Determining the Location of Tektins Within a Vital Molecular Machine

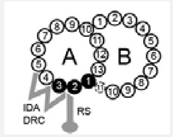
Experiments performed in the GCD lab of Dr. Richard W. Linck

Poster and project work by Michael Ries; CBS biochemistry major and chemistry 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]



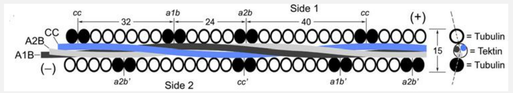
Defying Dogma

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 protofilament, 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]



Right: A depiction of the dynein-spoke-tektin filament model

Project Aims

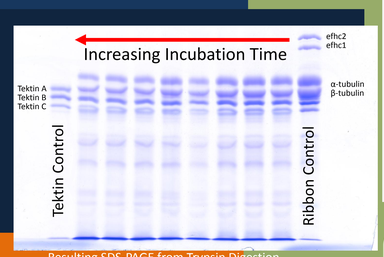
- To determine the location of the tektin filament within the ribbon
- To determine the location of the ribbon within the flagellum
- To determine how dynein and tubulin interact with the tektin filament
- We hoped that the results of this project would also further our confidence in the dynein-spoke-tektin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

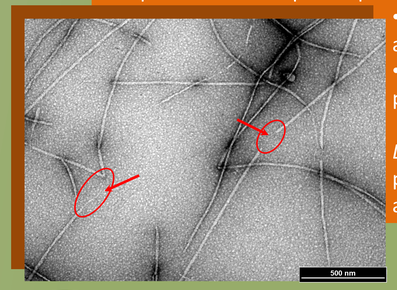
Strongylocentrotus purpuratus

2 Partial Digestion of Tubulin Flanking Intact Ribbon -
- Prepare 2 concentrations of proteases (including trypsin) to partially digest flanking tubulin
- 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



Resulting SDS-PAGE from Trypsin Digestion

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 1200 TEM at 60,000X 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

Mass Spectrometery Instrument

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 speciation [7]

It'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 Christna Ouche for his patience, time, and help that he has given me throughout this entire project.

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