

Investigating Rab5 in APP-mediated axonal transport.

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Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder that causes neuronal cell death in the hippocampus which is the part of the brain involved in memory. AD is caused by protein fragments that are formed by abnormal cleavage of the amyloid precursor protein (APP). Studies have shown that the early endo-lysosomal pathway is disrupted in AD. However, the mechanism by which this occurs is still unknown. Rab5 is part of the Ras superfamily of GTPases. Rab5 is located in the plasma membrane of early endosomes and is likely required for their transport in neurons. We examined the effects of Rab5 activity on the axonal block phenotype caused by expression of full-lengths human APP (APP695) in neurons of *Drosophila* 3rd instar larvae. We determined that expression of dominant negative Rab5 greatly reduced the number of axonal blocks in APP695 larvae. Expression of active and wild-type Rab5 also reduced APP-mediated axonal blocks. This data suggests that APP genetically interacts with Rab5 and that APP mediate axonal transport requires active Rab5. This suggests that Rab5 may be a target for therapeutics aimed at alleviating the effects of AD.

Expression of human APP causes axonal blocks

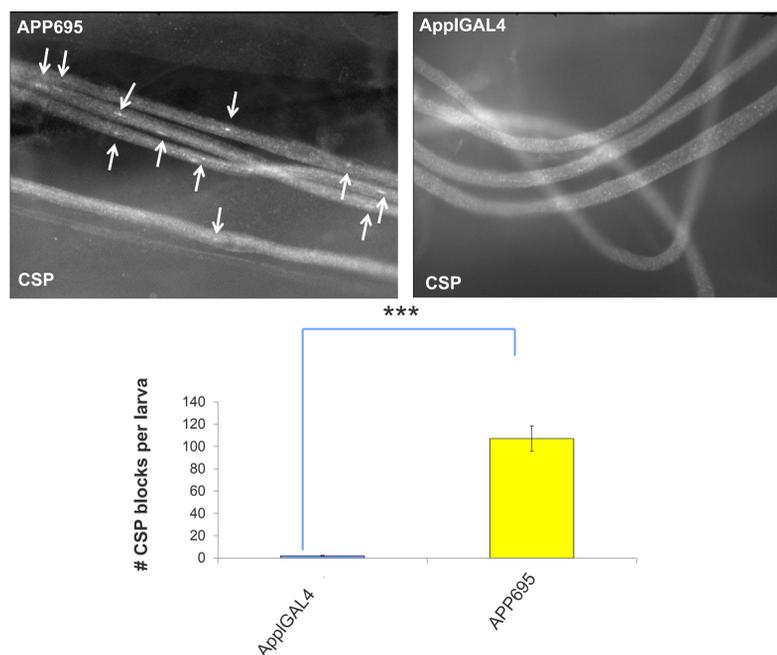


Figure 1: Larvae expressing human APP695 shows axonal blocks compared to WT larvae (AppGal4). The average amount of cysteine string protein (CSP) blocks per third instar *Drosophila* larvae shows many blocks. The average number of axonal blocks in AppGal4 was 2 and 107 in APP695. The p-value was calculated using student's t-test. The images are of axons inside the third instar larvae. The arrows in the pictures point to CSP blocks inside axons. N=5 for AppGal4, N=8 APP695, (***) $p \leq 0.0005$.

Expression of WT Rab5, constitutively active (CA) Rab5 or dominant negative (DN) Rab5 has no effect on axonal transport

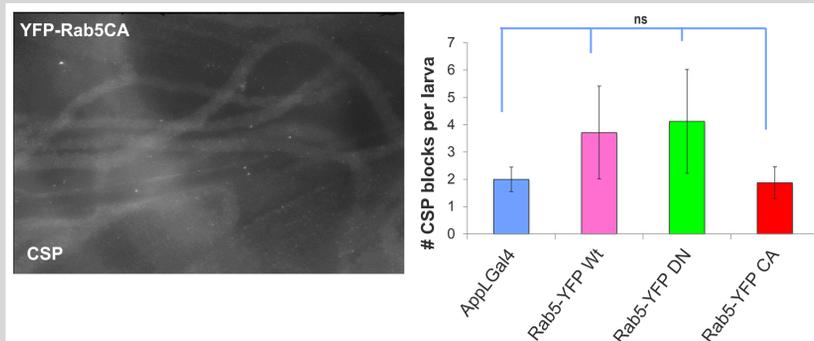
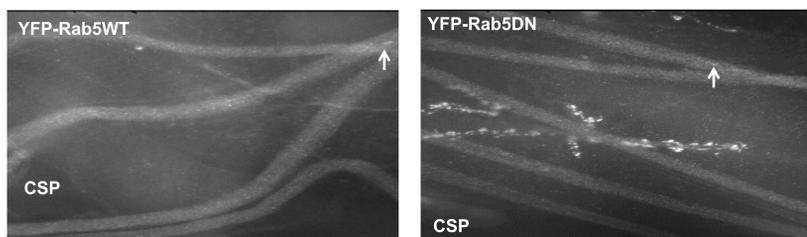


Figure 2: Expression of Rab5 has no effect on axonal transport. DN stands for dominant negative, CA means constitutively active, and WT is wild type. The graph represents the average amount of CSP blocks per third instar *Drosophila* larvae. The p-value was calculated using student's t-test. The average number of axonal blocks per third instar larvae for YFP-Rab5WT=3.7, YFP-Rab5DN=4.1, and YFP-Rab5CA=1.9 blocks. N=8 for YFP-Rab5CA, N=8 for YFP-Rab5DN, and N=5 for YFP-Rab5WT, (ns=no significance).

Expression of Rab5 rescues APP-mediated axonal transport defects

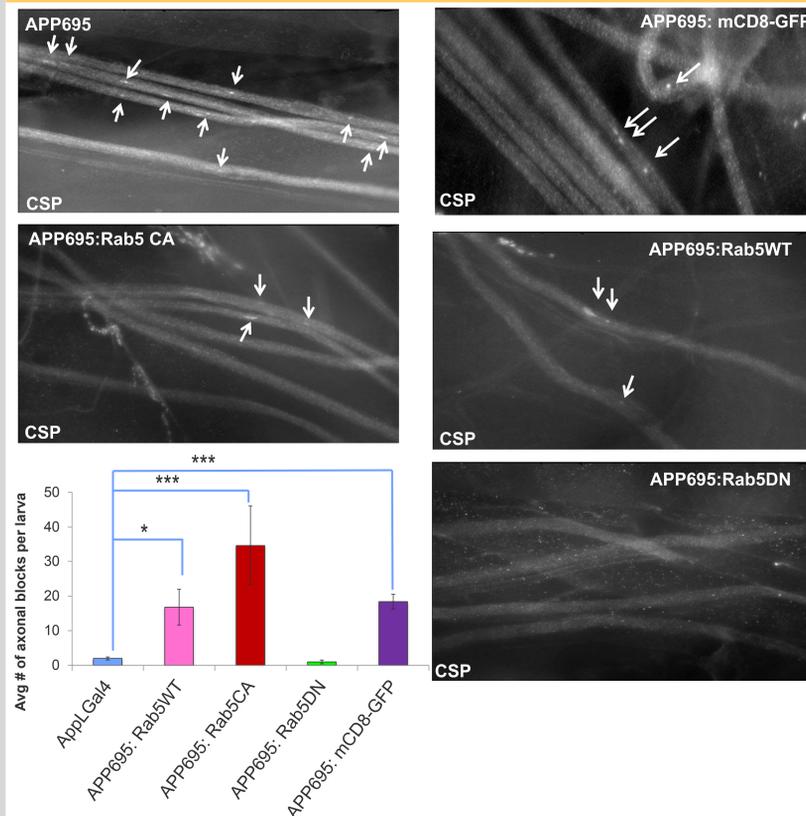


Figure 3: Expression of Rab5 rescues APP-mediated axonal blocks. The graph represents the average number of axonal blocks per third instar *Drosophila* larvae. The average number of axonal blocks per larvae was APP695:Rab5DN=2.8, APP695:Rab5WT=16.8, APP695:Rab5CA=34.6, and APP695:mCD8-GFP=18.4. The asterisks above the graph represent a significant p-value ($p \leq 0.05$). The p-value was calculated using student's t-test. The number of larvae tested for the experiment were 8 for APP695xRab5DN, 5 for APP695xRab5CA, 5 for APP695xRab5WT, and 5 for APP695:mCD8-GFP. (***) $p \leq 0.0005$.

Expression of human APP causes larval behavioral defects

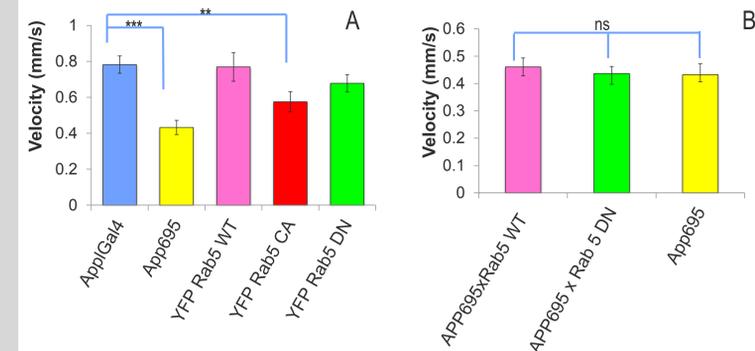
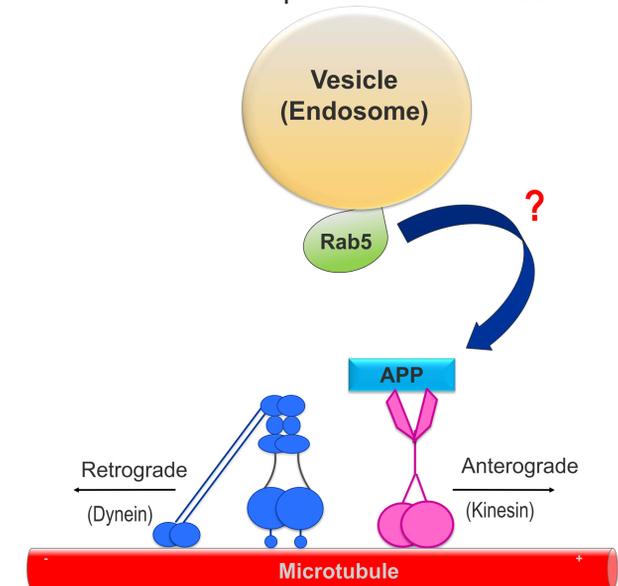


Figure 4A and 4B: Velocities of third instar larvae were quantified. Each larvae had two trials and had to crawl 50 mm in three minutes or less. Larvae expressing APP695 had the lowest average velocity of 0.43 mm/s. The p-value was calculated using student's t-test. N=10 for AppGal4, N=12 for APP695, N=12 for YFP-Rab5CA, N=12 for YFP-Rab5DN, and N=10 for YFP-Rab5WT, (ns=no significance, ** $p \leq 0.005$, *** $p \leq 0.0005$). In Figure 4B: The larvae express both APP695 and Rab5. There was no significant difference in the p-values when compared to APP695. N=10 for APP695:Rab5 WT and N=9 for APP695: Rab5 DN.

Conclusions

- Expression of APP695 causes axonal blocks in *Drosophila* larvae
- Rab5 WT, CA, and DN expression has no effect on axon transport.
- Expression of inactive Rab5 rescues APP-mediated axonal blocks in larvae expressing APP695, suggesting that excess of Rab5.DN can alleviate APP-mediated axonal transport defects.

Model of potential APP Rab5 Vesicle



Future work

- Is Rab5 part of the APP vesicle?

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