Beta 2 Tubulin Amino Acids Required for Spermtail Axoneme Function
Evolutionary change in organism traits are primarily caused by random genetic mutations in their amino acid codons that end up altering the proteins produced. The main question of researchers is how change occurs that will give a protein a new function without detrimentally affecting the original function of the protein in the organism. Beta 2 tubulin in Drosophila is an ideal model to study this question because it has a very sensitive structure/function relationship. Drosophila contains two main types of tubulin: Beta 1 which is found in the majority of cells and testes specific Beta 2. These proteins differ in only a few amino acids, however Beta 1 is unable to support the function of Beta 2.

The proposed studies will investigate how Beta 2 can modify the spermatid's ability to support the new function. We will perform a synergetic interaction between amino acids 29, 55, and 57 and then attempt to see if this allows for a new function to be created. The researchers will test the hypothesis that there is a synergistic interaction between Beta 2 amino acids 55 and 57 on Beta 2' function in Drosophila. The researchers will test the function of Beta 2's carbohydrate terminus, amino acids 381-446, by exchanging them into the Beta 1 protein. It could not replace Beta 2 function; however, spermatid tail length was closest to that of wild type flies indicating that these residues carried their function into the Beta 2 protein in an additive manner. However, Beta 1 with Beta 2 amino acids at locations 55 and 57 along with the Beta 2 carbohydrate tail lost Beta 2 function in its absence. It indicates that the function of 55 and 57 depends on Beta 2 specific amino acid interactions -- a synergism. Protein crystallography of the Beta 2 tail reveals that the tail is unique in that it has a unique structure (29, 55, 29, 57) and phylogenetic analyses show that the Beta 2 amino acid identities at these sites are unique among the more than 100 beta tubulins. This information led to the hypothesis that there is a synergistic interaction between Beta 2 amino acids 55, 57, and 29, providing a unique Beta 2 motif which will not function without having all the correct amino acids in place to complete the synergism. Study of this unique synergistic interaction is the main focus of this research project.

The first premise addresses the availability of choices in particular features based upon genetic mutations in the organism's DNA sequence. Some features, including proteins, may admit of change more readily than others, and this will influence the evolutionary process. The second premise addresses the competition among organisms provided with different phenotypes for the occupation of particular ecological niches. However competition would not be possible if the dynein choices had been available with which to compete. It is generally accepted that evolution is the force behind the generation of new species; however the exact roles of each premise are not fully understood.

The research team is using Drosophila as a model to investigate the location of the Beta 2 tubulin. The researchers will study the transgenic fly that can lack the Beta 2 tubulin. Their goal is to determine how changes occur that will give a protein a new function without detrimentally affecting the original function.

Evolutionary changes in proteins occurs due to alterations in the amino acid coding sequences of an organism's genetic code. Some changes may have equal effect, some may have equal variable; some are even more altered than others. Here we seek to determine why Beta 2 protein does not admit of harvestable variation, by testing if there is a unique Beta 2 amino acid synergism that is fundamental to Beta 2 function that, due to being a synergism, is resistant to evolutionary change.

Methods

A plasmid construct (Fig. 3) containing a Beta 1-tubulin along with Beta 2 codons at locations 29, 55, 57, and 381-446 and designated TARGARC. The TARGARC construct was then introduced to an offsite location (Rainbow Transgenics, CA) to be transformed into W1118 (white) flies. The p-element vector contained the white+ gene that generates a red eye phenotype, so the transformed flies containing the TARGARC insert could be distinguished due to the expression of a red eye phenotype.

To ensure that TARGARC is the sole source of beta tubulin in the testes, specific cross were done using a "K" designated chromosome that contains a null copy of the Beta 2 tubulin. Virgin flies containing the TARGARC insert were mated with a stock of opposite sex K/TM3 flies (TM3 is a balancer chromosome as specified above). The resulting progeny were then used to disperse the TARGARC construct. No progeny were observed from the cross suggesting that the flies did not contain the TARGARC construct.

Discussion

The largest surprise from the results obtained was the complete lack sperm found in the testes of TARGARC flies. Based on previous results from other tests of Beta 2 amino acids in a Beta 1 background, some resemblance of sperm was found, regardless of whether or not they were full length or motile. The whole hypothesis for the TARGARC vector was based on previous work that they were slowly increasing the length and improving the structure of the axonemal until the potential synergism was found. We believed we would see improved function in spermatogenesis, and certainly expected some spermatogenesis function, given prior tests of chimeric tubulin. The gel electrophoresis and the PCR test confirmed that the flies did not contain the TARGARC insert. That is a surprising result that was not a good one could yield promising results once the transformation issue is resolved. The most promising result is that the addition of Beta 2 tubulin does not affect the ejaculate or the spermatozoon motility. If the addition of Beta 2 tubulin did not affect the ejaculate or the spermatozoon motility, then it would be expected that the flies would be able to produce some resemblance of sperm despite having two copies of Beta 2. However, no sperm were found and these testes very closely resembled those of their white eye counterparts. Some pre-meiotic spermatids were seen attempting to undergo meiosis and form sperm (Fig. 5b), but they were unable to complete the process.