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Studying the Evolution of Beta 2 Tubulin in Dipterans

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Squashing Fly Heads

Drosophila melanogaster: Anastrophula suspense (true fruit fly, 80MY since sharing a common ancestor with D. melanogaster, Musca domestica (housefly, 110 MYA), and Chrysops spp. (deerfly, 130MYA). We construct this tree by plotting changes in the Beta 2 sequence on a fly phylogeny (130MYA). We can do this by using a PCR approach to clone the Beta 2 gene in D. melanogaster. A second round of dilution of the stock House Fly DNA yielded DNA template, no DNA bands were present in the gel.

Discussion:

At first, no results were obtained from PCR. After troubleshooting, it was determined that poor reagents were responsible for the lack of results. New dNTP and polymerase were obtained and proper PCR samples soon followed. With the first 9 House Fly PCR experiments (combining each of the possible 3' and 5' primers) each containing 1/1 (no dilution from stock) concentration of DNA template, no DNA bands were present in the gel. The second round of PCR used a 1/1000 dilution of the stock House Fly DNA yielding DNA bands that were less distinct than the DNA ladder. The bands were broad, but still showed signs of sequences in the desired length range of 1.0-1.5 Kb. For the future, the PCR DNA sample that yielded the bands can be used as a DNA template in a second round of thermocycling in an attempt to brighten the 1.0-1.5 bands.

Methods:

Primer Design

Two sets of PCR primers were generated from existing Beta two tubulin sequences. The first set, “Drosophila” primers were made from consensuses 5' and 3' sequences generated from Drosophila spp. sequences: D. melanogaster, D. yakuba, D. ananassae, D. virilis, D. willistoni, D. persimilis, D. pseudoobscura, D. simulans, D. sechellia, D. mauri, D. erecta, D. grimshawi. The second set, “Dipteran” primers, were generated from consensuses Drosophila spp., Aedes aegypti, and Anopheles gambiae sequences.

Dip 5': attatgaacatgctgtaaatgtgcyaYWtBc
Dip 3': attatgaacatgctgtaaatgtgcyaYWtBc

Introduction:

How do proteins evolve while maintaining their function? Previous studies (Raff et al. 2000, Nielsen et al. 2001) find a highly stringent structure/function relationship between the Drosophila melanogaster testsis-specific tubulin Beta 2 and the spermatid axoneme, such that small changes in the Beta 2 protein render it unable to generate a motile axoneme. This raises the question, how does Beta 2 evolve while maintaining its function?

Previously, we found that in fact this protein has not evolved at a single amino acid site for 60 million years in the genus Drosophila (Nielsen et al. 2006, Nielsen et al. 2010). However, if you look back further in time, D. melanogaster and A. gambiae have 40 amino acid differences in their Beta 2 amino acid sequences, and shared a common ancestor approximately 220 MYA. This indicates that this protein has been able to evolve in its history. Given its lack of evolution in Drosophila, we would like to know the nature of its evolutionary change, which would provide clues as to how it was able to evolve, and why it is no longer evolving in Drosophid is.

We can do this by using a PCR approach to clone the Beta 2 gene in relatives of Drosophila and identify changes in the coding sequence. We can interpret the function of evolving amino acids based on their role in the folded protein (Nogales et al. 1999), and ultimately can express these Beta 2 homologs in D. melanogaster to determine if they are capable of supporting its spermatid axoneme. If they can, we can conclude Beta 2 evolved along a narrow pathway that maintained its function. If not, we can conclude Beta 2 co-evolved with other proteins in the spermatid.

Results:

At first, no results were obtained from PCR. After troubleshooting, it was determined that poor reagents were responsible for the lack of results. New dNTP and polymerase were obtained and proper PCR samples soon followed.

DNA preparation

DNA from flies of each species was obtained using the squash buffer method (Gloor et al. 1993). Briefly, 1 fly is ground with a pipet tip in 20ul 10 mM Tris-Cl pH 8.2, 1 mM EDTA, 25 mM NaCl, and 200 uM Proteinase K. This is incubated at room temperature for 20 min. to allow the Proteinase K to digest the tissue and release genomic DNA into solution, followed by 2 minutes at 95C to inactivate the Proteinase K.

PCR

We first worked with House Fly DNA templates in both 1/1 and 1/1000 concentrations. For each DNA template, 9 different 3' and 5' Primer combinations were used in PCR thermocycling. The 3' primers were Dros 3', Dipteran (dip) Combo A, dip Combo G, dip revAgB2, and dip revAgB2. The 5' primers combined were dros 5', dip Combo geo, dip triple combo, and dip quad combo.

1 microliter of 3' was combined with 1 microliter of each of the 5' primers. They were then added to a mix of 5ul 1X standard buffer, 1 ul dNTP, 1ul DNA template, .25ul taq polymerase and water up until 50ul total. The 9 tubes were placed in the thermocycler and run with temperatures of 95, 60, and 75 degrees Celsius. Afterwards, DNA samples were loaded into an agarose gel along with a DNA ladder of known band lengths. Gel electrophoresis was then run at approximately 110 millamps. The gels were then imaged under UV light to compare band lengths of the 9 samples with the standard DNA ladder.

Studying the Evolution of Beta 2 Tubulin in Dipterans

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Abstract: Mosquitoes (e.g. Aedes aegypti and Anopheles gambiae) and fruit flies (e.g. D. melanogaster) are two very distant relatives in the order Diptera (flies) that shared a common ancestor 220 million years ago that had the Beta 2 tubulin gene in its genome. From that time to present, the Beta 2 gene has evolved to its present form their respective lineages. We know that the protein sequence for Beta 2 in D. melanogaster and Anopheles gambiae differ at 40 amino acids. Here, we aim to construct a phylogenetic tree that shows how genetic changes occurred in its evolutionary history since mosquitoes and flies shared a common ancestor. This is done by combining different sets of 3' and 5' primers constructed from the Anopheles gambiae and D. melanogaster Beta 2 sequences in PCR, and using PCR to clone Beta 2 in fly species closely related to D. melanogaster: Anastrophula suspense (true fruit fly, 80MY since sharing a common ancestor with D. melanogaster, Musca domestica (housefly, 110 MYA), and Chrysops spp. (deerfly, 130MYA). We construct this tree by plotting changes in the Beta 2 sequence on a fly phylogeny with a complete tree, we can better understand the nature of amino acid changes that allowed Beta 2 to evolve while maintaining its function. We can then express the Beta 2 gene from these species in Drosophila melanogaster, to determine if they support its spermatid and thereby indicate whether this protein evolved by a narrow evolutionary pathway, or co-evolution with other sperm proteins.