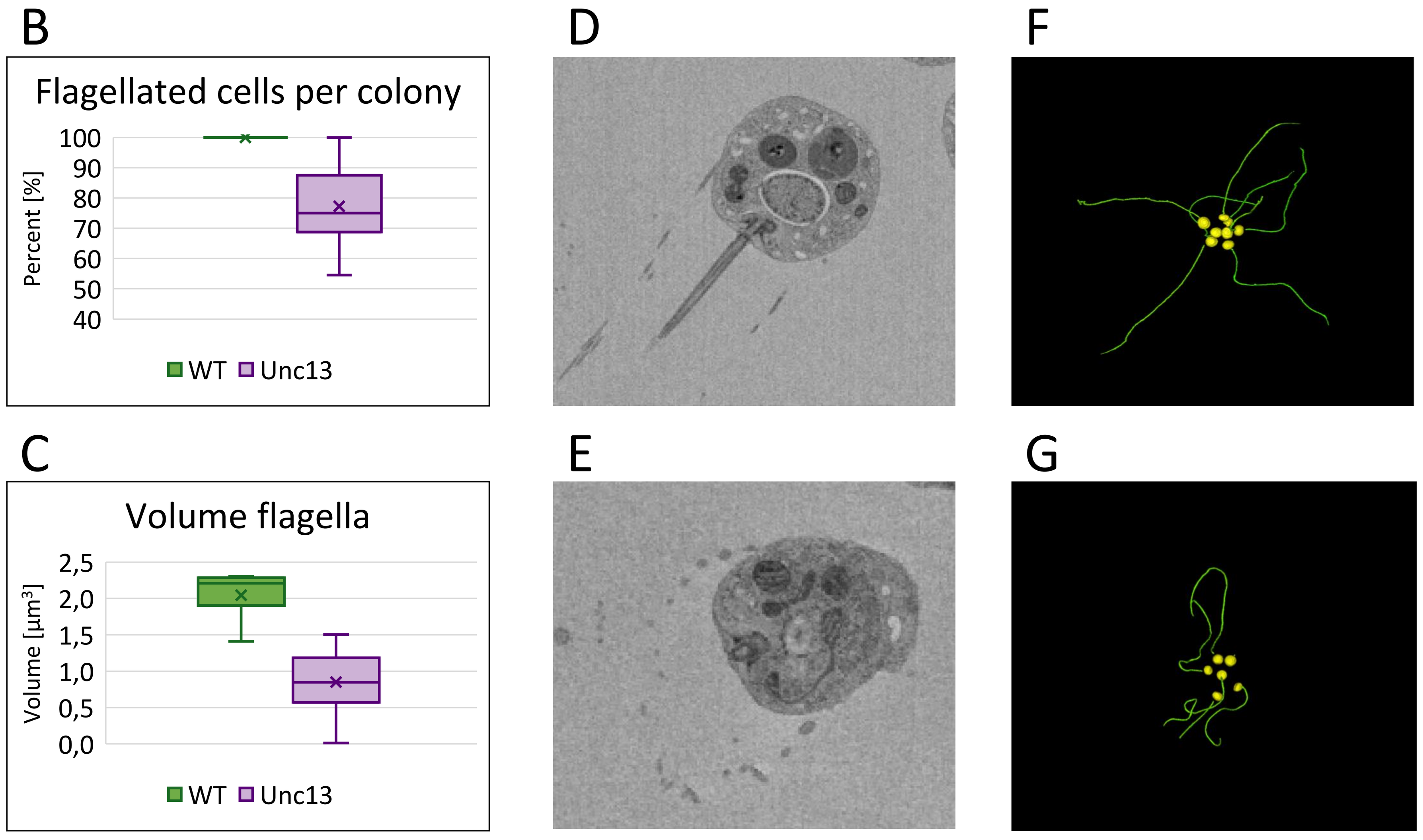

Figure 1. ΔUnc13 colonies show fewer and smaller flagella. (A) Overview of the number and average volume of full reconstructed flagella in *S. rosetta* colonies. (B) Percentage of flagellated cells per colony. (C) Flagellar volume measurements of fully reconstructed flagella in each colony. (D–E) Electron micrographs of a wild-type (WT) colonial cell (D) and a ΔUnc13 colonial cell (E). (F–G) 3D reconstructions of a WT colony (F) and a ΔUnc13 colony (G). Green: flagella, yellow: nuclei

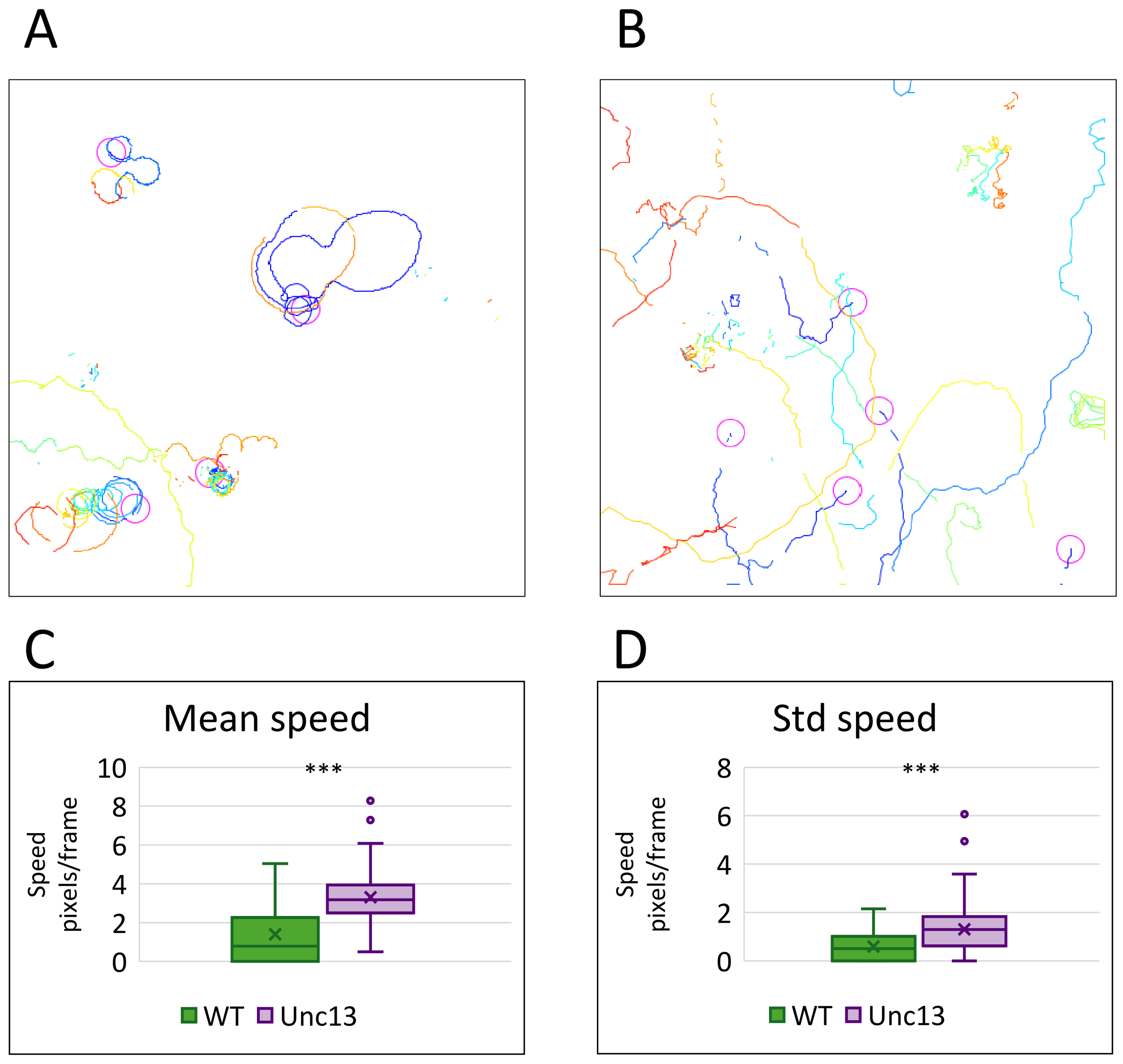

Figure 2. ΔUnc13 have a stochastic and faster movement. (A-B) Trajectories of WT colonies (A) and ΔUnc13 colonies (B). (C) Mean speed per colony over the course of imaging shows a significant increase in mutant colonies, $P<0.001$. (D) Standard deviation speed per colony over the course of imaging shows a significant increase in mutant colonies, $P<0.001$.

Conclusion

The electron microscopy shows that in colonies, some ΔUnc13 cells lack a flagellum and those that are present have a reduced volume, idicating shorter flagella. Furthermore, the live imaging shows a mobility change in the ΔUnc13 colonies. These findings are consistent with Unc13 playing an important role in formation or maintenance of the flagella in *S.rosetta*, providing valuable insights into the evolution of synaptic proteins[3].

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