Understanding Parkinson’s Disease Pathology in Yeast: A Role for Endocytosis

Maiwase Tembo

Biology Department, Lake Forest College
Advisor: Dr. Shubhik DebBurman
Road Map

• Introduction
• Hypothesis
• Aims
• Results
• Conclusions
• Future Studies

http://gailbrokaw.files.wordpress.com/2009/02/j0438492.jpg
Neurodegenerative Diseases

- Alzheimer's Disease
- Huntington's Disease
- Prion Diseases
- Lou Gehrig's Disease
- Parkinson's Disease
Parkinson’s Disease

- Hypokinetic movement disorder
- Second most common neurodegenerative disease
- 7 million worldwide
- 1 million in the US
- No known cure
Pathology

Cut section of the midbrain where a portion of the substantia nigra is visible.

Substantia nigra

Diminished substantia nigra as seen in Parkinson's disease.

Alpha Synuclein

http://catalog.nucleusinc.com/generateexhibit.php?id=13231
http://www.idsia.ch/NNcourse/brain.html
Alpha Syn imbalance

Lewy bodies

Protein Imbalance
What’s causing the imbalance?

- Degrading too little protein?
- Making too much Protein?
- Both?
Response to misfold

Protein Folding

Misfolded Protein

Re-fold

Cytoplasm & Nucleus

Plasma membrane
Extracellular

Proteasome

Lysosome
Alpha Synuclein

Extracellular fluid

Cytoplasm

Presynaptic terminals
Response to misfold

Protein Folding

Misfolded Protein

Cytoplasm & Nucleus

Re-fold

Plasma membrane
Extracellular

Proteasome

Lysosome
Lysosomal Degradation

- Phagocytosis
- Endocytosis
- Autophagy
Previous Work

**Autophagy** — Ray Choi, Alina Konnikova, Daniel Sanchez, Kayla Ahlstrand and Pete Sullivan

**Endocytosis** — Jessica Price, Mithaq Vahedi, Alexandra Ayala, Jaime Perez and Madhavi Senegolage, Rida Khan
Lysosome
Early Endosome
Late Endosome
MVB
vps 34
vps 27
vps 23
vps 28
vps 37
mvb 12
vps 22
vps 25
vps 36
vps 2
vps 20
vps 24
vps 32
vps 4
vps 60
vta 1
doa 4
Cytoplasm
Pre-ESCRT
ESCRT-I
ESCRT-II
ESCRT-III
Post-ESCRT
Endosome lumen
Hypothesis: Impaired endocytosis affects alpha-synuclein pathological properties

- Endocytosis intact
  - + endocytosis
  - Does α-synuclein localization change?
  - Does α-synuclein accumulate more?
  - Are more cells toxic?
- Endocytosis gene deletion
  - - endocytosis

A Genetic Approach
Compromising Endocytosis
Model organisms

- Yeast
- Mice
- Worms
- Flies
- Primates
Why Yeast?

- Inexpensive
- Easy to grow
- Easy to manipulate genes
- Make, fold, and degrade proteins just like humans
- Endocytosis is conserved between humans and yeast

Budding Yeast
## Endocytosis Summary

<table>
<thead>
<tr>
<th>Strains</th>
<th>Change in Localization</th>
<th>Increased Accumulation</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pre-ESCRT</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vps34</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>vps27</td>
<td>Yes</td>
<td>None</td>
<td>No</td>
</tr>
<tr>
<td><em>ESCRT-I</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vps23Δ</td>
<td>No</td>
<td>None</td>
<td>No</td>
</tr>
<tr>
<td><strong>vps28Δ</strong></td>
<td>Yes</td>
<td>Yes</td>
<td><strong>Yes</strong></td>
</tr>
<tr>
<td>vps37Δ</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>mvb12Δ</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><em>ESCRT-II</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vps22Δ</td>
<td>Yes</td>
<td>None</td>
<td>No</td>
</tr>
<tr>
<td>vps25Δ</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>vps36Δ</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><em>ESCRT-III</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vps2Δ</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>vps20Δ</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>vps24Δ</td>
<td>No</td>
<td>None</td>
<td>No</td>
</tr>
<tr>
<td>vps32Δ</td>
<td>Yes</td>
<td>None</td>
<td>No</td>
</tr>
<tr>
<td><em>Post-ESCRT</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vps4Δ</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>vps60Δ</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>doa4Δ</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>vta1Δ</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

**Key**

- Localization : 13
- Accumulation: 12
- Toxicity: 1

**vps28Δ**
Does synuclein concentration in \textit{vps28Δ} affect this toxicity?

Higher concentration will result in:
- More aggregates, more cytoplasmic
- More accumulation
- More toxicity
Aims -> Predictions

LOW CONC

vps28 WT
vps28Δ WT

HIGH CONC

vps28 WT
vps28Δ WT
Question 1: More aggregates?

http://biology.unm.edu/ccouncil/Biology_203/Images/Microscopes/microscope6.jpeg
Question 1: More aggregates?
Question 2: More accumulation?

1. Cell makes proteins
2. Break up cells and obtain proteins
3. Transfer to gel
4. Add antibody
Question 2: More accumulation?

vps28
Alpha syn Concentration

Ladder | L | H | L | H

Alpha Syn
PGK
Question 3: More toxicity?

TEST: SPOTTING

Control  Test  Control  Test

No Toxicity

Toxicity
Question 3: More toxicity?

<table>
<thead>
<tr>
<th>vps28</th>
<th>Alpha Syn</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>L</td>
</tr>
<tr>
<td></td>
<td>H</td>
</tr>
<tr>
<td>-</td>
<td>L</td>
</tr>
<tr>
<td></td>
<td>H</td>
</tr>
</tbody>
</table>

YES!
Conclusion

Alpha synuclein concentration ➤ Toxicity

• More cytoplasmic
• More accumulated More Toxic

Future Studies:
• Fission Yeast
• More repeats
Importance

Phagocytosis

Endocytosis

Autophagy

Plasma Membrane
Importance
I would like to thank:

• Dr. DebBurman

• The DebBurman lab
  - Madhavi Senagolage
  - Natalie Kukulka
  - Katrina Campbell
  - Galina Lipkin
  - Michael Herriges