ALZHEIMER'S DISEASE

Presenilins and Calcium Signaling—Systems Biology to the Rescue

Ilya Bezprozvanny*

Mutations in presenilins result in familial Alzheimer's disease (FAD). Presenilins encode a catalytic subunit of the γ-secretase complex, and FAD mutations in presenilins alter γ-secretase activity. Many FAD mutations in presenilins also affect intracellular calcium signaling. To explain these results, it was proposed that presenilins also function as endoplasmic reticulum (ER) calcium leak channels and that this function is disrupted by FAD mutations. Although this hypothesis has been controversial, new research supports the calcium leak channel hypothesis. One group reported the presence of putative ion-conduction pore in the high-resolution crystal structure of bacterial presenilin homolog PSH1. Another group identified an essential role for presenilins in mediating ER calcium leak in a cell-based screen for calcium homeostasis modulators. These results should enable the field to move forward and to focus on exploring connections between FAD mutations in presenilins, changes in γ-secretase and ER Ca^{2+} leak functions, and development of the disease.

Presenilin 1 (PS1, encoded by *PSEN1*) and presenilin 2 (PS2, encoded by *PSEN2*) are 50-kD proteins that contain nine transmembrane domains and reside in the endoplasmic reticulum (ER) membrane. The assembly of presenilins with nicastrin, APH-1, and PEN-2 forms the γ-secretase complex, which is transported to the cell surface and endosomes, locations where it cleaves several substrates, including the amyloid precursor protein (APP). More than 180 missense mutations identified in the *PSEN1* gene and 20 mutations in the *PSEN2* gene result in familial Alzheimer's disease (FAD). γ-Secretases generate amyloid-β (Aβ) peptide, the main constituent of the amyloid plaques in the brains of both FAD and sporadic AD patients, and most attention of the Alzheimer's disease (AD) field has been focused on studies of the γ-secretase function of presenilins. Increasing evidence suggests that presenilins also have γ-secretase–independent functions. One of these functions is related to calcium (Ca^{2+}) signaling. The connection between FAD mutations in presenilins and abnormal Ca^{2+} signaling was initially observed in studies with fibroblasts from FAD patients almost 20 years ago (*1*) and has been replicated many times in various experimental

Department of Physiology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX 75390, USA; Laboratory of Molecular Neurodegeneration, St. Petersburg State Polytechnical University, 195251 St. Petersburg, Russia.

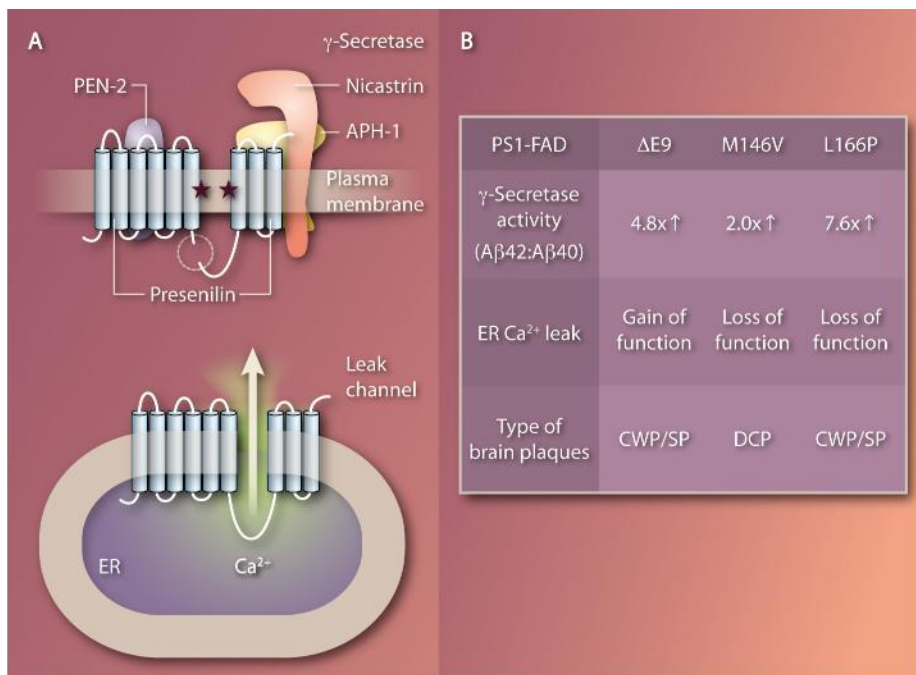*Corresponding author. E-mail: ilya.bezprozvanny@utsouthwestern.edu

systems. However, a mechanistic explanation for these findings has been lacking.

Three different models have been proposed to connect presenilins to Ca^{2+} signaling: (i) Presenilins function as passive low-conductance ER Ca^{2+} leak channels (*2, 3*), (ii) presenilins activate the inositol(1,4,5)-trisphosphate receptor (InsP$_3$R) (*4*), or (iii) presenilins enhance the activity of the ER Ca^{2+} pump (*5*). Because these three groups used similar experimental approaches and methods but came to different conclusions, these studies resulted in controversy (*6*). Considering that most of the AD field favored presenilin acting as a γ-secretase, this unresolved controversy marginalized the Ca^{2+} signaling function of presenilins.

New perspectives to this conundrum were provided in studies that used different approaches. One breakthrough came from determination of the crystal structure of archaeal presenilin homolog PSH1 (*7*), which provides the first atomic resolution information about the three-dimensional structure of presenilins. The resolution of the structure is sufficiently high to visualize a large, water-filled hole that traverses the entire protein across the lipid bilayer. The hole is surrounded by transmembrane domains 2, 3, 5, and 7 and is large enough to allow passage of small ions (*7*). Mutagenesis data from mammalian presenilins suggested that the ion-conducting pore of presenilins is lined by residues of transmembrane domain 7, but not transmembrane domain 6 (*8*), which is consistent with the structure of PSH1. Although much

additional work is needed, the water-filled cavity in the PSH1 structure is a likely candidate for the ion-conducting pore in the presenilin Ca^{2+} leak channel.

The second breakthrough came from the application of a systems biology approach (*9*). Bandara *et al.* set out to develop a quantitative model of cellular Ca^{2+} homeostasis by performing single-cell Ca^{2+} imaging studies and identifying a set of differential equations that describes major Ca^{2+} pumps and leak currents in human embryonic kidney 293 cells. They transfected 250 candidate short-interfering RNAs (siRNAs) into the cells and used the mathematical model to quantify the effects of knockdown on Ca^{2+} pump and leak rates, which resulted in the identification of proteins involved in the elusive ER Ca^{2+} leak pathway. Knocking down PS2 or ORAI2 dramatically reduced ER Ca^{2+} leak rate, and knocking down PEN-2, encoded by *PSENEN*, greatly increased ER Ca^{2+} leak rate. Knockdown of PSENEN would inhibit proteolytic processing of presenilins and thus increase the holoprotein form of the protein, which is the form of presenilins that functions in ER Ca^{2+} leak (*2*). Thus, enhanced ER Ca^{2+} leak resulting from PEN-2 knockdown most likely reflects the accumulation of the presenilin holoprotein in the ER.

These two independent lines of evidence (*7, 9*) appear to support the "ER Ca^{2+} leak channel" hypothesis (*2*). This hypothesis states that presenilins exist in two different states and play different roles in different subcellular compartments. Cleaved (or "mature") presenilin forms a γ-secretase complex together with nicastrin, APH-1, and PEN-2, which is located in endosomal compartments and at the plasma membrane (Fig. 1A). Holoprotein (or "immature") presenilin forms the ER Ca^{2+} leak channel in the ER (Fig. 1A). Which function is affected by FAD mutations in presenilins? Different mutations may affect different functions. Some mutations in presenilins, such as the PS1-ΔE9 mutation, primarily affect γ-secretase function and result in highly increased ratio of Aβ42 to Aβ40 (Fig. 1B). Other mutations, such as the PS1-M146V mutation, primarily affect ER Ca^{2+} leak function and result in ER Ca^{2+} overload (*3*) (Fig. 1B). Some mutations, such as the PS1-L166P mutation, affect both functions, which result in ER Ca^{2+} dysregulation and increase the Aβ42:Aβ40 ratio (Fig. 1B) (*3*). Clinical phenotypes of presenilin mutant families are quite het-

Fig. 1. γ-Secretase and ER Ca²⁺ leak function of presenilins—implications for Alzheimer's disease. (**A**) γ-Secretase complex is formed by cleaved presenilin, nicastrin, APH-1, and PEN-2 and located in the endosomal compartments and at the plasma membrane. The Ca²⁺ leak activity is mediated by the holoprotein form of presenilin in the ER. Stars represent catalytic aspartates mediating proteolytic activity of γ-secretase. (**B**) The effect of PS1-FAD mutations on the γ-secretase and ER Ca²⁺ leak functions of presenlin. PS1-ΔE9 mutation has a strong effect on Aβ42:Aβ40 ratio. PS1-M146V has main effect on ER Ca²⁺ leak function. PS1-L166P mutation affects both γ-secretase and ER Ca²⁺ leak functions of presenilins. GOF, gain of function; LOF, loss of function; CWP/SP, cotton wool plaques with spastic paraparesis; DCP, dense core plaques.

erogeneous (*10*), and the fact that various point mutations affect either one or both functions may help to explain this heterogeneity. Mutations that have strong effects on γ-secretase function of presenilins (such as PS1-ΔE9 and PS1-L166P) appear to segregate with cotton wool plaques and spastic paraparesis phenotype, whereas mutations that affect primarily ER Ca²⁺ leak function (such as PS1-M146V) do not (Fig. 1B) (*3*).

Obviously, additional basic and clinical studies are needed to test and develop these ideas, but the two recent publications (*7, 9*) should facilitate the exploration of how FAD mutations in presenilins change γ-secretase and ER Ca²⁺ leak functions, and contribute to the development of AD.

References and Notes

1. E. Ito, K. Oka, R. Etcheberrigaray, T. J. Nelson, D. L. McPhie, B. Tofel-Grehl, G. E. Gibson, D. L. Alkon, Internal Ca²⁺ mobilization is altered in fibroblasts from patients with Alzheimer disease. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 534–538 (1994).

2. H. Tu, O. Nelson, A. Bezprozvanny, Z. Wang, S.-F. Lee, Y.-H. Hao, L. Serneels, B. De Strooper, G. Yu, I. Bezprozvanny, Presenilins form ER Ca²⁺ leak channels, a function disrupted by familial Alzheimer's disease-linked mutations. *Cell* **126**, 981–983 (2006).

3. O. Nelson, C. Supnet, H. Liu, I. Bezprozvanny, Familial Alzheimer's disease mutations in presenilins: Effects on endoplasmic reticulum calcium homeostasis and correlation with clinical phenotypes. *JAD* **21**, 781–793 (2010).

4. K. H. Cheung, D. Shineman, M. Müller, C. Cárdenas, L. Mei, J. Yang, T. Tomita, T. Iwatsubo, V. M. Lee, J. K. Foskett, Mechanism of Ca²⁺ disruption in Alzheimer's disease by presenilin regulation of InsP3 receptor channel gating. *Neuron* **58**, 871–883 (2008).

5. K. N. Green, A. Demuro, Y. Akbari, B. D. Hitt, I. F. Smith, I. Parker, F. M. LaFerla, SERCA pump activity is physiologically regulated by presenilin and regulates amyloid beta production. *J. Cell Biol.* **181**, 1107–1116 (2008).

6. D. Shilling, D. O. Mak, D. E. Kang, J. K. Foskett, Lack of evidence for presenilins as endoplasmic reticulum Ca²⁺ leak channels. *J. Biol. Chem.* **287**, 10933–10944 (2012).

7. X. Li, S. Dang, C. Yan, X. Gong, J. Wang, Y. Shi, Structure of a presenilin family intramembrane aspartate protease. *Nature* **493**, 56–61 (2013).

8. O. Nelson, C. Supnet, A. Tolia, K. Horré, B. De Strooper, I. Bezprozvanny, Mutagenesis mapping of the presenilin 1 calcium leak conductance pore. *J. Biol. Chem.* **286**, 22339–22347 (2011).

9. S. Bandara, S. Malmersjö, T. Meyer, Regulators of calcium homeostasis identified by inference of kinetic model parameters from live single cells perturbed by siRNA. *Sci. Signal.* **6**, ra56 (2013).

10. A. J. Larner, M. Doran, Clinical phenotypic heterogeneity of Alzheimer's disease associated with mutations of the presenilin-1 gene. *J. Neurol.* **253**, 139–158 (2006).