Transferring the *Presenilin* Gene from *Arabidopsis thaliana* to *Drosophila melanogaster*

Tara M. Kiss and Christopher J. Jones,
Department of Biology, Moravian College, Bethlehem, PA

Abstract

The presenilin gene products play major roles in development, cell signaling, and cellular transport. In humans, mutant forms of the presenilin protein have been implicated in the development of early-onset familial Alzheimer's disease. Homology among the *presenilin* genes has been discovered across many species, which indicates conservation of this gene. These homologous proteins either play or are speculated to play similar roles to presenilin in humans.

In order to understand presenilin's similarities between different organisms, the *presenilin* gene from *Arabidopsis thaliana* (thale cress) has been transferred to a newly created vector and will be inserted into *Drosophila melanogaster* embryos. The insertion of the *Arabidopsis presenilin* gene into these embryos will allow for its functional characterization in *Drosophila* by observing its phenotypic effects.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder in humans, which leads to memory loss, personality change, and dementia. Physiologically, AD is characterized by the presence of amyloid plaques and neurofibrillary tangles in the brain and central nervous systems of afflicted individuals (*1*). Four genes have been linked to familial Alzheimer's disease: *β-amyloid precursor protein* (APP), *presenilin 1* (PS1), *presenilin 2* (PS2), and *apolipoprotein E* (*ApoE*). Familial Alzheimer's disease accounts for approximately 10% of all AD cases and shows an autosomal dominant inheritance pattern (*2*). The presenilin proteins are important therapeutic targets for AD treatment because of the role they play in the production of the β-amyloid peptides (Aβ) that lead to the amyloid plaque formation seen in AD patients. The presenilin gene products also play roles in development, brain aging, vesicle transport, and are implicated in cell signaling pathways, such as the Notch and SPP (signal peptide peptidase) pathways.

Homology among the *presenilin* genes is seen across species, including humans, *Drosophila melanogaster*, *Arabidopsis thaliana* (thale cress), *Caenorhabditis elegans* (nematode), and *Mus musculus* (mouse). The regions of homology indicate evolutionary conservation of these genes across species, signaling the importance of the role(s) that presenilin plays. Human PS1 and PS2 share a 65% amino acid sequence homology overall and a ~90% homology in the trans-membrane domains of the protein (*2, 3*). PS1 and PS2 are both ~52% homologous to *Drosophila* presenilin and PS1, PS2, and *Drosophila* presenilin are all ~29% homologous to *Arabidopsis* presenilin (*4*).

In order to understand presenilin's similarities between different organisms, the *presenilin* gene from *Arabidopsis* has been isolated and a newly created vector containing the *presenilin* gene insert will be injected into wild type *Drosophila* embryos. The insertion of the *Arabidopsis presenilin* gene into these embryos will allow us to analyze the functional characterization of the presenilin protein in *Drosophila* by observing phenotypic effects on development, morphology, and behavior.

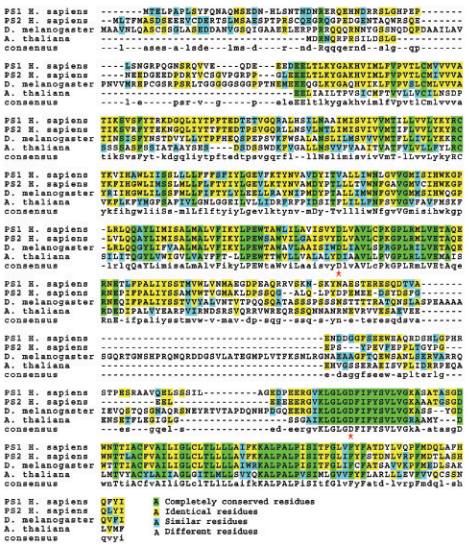


Figure 1. Amino acid multiple sequence alignment of PS1 *H. sapiens* (NP_015557, presenilin 1 isoform 1-463), PS2 *H. sapiens* (NP_000438, presenilin 2 isoform 1), *D. melanogaster* (NP_524184, G18803-PA, isoform A), and *A. thaliana* (NP_180551, presenilin family protein)

Results

Plasmid Construction

The plasmid pAdZ was created two years ago by Amrita DeZoysa, a former Moravian College student. She excised the *Arabidopsis presenilin* gene from a bacterial artificial chromosome (BAC) and ligated the gene fragment into the vector LITMUS 28i (*5*). I digested pAdZ with XbaI to excise the *presenilin* gene insert and HindIII to prevent recircularization of the remaining plasmid. The gene insert was gel purified. pP{UAST} was also digested with XbaI and dephosphorylated to prevent recircularization. The purified insert was ligated into the digested pP{UAST} using T4 DNA ligase. The ligated product was used to transform XL1blue *Escherichia coli* cells and these cells were grown up on standard agar plates supplemented with ampicillin. Bacterial colonies were selected based on ampicillin resistance and screened to determine the presence of the plasmid pAtP, which is the product of the ligation of the *presenilin* gene insert and pP{UAST}. Colonies that were selected were grown in Luria broth (LB) containing ampicillin, and plasmid DNA was isolated and digested with EcoRI. The digested DNA samples were run on an agarose gel. Two colonies were found to contain the presenilin gene insert: one contained the gene in the incorrect orientation relative to the transcription start site and the second contained the gene in the correct orientation. This latter plasmid was further digested with BamHI and XhoI to confirm the insert was in the correct orientation; this plasmid was named pAtP (See figure 4).

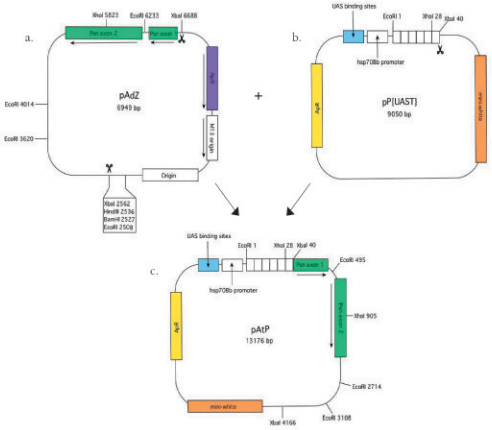


Figure 2. Overview of the plasmid construction.
a. pAdZ, b. pP{UAST}, c. pAtP

Plasmid Injection

Currently, preparations are being made for injection of pAtP into wild type *Drosophila* embryos according to the *Drosophila* germline transformation procedure (*6*). In this procedure, the prepared embryos are injected with the pAtP using a micro-manipulator, as in Figure 3. A helper plasmid called pTURBO will be co-injected with pAtP. pTURBO carries the gene for P-transposase, which is necessary for incorporation of the *Arabidopsis presenilin* gene insert into the *Drosophila* cells. The incorporation of the *presenilin* gene insert into the *Drosophila* cells is essential for further characterization of the gene's effect in the fly.

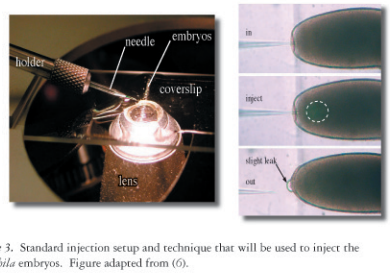
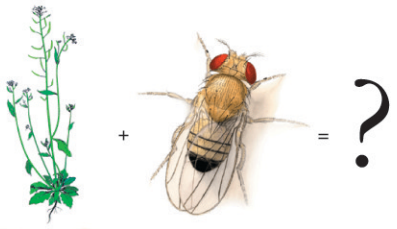


Figure 3. Standard injection setup and technique that will be used to inject the *Drosophila* embryos. Figure adapted from (*6*).

Phenotypic Characterization

After successful injection and incorporation of pAtP into the embryos, they will be continually observed throughout development. The objective is to determine if the flies are viable with the additional *Arabidopsis presenilin* gene. If the flies are viable then the next question is what phenotypic effects does gene substitution produce in the organism?



Discussion

Figure 4 shows the digested samples of pAtP DNA. Cutting pAtP with EcoRI, BamHI, and XhoI produces DNA fragments that are the approximate size expected from the predicted pAtP sequence. pAtP uncut appears to be ~9.1 kb, cut with EcoRI produces a 10.1 kb, a 2.2 kb, a 0.49 kb, and a 0.39 kb fragment, cut with BamHI produces a 7.7 kb and a 5.4 kb fragment, and cut with XhoI produces a 12.3 kb and a 0.88 kb fragment. This gel confirms that the plasmid isolated is the desired one and also that the presenilin gene insert from pAdZ is in the correct orientation for transcription.

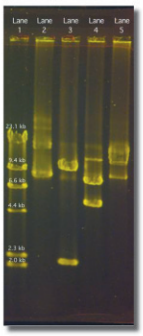


Figure 4. 0.8% agarose gel showing evidence of pAtP isolation. Lane 1 λ-HindIII standard, lane 2 pAtP uncut, lane 3 pAtP cut with EcoRI, lane 4 pAtP cut with BamHI, and lane 5 pAtP cut with XhoI.

Currently preparations are being made to start the injection phase, which will then allow for the phenotypic characterization. The injection of the *Arabidopsis presenilin* gene into wild type *Drosophila* embryos will produce flies that contain two versions of the *presenilin* gene, their own and the *Arabidopsis* version. The first question that can be answered is what effect does the second version of presenilin have on development, morphology, and behavior? If these flies are viable with both versions of the *presenilin* gene, then through various crosses the *Drosophila presenilin* gene can be removed leaving only the *Arabidopsis presenilin* gene in the flies. The second question that would then be able to be answered is what effect does substitution of the *Drosophila presenilin* gene with the *Arabidopsis presenilin* have on development, morphology, and behavior? By answering both of these questions, we will be able to further understand the divergent evolutionary connections between *Drosophila* and *Arabidopsis*.

References

1. D. I. Dominguez, B. DeStrooper, *Trends in Pharmacological Sciences* **23**, 324 (2002).
2. P. E. Fraser et al., *Biochimica et Biophysica Acta* **1502**, 1 (2000).
3. W. Xia, M.S. Wolfe, *J. of Cell Sci.* **116**, 2839 (2003).
4. ClustalW, Biology Workbench, San Diego Supercomputer Center, http://workbench.sdsc.edu.
5. A. DeZoysa, Honors Thesis, Moravian College (2003).
6. N. Gompel, *Drosophila* Germline Transformation, (2003) http://www.molbio.wisc.edu/carroll/methods/Miscellaneous/injection.pdf.

Acknowledgments

Moravian College Honors Program
Student Opportunities for Academic Research (SOAR) Program
Amrita DeZoysa (Moravian College, 2003)
Gina Cain, StudioSouth Designer

MORAVIAN COLLEGE
Biology Department, Bethlehem, PA
1742