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"A Drosophila model for ALS based on TBP-43"

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurological disorder characterized by selective degeneration and death of motor neurons in the cerebral cortex, brain stem and the spinal cord. This disease, which starts during adulthood, results in progressive paralysis and is fatal within a few years, usually due to respiratory failure. A small percentage (10%) of all ALS cases are inherited (familial ALS, FALS) and have been linked to a number of genes including superoxide dismutase (SOD1), senataxin, ALsin and P150 dynactin. However, 90% of the known ALS cases are sporadic (SALS) and remain poorly understood. While animal models have been useful in the patho-physiological characterization of FALS, they have fallen short of providing insights into the predominant SALS cases, which are likely due to mutations in several, yet to be discovered genes.

As with other neurodegenerative disorders, ALS pathology includes ubiquitin positive cytoplasmic bodies, although it remains unclear if these have a toxic or protective role. Recently, TBP-43 has been reportedly found in all but SOD1-positive ALS inclusions. In addition, mutations in TBP-43 have been identified in both FALS and SALS cases. Thus TBP-43 has emerged as a common denominator for the majority of ALS cases known to date. TBP-43 is a highly conserved heterogeneous ribonucleoprotein, demonstrated to bind UG-rich sequences and regulate mRNA splicing. Proteomic analyses of ALS cytoplasmic inclusions have identified a 28 kDa TBP-43 fragment, which corresponds to the C-terminus domain of the protein and accumulates in ALS inclusions together with the full length TBP-43. Interestingly, the majority of mutations associated with ALS are also found in the C-terminal region, suggesting that this domain may be involved in the pathogenesis of ALS.

In recent years, the fruitfly *Drosophila* has emerged as a premiere model for studying human neurological and neurodegenerative disorders. The abundance of genetic and molecular tools available, together with the high degree of conservation between *Drosophila* and human disease genes have led to the discovery of novel pathways and therapeutic approaches for several human diseases including Fragile X syndrome, ataxias and RNA mediated neurodegeneration. Here we plan to fully exploit these advantages of the fly system and establish a *Drosophila* model for ALS based on both loss of function and overexpression of TBP-43 in the nervous system.

Aim 1. Determine the function of TBP-43 in the nervous system by studying the loss of function phenotypes of the *Drosophila* homologue, TBPH-2.
We will use the larval neuromuscular junction (NMJ) as a model system for determining the neuronal phenotypes due to loss of TBPH-2 during development. These experiments

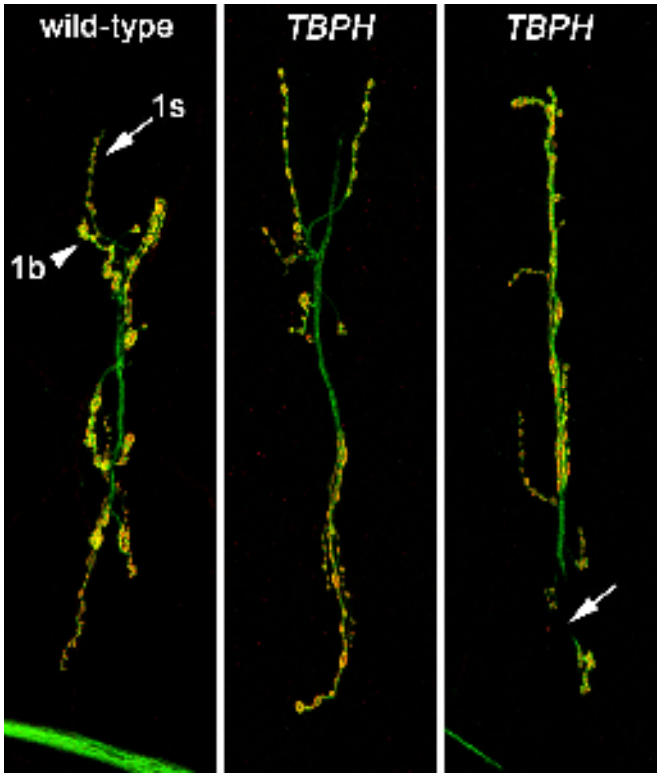
will allow us to determine if TBPH-2 is required during neural development, specifically in motor neuron function.

Aim 2. Identify novel therapeutic targets for ALS through genetic and drug screens in *Drosophila*.

We will take advantage of the power of *Drosophila* genetics to screen for dominant rescue or enhancement of the TBP-43 neurodegenerative phenotypes in the nervous system. These experiments will lead to the identification of novel genes that, when mutated, can alleviate or enhance the effects of TBP-43 in neurons. Any new gene identified in these genetic screens will be further validated by testing for genetic interactions with TBPH-2 in motor neurons. We also propose to use a collection of the 2,000 FDA approved drugs to test for modulating the TBPH phenotypes in *Drosophila*. All candidate drugs will be further tested and validated by examining their effects on motor neurons in the absence of TBP-43 function or in the presence of cytoplasmic inclusions due to TBP-43 overexpression.

In summary, this proposed effort aims to establish a *Drosophila* ALS model using both loss and gain of function paradigms. The critical feature of this model is the use of TBP-43, which has been identified as a major component in most ALS-specific cytoplasmic inclusions. Through the use of genetic screens we are well positioned to identify novel genes that cause unknown FALS as well as SALS, which represent the majority of ALS cases. In addition, our pharmacological approach is likely to uncover therapeutic agents that will target the TBP-43 containing cytoplasmic inclusions, a common denominator for most ALS cases known to date. Furthermore, since cytoplasmic inclusions are a hallmark of human neurodegenerative disorders, we may discover approaches that are applicable to diseases other than ALS.

Preliminary quantitative analyses of the TBPH mutant neuromuscular junctions (NMJs) indicate a decreased numbers of synaptic boutons when compared to wild-type (both 1b and 1s, see figure below). The arrow in the right panel points to a possible site of neurodegeneration. Additional NMJ preparations have been made and are currently being imaged and quantified.


wild-type
TBPH
TBPH
1s
1b