Transformation of *Drosophila* with mutated presenilin via ΦC31 integrase-mediated cassette exchange

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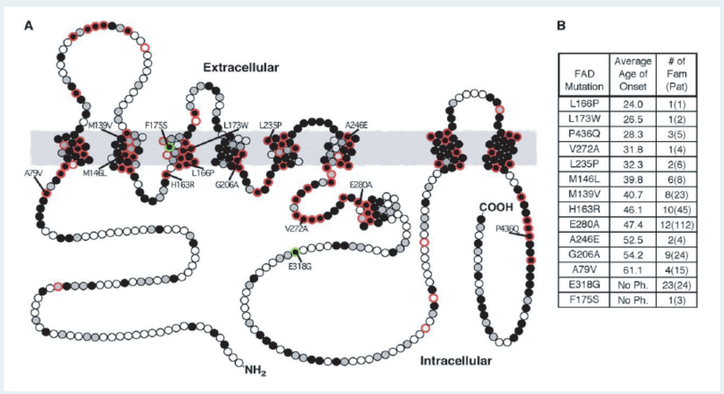
Introduction

Alzheimer's disease (AD) is the most common form of dementia in man and womenover 60. Familial AD is an early-onset form of dementia that has been linked to mutations in *PS1* and *PS2* genes. PS1 codes for presenilin protein, which acts as a catalytic subunit of gamma-secretase. Cleavage of amyloid precursor protein (APP) and Notch receptor by presenilin is essential for cell development. Missense mutations in *PS1* cause excess cleavage of APP, which results in accumilation of amyloid beta plaques in the intercellular matrix between the neurons, a hallmark of Alzheimer's disease.

Clinical heteregeneity in FAD has been observed by the age of onset ranging from 24 to 65 (Figure 1). Seidner et al. attempted to correlate the FAD age of onset to the function of presenilin using transgenic *Drosophila*. Fourteen transgenic *Drosophila* lines were created by interoducing different mutated *Psn*, a *PS1* homolog in *Drosophila*, randomly into flies with psn null genetic background. Results of the study revealed that Notch activation and morphology of transgenic *Drosophila* correlate with the FAD age of onset in humans. Upon statistical analysis, the mutations were categorized as strong, intermediate, and weak based on their effect on the organism (Seidner et al. 2006).



FAD Mutation	Average Age of Onset	# of Fam (Pat)
L166P	24.0	1(1)
L173W	26.5	1(2)
P436Q	28.3	3(5)
V272A	31.8	1(4)
L235P	32.3	2(6)
M146L	39.8	6(8)
M139V	40.7	8(23)
H163R	46.1	10(45)
E280A	47.4	12(112)
A246E	52.5	2(4)
G206A	54.2	9(24)
A79V	61.1	4(15)
E318G	No Ph.	23(24)
F175S	No Ph.	1(3)

Figure 2. Mutations in Familial AD presenilin selected for analysis by Dr. Mark Fortini (Seidner et al., 2006). A. Human presenilin protein with missence mutations that have been mimicked in transgenic *Drosophila*. B. Fourteen mutations characterized by the average age of onset and number of families and patients affected. E318G and F175S produced no phenotype (No Ph.)

Project goals

The purpose of the following research was to examine behavioral changes, if any, that are caused by mutations in *Drosophila Psn*. Specifically, the learning and memory abilities of mutant flies were to be examined using courtship conditioning. After obtaining ambiguous results from behavioral studies, likely due to position effect of random transgene insertion, the goal of the project shifted to creating transgenic *Drosophila* lines with mutated *Psn* inserted at the same exact location. Site-specific transformation could be achieved via ΦC31 integrase-mediated cassette exchange (Figure 2), recently designed by Bateman and colleagues.

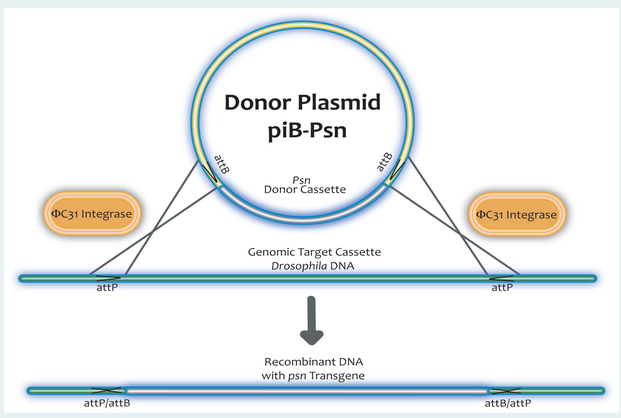


Figure 2. ΦC31 integrase initiates recombination between *attB* and *attP* sites resulting in insertion of the *Psn* cassette from the donor plasmid at the target cassette position within the *Drosophila* genome.

Results

Courtship conditioning of trangenic *Drosophila* lines created by Seider and his collegues was not successful since no learning occured in wild-type *Drosophila*. Instead, courtship rituals were studied but revealed no uniform pattern in behavioral abnormalities (Figure 3).

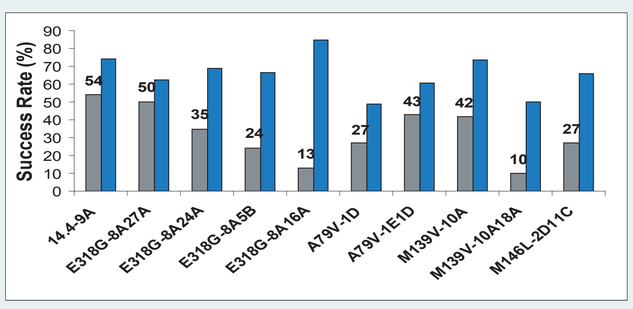


Figure 3. Copulation success. A Mann-Whitney test showed significant differences in copulation success (blue) in A79V-1D and M139V-10A18A mutants compared to flies carrying wild-type *Psn* (14.4-9A). The grey bars and the value above them represent the total number of copulations within each line.

Figure 4. LacZ staining of larval and adult brains of target site *Drosophila* strains. A. F0712.3.12 line, target site at 38F1. B. F08016.6M line, target site at 37B7 C. M0712.3.12 line, target site at 38F1.

LacZ staining was performed on target lines with integrated *lacZ* to determine which target line has the highest transgene expression level in the brain. Negative controls, *w*1118 and UAS-LacZ, showed no staining in either adult or larval brains (Figure 4). Positive control flies *elav*c155/UAS showed slight staining in adults and no visible staining in larval brains (not shown). Target line with insert at 37B7 produced the most staining (Figure 5) and will be used for future transformations.

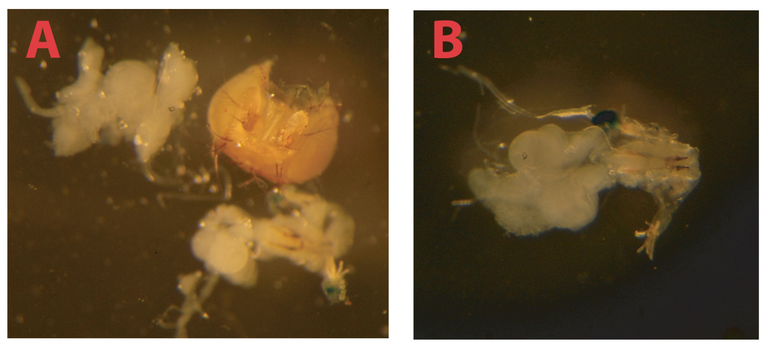


Figure 5. LacZ staining of negative controls. A. Larval and adult brain of negative control, *w*1118. B. Larval brain of UAS-LacZ.

Recombination frequencies have been improved with the introduction of an endogenous source of ΦC31 integrase, as described by Bischof et al., 2007. To make use of this technology, new fly lines carrying both target cassettes and *ΦC31* gene were created using traditional genetic crosses.

Construction of donor plasmid containing *Psn* flanked by two *attP* sites proved to be challenging due to some technical difficulties. The research is currently ongoing in attempt to resolve these problems and to ligate the piB-Psn construct.

Future Directions

Once piB-Psn donor plasmid is ligated, site-drected mutagenetis will be used to make missense mutations within the *Psn* gene. Mutated Psn will then be integrated by injecting embryos of the target line carrying endogenous source of ΦC31 with mutated piB-Psn constructs. Upon successful *Drosophila* transformation, functional copy of *Psn* will be removed from the genome using traditional genetic crosses.

Finally, learning and memory abilities will be explored and compared to those of wild-type *Drosophila*. Instead of courtship conditioning, different sensory modalities, such as vision or olfactory, may be used in the behavioral studies. Moreover, other characteristics, including morphology, longevity, and fertility may also be studied.

References

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Acknowledgements

I would like to sincerely thank my advisor Dr. Christopher Jones, who has helped me every step of the way and who continues to be a great mentor, the Student Opportunities for Academic Research and Honors Project programs at Moravian College for providing support, and Drs. Mark Fortini and Jack Bateman for their generosity and help in enabling me to conduct my research.