Determining the Location of Tektins Within a Vital Molecular Machine

Experiments performed in the GCD lab of Dr. Richard W. Linck

Poster and project work by Michael Ries; CBS biochemistry major and minor minor

**Flagellar/Ciliary Structure**

**Familiar Structures:**
- The tail of an animal’s sperm is an easily recognized flagellum
- Non-motile cilia are found in the human eye and facilitate vision
- Cilia clear lungs and bronchioles of mucus

**The Axoneme:**
- It is the “skeleton” of a cilium/flagellum
- Composed of a cylindrical arrangement of 9 doublet microtubules [1] one doublet tubule shown below (A and B microtubules)
- Doublet microtubules are highly conserved evolutionarily, denoting their importance in nature
- A very stable ribbon of 3 protofilaments (shown below as protofilaments 1, 2, and 3 of tubule A) remains after exposure of doublet microtubules with a 0.5% Sarkosyl detergent
- The ribbon has been shown to be composed of tubulin heterodimers and tektins A, B, and C [2]

**Defying Dogma**

Currently two models exist to explain the location of tektin within the axoneme. It’s unknown which of these is correct.

**Tektin is Just Stuck In Between:**
- Known as the dynein-spoke-tektin filament model [2] shown in below and above images
- In this model protofilament 2 of the A doublet microtubule is composed of tektins, not tubulin

**Or Tektin’s Underlying Secret:**
- Prevailing view by most scientists
- Known as the partition model, in which the tektin filament is not one of the 3 protofilaments of the ribbon
- The tektin filament is instead an accessory filament that lies on the inner surfaces of protofilaments 11-13 [3]

**One Truth:**
- Current evidence supports the dynein-spoke-tektin filament model:
  - Isolated tektin filaments retain a fraction of inner dynein arms (IDA) located as depicted in the figure above [2]
- One of the protofilaments in the ribbon appears to be of less mass than a tubulin protoflament, denoting its different structure [4]
- Polyclonal tektin antibodies do not label along the surface of the ribbon but only label when the tektin filament extrudes from the center of the ribbon [5]

**Project Aims**
- To determine the location of the tektin filament within the ribbon
- To determine the location of the ribbon within the flagellum
- To determine how dynein and tubulin interact with the tektin filament
- We hoped that the results of this project would also further our confidence in the dynein-spoke-tektin filament model

**Methods**

1. **Prepare Samples: Ribbon Purification** -
   - Collect sperm from Strongylocentrotus purpuratus (sea urchins)
   - Isolate axonemes from sperm tails with Triton-X detergent solution
   - Purify doublet tubules by dialysis
   - Purify ribbons with 0.5% Sarkosyl detergent
   - The ribbon has been shown to be composed of tubulin heterodimers and tektins A, B, and C [2]

2. **Partial Digestion of Tubulin Flanking Intact Ribbon** -
   - Prepare 2 concentrations of proteases (including trypsin) to partially digest flagelkubulin
   - Incubate ribbons in protease and salt for increasing times
   - Run an SDS-PAGE gel of the results to allow for visualization of partially digested ribbons

3. **Visualize Intact Filaments By TEM** -
   - Layer copper grids with plastic membrane
   - Evaporate carbon film on top of plastic membrane
   - Prepare several samples of partially digested ribbons with negative stain
   - Take digital images of ribbons with the use of a JEOL 1000 TEM at 50,000X magnification
   - Attempt to locate intact tektin filaments with possible partially bound tubulin fragments
   - Left: A micrograph of ribbons. Tektin filaments protrude from the ribbon in the red circled areas.

4. **Identify Loosely Bound Tubulin Fragments** -
   - Utilize mass spectrometry to identify remaining loosely bound tubulin fragments left after partially digesting ribbons
   - Compare mass spectrometry results to known crystallographic structure of α and β tubulin proteins
   - Deduce whether the tektin filament binds to the sides of the tubulin heterodimers (as in the dynein-spoke-tektin filament model) or binds along the inside surface of the tubulin heterodimers (as in the partition model)
   - Consequently determine the correct model

**Conclusions**

- **Proteolytic enzymes used were chymotrypsin, trypsin, papain, and subtilisin**
- **Chymotrypsin and trypsin did not digest ribbons enough**
- **Subtilisin digested the tubulin protofilaments almost completely but also partially digested the tektin filament**
- **Papain completely digested the ribbons at the concentration we used**
- **There is no discernable difference between partially digested ribbons and normal ribbons viewed under TEM**
- **Further optimization of the proteolytic experiment is required for subsequent mass spectrometric analysis to be continued**

**Why Tektin?**

The Old Have Wisdom to Give
- Tektins have been evolutionarily conserved for 850 million years [6]
- This points out their importance in biological systems
- Tektins have proven to be an invaluable tool in the phylogenetic analysis of insect speculation [7]
- Idea's Everywhere
- Tektin coding DNA has been sequenced in mammals, fish, sea urchins, insects, and nematodes
- Tektins occur in all eukaryotic organisms able to produce flagella or cilia [8]

Tektin's Potential Role in Disease
- Tektins are vital in the formation of working cilia [2]
- Mutations in tektin in mice leads to male infertility and other ciliopathies [9]
- Polycystic kidney disease is caused by improper formation of cilia meaning tektins may have some role in this disease [10]

**Future Direction**

Other Proteases:
- Experimentation with papain at lower concentrations could cleave less tubulin
- The protease Glu-c endopeptidase is promising in that it may partially digest ribbons to the extent we require

Entirely New Avenues:
- Using a tektin or tubulin affinity column to isolate the binding domains of tubulin
- Using mass spec to identify IDA fragments that bind to tektin

**Mass Spectrometry Stoichiometry**:
- Use of mass spec to quantify the ratio between tubulin and tektin, where a ratio of 2:1 will support the dynein-spoke-tektin model

**Acknowledgments**

I would like to thank Dr. Richard Linck for offering me the opportunity to complete this UROP in his laboratory and also for his guidance during the project. I would also like to thank Christina Ouche for his patience, time, and help that he has given me throughout this entire project.

**References**