Complimenting a Chlamydomonas reinhardtii Mutation Using Cell Penetrating Peptides

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Chlamydomonas reinhardtii is a photosynthetic model organism most notable for its easily manipulatable genetics. C. reinhardtii uses flagella to swim and optimize its growth conditions in the light. We plan to use cell penetrating peptides (CPPs) to compliment C. reinhardtii that is affected with the IFT46 mutation. Cell penetrating peptides are short peptides that can move across a cell membrane. The novel CPP that we are using is called TaT-CaM. It consists of the trans-activator of transcription (TaT) and the calmodulin domain (CaM) that binds to a calmodulin binding site (CBS) engineered into our protein of interest, IFT46. IFT46 is an Intraflagellar Transport Protein (IFT) required for flagella assembly. The IFT46-1 mutant causes paralyzed flagella. Previous results have shown TaT-CaM is an effective way to deliver protein into C. reinhardtii. To complement the IFT46 mutation using CPP, we must express the CBStagged IFT46 protein (CBS-IFT46). I have transformed bacterial competent cells with the CBS-IFT46 plasmid, attempting to make the competent cells express the protein. I have performed restriction digests to ensure the CBS-IFT46 sequence was properly inserted into the vector. The digest results indicate the DNA was inserted into the vector correctly, but I have been unsuccessful in getting the bacterial cells to express the CBS-IFT46 protein. In the future, we will send samples of the clone plasmid to be sequenced and potentially a different expression vector will be used. Completion of this work will show that CPPs have novel use, allowing efficient protein introduction into the cell.

Keywords: Flagella, Algae, *Chlamydomonas*, Mutation, Cell Penetrating Peptide, CPP, *Chlamydomonas reinhardtii*, *C.reinhardtii*, IFT46, TaT-CaM.