Characterization and Localization of *Paramecium* Pawn A: a Calcium Channel Regulator.

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Paramecium, a unicellular organism, swims forward in normal culture medium and occasionally swims backward for short periods. Wild type cells perform long backward swimming in depolarizing solutions [e.g. $BaCl_2$]. Some mutants of *Paramecium* do not swim backward at all due to the absence of voltage gated Ca^{2+} current. These cells are called Pawns. Till now three genes [pawn A, B, C] have been found to be mutated in Pawn cells and only pawn A and B were cloned. We used RNAi feeding method with a pawn A construct (GSPATG00010883001) in the RNAi vector L4440 to down regulate Pawn A in wild type cells and observed the swimming behavior. We found that we could produce a Pawn phenotype with RNAi. We expressed the Flag tagged Pawn A to follow the protein product to find its location. C terminal 3X Flag tagged pawn A construct was microinjected into the macronuclei of pawn A mutant cells in order to test whether the cloned sequence rescued the wild type phenotype and to find the location of functional Flag tagged protein product by immunostaining. The cells showed intracellular and surface immuno- fluorescence. Western blots probed with an anti Flag antibody, showed specific staining in pellicle- rich and endoplasmic reticulum- rich fractions. We performed Glucose 6 phosphatase assay to determine the efficiency of the fractionation techniques. As Pawn A is not a channel protein, our aim is to find its association with voltage gated Ca^{2+} channel.

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