Testing the Role of GSKβ and Presenilin(PSN) in Axonal Transport.

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ABSTRACT

All neurons in a human brain are constantly transporting proteins, which is vital for the survival of neurons. A person who has Alzheimer's disease develops neuronal dysfunction resulting in plaques of Aβ (Amyloid beta), synaptic lesions and neuronal apoptosis. It has been suggested that the plaques containing Aβ accumulate due to abnormalities in the cleavage of Amyloid Precursor Protein (APP) by Presenilin (PSN), which is the catalytic component of the gamma secretes complex. Previously, APP was found to function as a receptor for kinesin-1 mediated transport. Recently, both PSN and GSK-3β have been implicated in kinesin-1 transport. Using Drosophilia, as our model system we investigated how PSN and GSK-3β function in axonal transport. In Drosophila, mutations of motor proteins show axonal blocks indicating axonal transport defects. Using this phenotype we first tested over expression of PSN and found no defect in axonal transport. We next over expressed active GSKβ, inactive GSKβ, and a kinase dead form of GSKβ. While both active GSKβ and the kinase dead GSKβ did not show blockages, the inactive GSKβ showed blocks. Furthermore, we tested if GSKβ and PSN genetically interact with each other. We found that reduction of PS with active GSKβ or inactive GSKβ showed blockages. We also tested if GSKβ interacts with motor proteins. We found that both reduction of kinesin, the anterograde motor and dynein, the retrograde motor with active GSKβ show axonal blocks. Taken together our observations indicate that PSN and GSKβ regulate axonal transport and affect both kinesin and dynein motors.

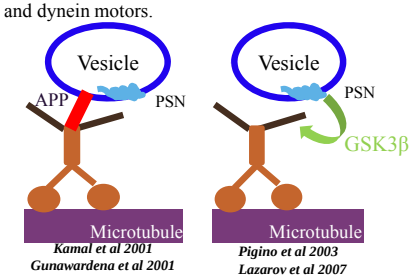

Figure 1:APP transports a subclass of vesicles that contain PSN (Kamal et al 2001). APP can function as a kinesin-1receptor during transport (Gunawardena et al 2001). PSN and GSK3β are thought to interact and regulate kinesin-based transport (Pigino et al 2003, Lazarov et al 2007).

GSKβ and PSN have a role in axonal transport.

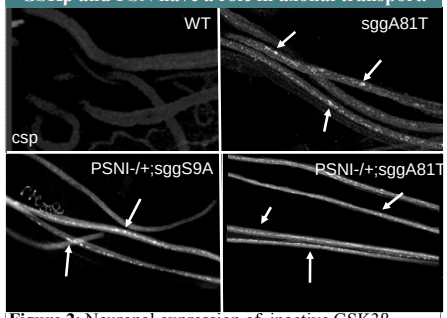

Figure 2: Neuronal expression of inactive GSK3β resulted in axonal blocks. The reduction of PSN with over-expression of active GSK3β and reduction of PSN with over expression of inactive GSK3β also resulted in axonal blocks, in contrast to heterozygous reduction of PSN and expression of active sgg alone These observations indicate that PSN and GSK3β function in axonal transport. Axonal blockages are observed using the synaptic vesicle marker CSP.

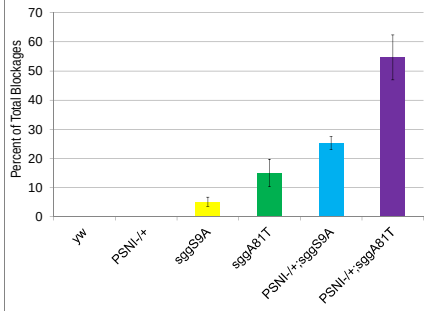

Table 1:Quantitative analysis indicates the extent of axonal blocks. Neuronal expression of inactive GSK3β shows a significant amount of blocks which are enhanced by reduction of PSN. This enhancement is much greater than what is observed with reduction of PSN with excess active GSK3β. ***N=4 larvae.***

PSN and GSKβ genetically interact with kinesin-1

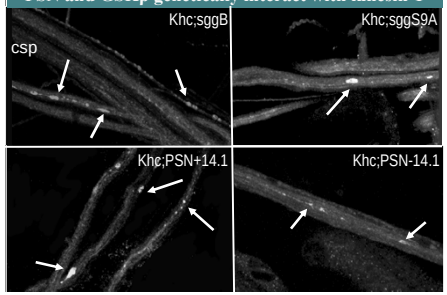

Figure 3: Genetic reduced of the kinesin with excess wild type GSK3β (sggb), active GSK3β(sggS9A), and two different isoforms of PSN resulted in blockages, indicating that GSK3β and presenilin interacts with the kinesin motor.

Quantification analysis of the extent of axonal transport defects in GSKβ and PSN larvae.

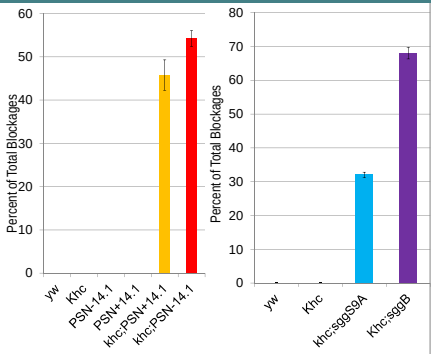

Table 2: Quantitative analysis indicates that the extent of axonal blocks in larvae expressing PSN with reduction of kinesin show a significant amount of blocks compared to larvae expressing GSK3β with reduction of PSN. These observations suggest that both PSN and GSK3β genetically interacts with kinesin and may regulate kinesin based axonal transport pathways. ***N=4 larvae.***

GSKβ Effect on NMJ development

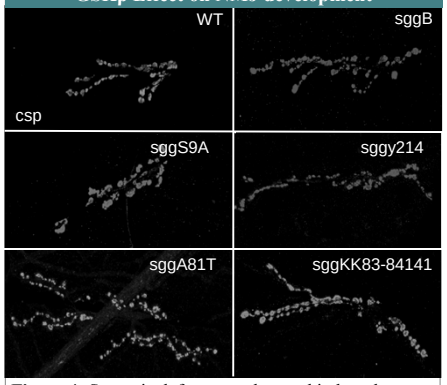

Figure 4: Synaptic defects are observed in larval synapses over expressing wild type GSK3β constitutively active GSK3β (sggS9A) ,constitutively inactive GSKβ (A81T, Y214) and a kinase dead form of GSKβ (KK83-84141) compared to wild type larvae. All larvae that are over expressing different forms of GSK3β show defects in NMJ development. NMJs were visualized using the pre synaptic markers CSP and HRP at muscle segments 6/7.

Quantification analysis of the extent of NMJ defects in GSKβ expressing larvae.

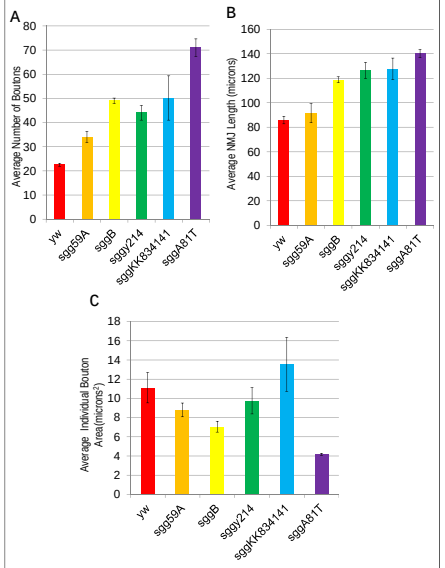

Table 3: Quantitative analysis of the extent of NMJ defects in GSK3β expressing larvae compared to wild type larvae. To evaluate NMJ morphology We compared several different parameters; the number of synaptic boutons**(A)**, the length of the synapse**(B)** and the size of single boutons**(C) .** We found that over expression of wild type, active, inactive, and kinase dead forms of GSK3β increase the number of boutons, and the NMJ length compared to wild type. However, expression of inactive sgg decreases the area of synaptic boutons compared to wild type. These observations indicate that GSK3β is essential for proper synaptic development. ***N=4 larvae.***

Conclusions

- Both PSN and GSK3β have a role in axonal transport and both affect the kinesin-1 motor protein.
- PSN and GSK3β genetically interacts with each other for axonal transport.
- GSK3β has a role in NMJ development.

Future Directions

- Does GSK3β and PSN affect the dynein motor?
- Does PSN have a role in GSK3's function in NMJ development?
- What other proteins interact with PSN and GSK3β for axonal transport and for NMJ development?

Acknowledgements

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