

# RNA-interference of *Chlamydomonas reinhardtii* RSP8

Matthew Greseth\*, Pinfen Yang<sup>†</sup>, and Chun Yang<sup>†</sup>

\*Carroll College Undergrad, Waukesha WI 53186

<sup>†</sup>Marquette University Biology Department, Milwaukee WI 53233

## Abstract

Motile cilia and flagella confer organisms superiority to mate, avoid predators, harvest food and respond to environmental stimuli. The structure responsible for the motility within these organelles is the highly conserved 9+2 axoneme, which consists of nine outer doublet microtubules surrounding two central singlet microtubules. Dynein arms anchored to the outer microtubules drive the microtubule sliding and consequently oscillatory beating. However, the molecular mechanism for regulating dynein activation and thus flagellar beating remains largely unknown.

Studies of *Chlamydomonas reinhardtii*, a biflagellate unicellular green algae, suggested that the radial spoke, a T-shaped molecular complex regulates motility mechanically by interacting with the central pair and outer doublets as well as chemically by binding second messengers and changing enzyme activity. A mutant, *pr25*, is defective in the gene encoding radial spoke protein (RSP) 11 that contains an R11a domain known for anchoring GMP-dependent protein kinase A to the kinase anchoring protein (AKAP). RSP11 binds the spoke AKAP. *pr25* mutants displayed wild type motility in fresh medium, but became paralyzed in exhausted medium. In contrast, wild type cells swim regardless. In addition to RSP11, RSP8, an armadillo repeat spoke protein, is dramatically reduced as well. To test that RSP11 regulates flagellar beating via RSP8, we use RNAi to knockdown RSP8. Previously, the conventional hairpin plasmids failed possibly due to gene methylation. New hairpin constructs and a paromomycin resistance cassette were inserted into the pHANNIBAL vector that was routinely used in RNAi of *Arabidopsis*. The single vector was transformed into *Chlamydomonas*. Western blots of flagellar minipreps reveals antibiotic-resistant clones with RSP8 reduced at least 60%, indicating the pHannibal vector is suitable for RNAi of *Chlamydomonas*. We are currently investigating the motility phenotype.

## Vector Based RNAi

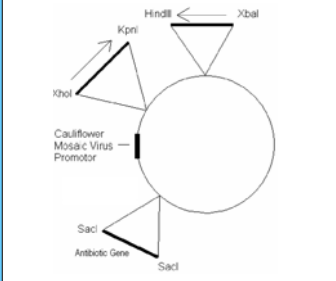


Fig. 1. To induce RNAi, a hairpin construct was designed and placed into the pHANNIBAL vector. The hairpin was created by placing two identical pieces from the desired gene into the vector in opposite orientation. pHANNIBAL contains the strong Cauliflower Mosaic Virus Promoter. To enable a single transformation, an antibiotic resistance gene was also placed in the vector.

## Radial Spoke and RSP8 Structure

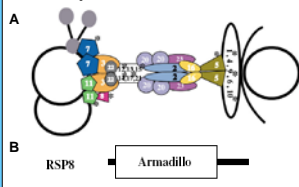


Fig. 2. A schematic of the radial spoke with relative positions of proteins (A). RSP8 is attached to RSP11 next to the doublet microtubules. A representation of what the protein structure of RSP8 is (B). The armadillo repeat is involved in protein-protein interactions and is thought to bind to RSP11. A 3-D image of an armadillo repeat (C) shows what RSP8 could look like.

## RSP8 Gene and Hairpin Constructs for RNAi

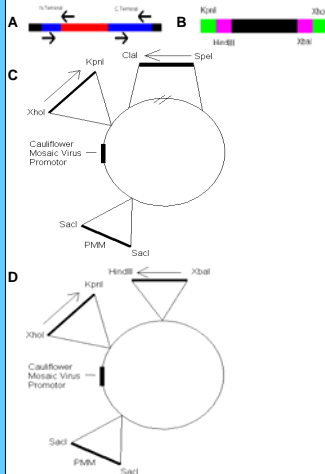


Fig. 3. Inserts were made from the RSP8 gene (A) from both termini. Using restriction enzyme directed-PCR, we generated inserts (B) with desirable restriction sites on both ends. Inserts were placed into pHANNIBAL as well the paromomycin resistance gene (C and D). Construct 1 (C) was generated by using a 300bp insert from the 5'UTR. Construct 2 (D) was also generated by using the same 300bp insert from the 5'UTR. Construct 3 (D) has the same design as Construct 2, only it contains a 500bp insert from the 3'UTR.

## Hairpin Confirmation Through Restriction Enzyme Digest

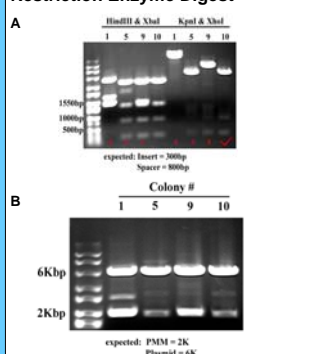


Fig. 4. Four colonies that contained construct C (Fig 3) were chosen from a slot lysis to undergo restriction enzyme digest. In the first digest (A), the four colonies were digested by XhoI & KpnI and XbaI & HindIII to confirm the presence of the two inserts that constitute the hairpin. Colony 1 does not have a band at 300bp or 800bp, therefore, it had no insert. Colonies 5, 9 and 10 had bands at 300 and 800. In the second digest (B), the same four colonies were digested by SacI to confirm the presence of the paromomycin resistance gene. All four colonies contained a band at 2Kbp which is the size of the paromomycin resistance gene. Colonies 1 and 9 contained brighter bands which suggests that they contain more than one paromomycin insert, while colonies 5 and 10 have only one insert. From these results, Colony 10 was used to transform into *Chlamydomonas* for RNA-interference because it has one PMM gene as well as the best results for the insert confirmation.

## PCR to Detect the Hairpin Constructs in *Chlamydomonas* Transformants

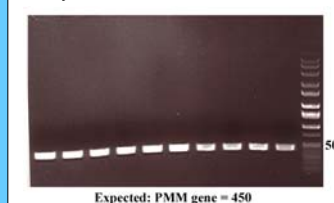


Fig. 5. This PCR confirms the presence of the Hairpin in the *Chlamydomonas* transformants. The PCR gave good results and showed that all the transformants sampled, a few from each construct, showed the presence of the paromomycin resistance gene. This inherently shows that the hairpin construct is in as well.

## Western Blot to Detect the Knockdown of RSP8

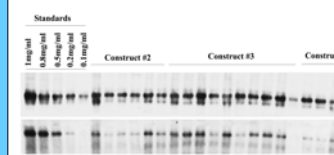


Fig. 6. Western Blot of *Chlamydomonas* transformants with all three constructs. When compared with the standard dilution lanes, two lanes from construct three have reduced amounts of RSP8. This shows confirmation that the RNAi technique worked.

## Conclusions

- Developed a construct for single transformation for hairpin and paromomycin resistance.
- Developed a technique that effectively induces RNAi in *Chlamydomonas*.

- Showed that RNAi knocked down RSP8.

- By combining the third construct and the technique used, other *Chlamydomonas* genes can be knocked down.

- The next step is to watch these two transformants to see if there is a phenotype change to help elicit the role of RSP8 in the axonemal structure.

## Acknowledgements

I would like to thank Cynthia Horst for her assistance in the onset of this project.

## References

Yang, et. al. 2005. Journal of Cell Science 119, 1165-1174