

The Effects Deletion Mutagenesis has on the Anchoring of a Putative Docking Domain, DPY-30

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Motile cilia and flagella are whip-like structures sweeping surrounding fluid for vital organs and diverse organisms. The motion is generated by the conserved 9+2 cytoskeletal axoneme inside these organelles. Previous studies using *Chlamydomonas*, a biflagellate single-cell green alga, indicate that a molecular complex, the radial spoke (RS) orchestrates the sequential activation of axonemal dynein motors to generate rhythmic beating and mediates motility changes induced by the 2nd messengers. My project tests the hypothesis that structural and calcium-binding modules are anchored through a novel DPY-30 domain to the scaffold molecule of RS, Radial Spoke Protein 3 (RSP3).

To test this, the strategy is to express the mutated RSP3 lacking the putative DPY-30 binding site in *Chlamydomonas* mutant missing RSP3. The prediction is that the transformants will express the truncated RSP3 but the flagella will be paralyzed due to the inappropriate assembly of DPY-30 proteins. First, Quick-change site directed mutagenesis was taken to delete from the RSP3 gene in a plasmid the two putative DPY-30 binding sites: amino acid a.a.# 262-285 and a.a.# 286-316. The PCR products were transformed into competent cells. Slot lysis, colony PCR and restriction digest revealed that only the aa#262-285 mutation was successful. The mutated plasmid was then transformed into an algal strain that does not express RSP3 and do not have RS. Upon acquiring the plasmid, the transformants clones were screened under a light microscope.

As predicted, all clones remained paralyzed. This result suggests that DPY-30 binding site is located around a.a. 262-285. To further determine the assembly deficiencies, we are growing up the transformant clones for biochemical analysis of RS complex. If the hypothesis is correct, the flagella of many clones will contain truncated RSP3 but the DPY-30 proteins will be deficient in quantity or stability.