

Potential Parallels in Presenilin Dependant γ -Secretase Cleavage: A Research Investigation of Notch, APP, and the EGFR

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Writers comment: As a part of my English 102B class, I was required to write a research proposal on any scientific topic that interested me. I chose to write about Presenilin because I have always been interested in Alzheimer's disease and because I had recently learned about Presenilin in a genetics class. Further, I found it really interesting that there may be a link between Presenilin activity and other integral membrane proteins, including the Epidermal Growth Factor Receptor (EGFR). This potential relationship fascinated me because I currently work with the EGFR in Dr. Tzipora Goldkorn's lab, at UC Davis. This assignment gave me a lot of practice in reading scientific articles and writing a technical work: two skills which will undoubtedly help me for years to come.

—*Jill Heidinger*

Instructor's comment: Jill wrote this proposal for my English 102B: Writing in the Biological Sciences class last winter. For her previous paper in the class, she wrote a literature review on the same topic; thus, the proposal assignment provided a logical next step. The "Background" section of this proposal sums up the relevant parts of her literature review in order to orient the readers, and then Jill builds on that knowledge by proposing a further experiment to expand our knowledge of how Presenilin acts in the cell. Although this assignment may not look like a grant proposal to NIH or NSF, it incorporates the thinking processes and writing strategies necessary in proposing a new project. I particularly admire Jill's ability here to lay out a clear project and to articulate what its results ought to be and what they will mean to this area of research.

—*Jared Haynes, English Department*

Introduction

Over 90 documented mutations in the *presenilin* I gene have been linked to the most severe cases of Alzheimer's (Selkoe, 2002). Presenilin function studies have thus focused on the relationship between Presenilin and the biogenesis of a particular protein fragment A β 42, believed to be responsible for many of the pathological symptoms of the disease. Studies suggest that Presenilin's activity as a γ -secretase—a multi-protein complex responsible for cleaving other proteins—may be responsible for the cleaving of the A β 42 fragment from its precursor, the Amyloid Precursor Protein (APP). Presenilin's role in a γ -secretase complex has led to questions about Presenilin's role in γ -secretase activity not only for the Amyloid Precursor Protein but also for other integral membrane proteins, such as Notch or the Epidermal Growth Factor Receptor.

Background

Most scientists agree that Presenilin plays some sort of a role in the γ -secretase cleavage of the Amyloid Precursor Protein and Notch. So far, there have been three major hypotheses. The first was that Presenilin “tags” molecules such as APP or Notch for posttranslational modulation so that the γ -secretase can recognize it. Others suggest Presenilin plays an important role as a transport vehicle for the proteins that need to be modified. Finally, the most recently supported hypothesis suggests that Presenilin acts as a γ -secretase itself (Schwarz and Thier, 1999). While at first this last hypothesis was met with much skepticism, current research findings suggest that this hypothesis may in fact be accurate after all. Since aspartate residues are known to be important in the active sites of γ -secretase complexes, two aspartate residues in Presenilin were mutated into alanine sites. As a result of these mutations, A β 40 and A β 42 failed to be cleaved and only residual amounts of APP-derived γ -secretase substrates, C83 and C9 were observed. This information indicates that Presenilin, and the aspartate residues in particular, are necessary for A β creation. (Kimberly et. al, 2000) Also, when Presenilin was labeled with photoaffinity tagging and then treated with a γ -secretase inhibitor, only Presenilin was bound by the inhibitor (Li et. al, 2000).

Recent evidence suggests that γ cleavage of the Notch and ERB-4 integral membrane proteins may be *presenilin* dependent in a similar

manner as γ -secretase cleavage of APP (Moehlmann et. al, 2002). It is now believed that a better understanding of how Presenilin acts as a γ -secretase with Notch or other integral membrane proteins could lead to a better understanding of how Presenilin might be capable of acting as γ -secretase of APP. γ -cleavage of Notch results in two fragments, just like the cleavage of APP (Moehlmann et al, 2002). Also, both Notch and APP seem to be cleaved on their C terminal ends (Lee et al., 2002). Since these similarities suggest that Presenilin may be important for cleaving Notch as well as APP, they may also help to explain why *Drosophila* flies expressing mutant Presenilin genes express a phenotype similar to Notch mutants (Schwarz and Thier, 1999). Further research into Presenilin's role as a γ -secretase in the Notch pathway and other integral membrane protein pathways will thus be influential in gaining a better understanding of Presenilin.

Project Summary

The role of Presenilin in cleaving Notch has helped to clarify the potential downstream effects of mutations in *presenilin*. The goal of our study is to link Presenilin's γ -secretase activity to the Epidermal Growth Factor Receptor (EGFR)—another intergral membrane protein—hopefully to link Presenilin to additional signaling cascades beyond that of Notch and APP. It is logical to suggest that Presenilin may be important in γ -secretase cleavage of EGFR as well as Notch and APP because ErbB4, an integral membrane protein in the same family as the EGFR, is also thought to be cleaved by Presenilin (Lee et al., 2002). If this relationship can be established, it will demonstrate Presenilin's importance in the intramembrane cleavage in yet another integral membrane protein. Since the EGFR is involved in numerous signaling cascades, a better understanding of EGFR cleavage could lead to a better comprehension of how *presenilin* mutations are involved in the degenerative symptoms associated with Alzheimer's disease.

Methodology

Our plan is to repeat the experiment done by Struhl and Greenwald (2001) with Notch, to correlate cleavage of the EGFR with Presenilin γ -secretase activity in *Drosophila*. The cleaved portion of Notch's intracellular domain is believed to act as a transcription factor inside the nucleus. Since the intracellular region of the EGFR is thought to be

involved in similar signaling, a similar experiment is logical (Lee et al., 2002). We plan to express the entire EGFR, the transmembrane domain only, and the intracellular domain of the EGFR (under control of a heat shock promoter) in wildtype and Presenilin mutant *Drosophila* wing imaginal disks. We will perform genetic crosses of these flies using the equipment and flies available to the Genetics 160L class at UC Davis. These crosses will be tedious, because we will first have to introduce the EGFR and EGFR fragments into the homozygous *presenilin* mutant flies. Once we obtain these mutants, we can expose the fly to heat, "turning on" the EGFR in the fly's wing. We will then observe the results of these crosses on phenotype of the fly, especially on neurogenesis.

Expected Results and Possible Implications

If Presenilin is responsible for cleavage of the EGFR, then having a fully active EGFR in a mutant background should show the same phenotype as the phenotype of the mutant *Presenilin* alone. On the other hand, if Presenilin is responsible for cleaving the EGFR into its transmembrane or intracellular domain, adding these fragments back into the mutant *presenilin* fly wing should result in a less severe phenotype than the mutant *presenilin* alone. If we see no gain of function in any of these three trials, we will be able to conclude that Presenilin is not needed to cleave the EGFR.

However, if we do see a gain of function upon addition of the EGFR fragments, we will then examine the effects of these double mutants on neurogenesis. Struhl and Greenwald (2001) showed with Notch that when Notch is constitutively active (the intracellular domain only), neurogenesis is suppressed. This suggests that Presenilin activity is required for the transducing activity of transmembrane domain, but not for that of the constitutively active intracellular domain (Struhl and Greenwald, 2001). Likewise, if Presenilin is involved in transduction of the cleaved intracellular domain of the EGFR into the nucleus, we will expect to see a suppression of neurogenesis with the constitutively active intracellular domain, but not with the full EGFR or transmembrane domain by itself. While further biochemical experiments will be needed to link Presenilin's activity as a γ -secretase to that of the EGFR, this experiment has the potential to establish a connection between the hypothetical cleavage region of the EGFR to Presenilin.

Identifying whether or not Presenilin is involved in γ -secretase cleavage of the Epidermal Growth Factor Receptor has important consequences. Cancer patients seem to have a greater accumulation of the cleaved portion of the EGFR in their cells' nucleuses, suggesting that cleavage of the EGFR may be important not only for Alzheimer's but cancer as well (Lee et al., 2002). Also of interest is the fact that a balance of the EGFR pathway has recently been shown necessary to maintain survival of adult neurons (Botella et. al, 2003). Thus, a better understanding of how Presenilin interacts with the EGFR (if it interacts at all) could lead to a better understanding of Alzheimer's disease, as well as a variety of cancers.

References

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