Genetic Clues to Alzheimer's Disease

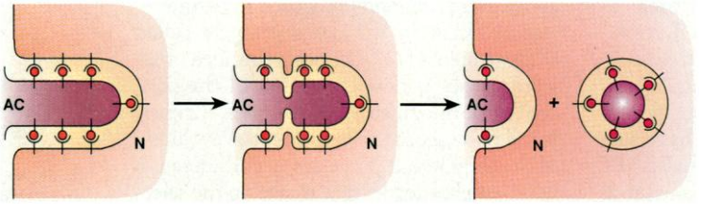
Nazneen N. Dewji and S. J. Singer

Patients with Alzheimer's disease (AD) exhibit progressive dementia and the gradual formation of extracellular neuritic plaques in the brain, particularly in the hippocampus and adjoining cortex. An important constituent of the neuritic plaques is β-amyloid, or Aβ (*1*), a set of oligopeptides of about 40 to 43 amino acids that are proteolytically derived from a much larger β-amyloid precursor protein (βAPP). This precursor, the product of a single gene, exists in several alternatively spliced forms that code for type I integral proteins of the plasma membrane, which span the membrane once (*2*). Aβ consists of about 28 amino acids of the large amino-terminal extracellular domain of βAPP plus the first 12 to 15 amino acids of its adjoining membrane-spanning region.

Genetic (autosomal dominant) forms of AD, called familial Alzheimer's disease (FAD), result in the relatively early onset of the disease. Three genes account for all the known cases of early onset FAD. The first of these genes is that for βAPP itself, located on chromosome 21, in which several point mutations within or close to the Aβ region are associated with FAD in different families (*2*). These FAD-causing mutations indicate that Aβ is somehow directly involved in the etiology of AD. Mutations in the gene for βAPP account for only a small fraction of cases of early onset FAD, however. The remaining cases of FAD are associated with mutations in two other genes: one on chromosome 14 that encodes the protein S182, a seven–transmembrane-spanning integral protein (*3*), and the other on chromosome 1, which encodes the protein STM2, another seven-transmembrane integral protein. STM2 is 67% homologous in amino acid sequence to S182 (*4*).

A plausible inference from these findings is that mutations in any of these three genes can accelerate the processes of Aβ formation and accumulation that are thought to be normally responsible for AD. Further, it is likely that these three proteins are all primarily and directly involved in those processes, although other as yet unknown components may participate.

βAPP-like proteins are found throughout the body, but the normal physiological functions of βAPP have not been definitively established. Neither do we know what purpose S182 and STM2 fullfil. Furthermore, it is unclear what mechanistic connection may exist among βAPP, S182, and STM2, the mutations of which collectively account for all known cases of early onset FAD. How is Aβ produced from βAPP physiologically, and how might S182 and STM2 participate in the process?



Cause of Alzheimer's disease? In this model, βAPP (crescents on stick) binds to S182/STM2 (red balls on stick), leading to a pinching off of a vesicle that eventually fuses with a multivesicular body. Aβ is then formed by degradation. (See text.) AC, auxiliary cell; N, neuron. Modified from figure 5A of (*6*).

Two developmental systems in which the cell-cell interactions are well defined may provide some hints: one in the *Drosophila* eye and the other in the vulva of *Caenorhabditis elegans*. Both of these systems have features that are strikingly similar to some of those in AD, and parallels may be profitably drawn from the eye and vulva to AD.

In the fully developed ommatidium of the *Drosophila* eye, R7 and R8 are neighboring neuronal photoreceptor cells that exist in a precisely arranged cell complex containing six other photoreceptors and auxiliary cells (*5*). During development, R8 is the first and R7 the last of the eight photoreceptor cells to become differentiated. R7 arises from the interaction of a pre-R7 epithelial cell with the neighboring R8 cell. Genetic analyses indicate that two genes, *sevenless* (SEV) and *bride of sevenless* (BOSS), are involved in this developmental interaction. The SEV protein, on the surface of the pre-R7 cells, is a type I integral protein tyrosine kinase, and the BOSS protein, on the surface of the R8 cell, is a seven–transmembrane-spanning integral protein. The two proteins bind to one another by means of their respective amino-terminal extracellular domains (*5*) and thus mediate the interaction of the pre-R7 and R8 cells and the subsequent signal transmission into the pre-R7 cell. Therefore, in this system, a key intercellular interaction in neuronal development is mediated by a type I integral protein on one cell binding to a seven–transmembrane-spanning integral protein on a neighboring cell.

As a consequence of the intercellular binding of the SEV and BOSS proteins in vivo, the two molecules in their entirety are incorporated into intracellular vesicles within the pre-R7, but not the R8, cell (*6*). These vesicles then fuse with multivesicular bodies (or secondary lysosomes), organelles of the pre-R7 cell that contain degradative enzymes. Once inside the multivesicular bodies, the intact SEV and BOSS proteins are likely broken down into proteolytic fragments. Similar vesicular internalization of two intercellularly interacting, cell surface proteins occurs in other developmental systems as well (*7, 8*) and may be a widespread phenomenon.

In the intercellular signaling system responsible for vulval development in *C. elegans* (*9*), one component, the protein product of the *lin-12* gene, is a type I, single–membrane-spanning integral protein. By genetic manipulation, the gene *sel-12* has been discovered, which when mutated corrects the developmental defect produced by a hypermorphic mutation in *lin-12*, suggesting that the proteins lin-12 and sel-12 interact with one another directly to produce development changes in vivo. Sel-12 shows substantial amino acid sequence homology to S182 and STM2, the seven–transmembrane-spanning integral proteins implicated in AD. These results provide another likely case of the direct binding of a type I integral membrane protein on one cell with a seven–membrane-spanning protein on another cell. In addition, the striking homology between sel-12 and S182 (and STM2) suggests that S182 or STM2 may also be directly involved in a developmentally significant intercellular interaction.

By analogy to these developmental systems, we propose the following scenario. One or more forms of the βAPP and S182 (or alternatively, the closely homologous STM2) proteins, like SEV and BOSS, may normally be components of an intercellular signaling system. In *Drosophila*, signaling requires the tyrosine kinase activity of the cytoplasmic domain of the SEV protein (*10*), but βAPP is not a tyrosine kinase; another as yet undescribed intrinsic or indirect activity of its cytoplasmic domain would have to provide the downstream signal. The βAPP protein on the surfaces of appropriate neurons and the S182 (or alternatively the STM2) proteins on the surfaces of neighboring auxiliary cells [perhaps the microglial cells discussed by Cras *et al.* (*11*)] in the hippocampus and other regions of the brain would bind

N. N. Dewji is in the School of Medicine and S. J. Singer is in the Department of Biology of the University of California at San Diego, 9500 Gilman Drive, La Jolla, CA 92093–0322, USA.

to one another specifically through their amino-terminal extracellular domains protruding from the cell membranes. This binding would mediate cell-cell adhesion (*12*, *13*) and transmit a signal into the neuron that ultimately causes certain normal responses. This normal signaling function, however, would not directly result in Aβ production. Instead, as a by-product of the intercellular interaction of βAPP and S182 (or STM2), perhaps by the process of mutual capping (*13*) of the two proteins into the membrane regions of cell-cell contact, vesicles would be pinched off the cell surfaces and incorporated into the interior of the neuronal cell (see figure). These vesicles would then fuse with multivesicular bodies inside the neuronal cell, where the βAPP would then be proteolyzed by enzymes in the multivesicular bodies, Aβ being a product of this proteolysis. The usual intracellular traffic between the lysosomal compartment and the plasma membrane would then release the Aβ from the neuronal cell, resulting ultimately in the formation of the extracellular neuritic plaques containing Aβ.

Previous proposals about the mechanisms of formation of Aβ in AD have suggested that it is produced by normal βAPP trafficking and turnover within neurons (*2*). Indeed, cultured cells expressing βAPP secrete Aβ in the conditioned media (*14*). However, a problem with these proposals is that βAPP turns over with a normal half-life of the order of one or a few hours (*15*), many orders of magnitude faster than neuritic plaque formation in AD. Furthermore, because βAPP is a ubiquitous cell surface protein of neurons, it is not clear how the selective deposition of the plaques in specific regions of the brain would occur. In addition, these proposals do not explain how S182 and STM2 contribute to the onset of AD.

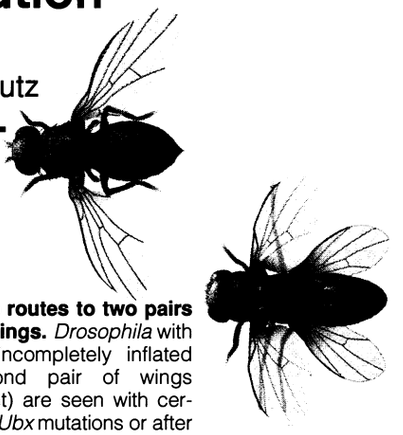
In contrast, our proposal provides direct roles for S182 and STM2 in AD, and for βAPP as a cell-cell adhesion molecule (*12*). It can explain selective production of Aβ in the hippocampus and adjoining cortex if the specific interaction between βAPP and S182 or STM2 required a particular form of regionally expressed βAPP [perhaps βAPP 695 (*16*)], S182, or STM2. The production of Aβ as a product of the cell-cell interaction system would be distinct from Aβ production by the normal turnover of βAPP on neurons and might therefore occur at a much slower rate, consonant with the usual late onset of AD. Finally, our proposal suggests new avenues of experimentation that may lead to a better understanding of the nature of AD.

References and Notes

1. G. Glenner and C. Wong, *Biochem. Biophys. Res. Commun.* **120**, 885 (1984).
2. D. J. Selkoe, *Annu. Rev. Cell Biol.* **10**, 373 (1994).
3. R. Sherrington *et al.*, *Nature* **375**, 754 (1995).
4. E. Levy-Lahad *et al.*, *Science* **269**, 970 (1995); E. I. Rogaev *et al.*, *Nature* **376**, 775 (1995).
5. H. Krämer, R. L. Cagan, S. L. Zipursky, *Nature* **352**, 207 (1991).
6. R. L. Cagan *et al.*, *Cell* **69**, 393 (1992).
7. P. J. Kooh, R. G. Fehon, M. A. T. Muskavitch, *Development* **117**, 493 (1993).
8. S. T. Henderson *et al.*, *ibid.* **120**, 2913 (1994).
9. D. Levitan and I. Greenwald, *Nature* **377**, 351 (1995).
10. M. A. Simon *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 8333 (1989).
11. P. Cras *et al.*, *Am. J. Pathol.* **137**, 241 (1990).
12. K. C. Breen *et al.*, **28**, 90 (1991).
13. S. J. Singer, *Science* **255**, 1671 (1992).
14. C. Haass *et al.*, *Nature* **359**, 322 (1992); P. Seubert *et al.*, *ibid.* **359**, 325 (1992); M. Shoji *et al.*, *Science* **258**, 126 (1992).
15. T. Oltersdorf *et al.*, *J. Biol. Chem.* **265**, 4492 (1990).
16. R. L. Neve *et al.*, *Neuron* **1**, 669 (1988).
17. This Perspective is dedicated to G. G. Glenner.

Selector Genes, Polymorphisms, and Evolution

Diethard Tautz



Two routes to two pairs of wings. *Drosophila* with an incompletely inflated second pair of wings (right) are seen with certain *Ubx* mutations or after exposing flies with a particular polymorphism in the *Ubx* gene to ether. Wild type, left.

Selector genes, master regulators of other genes, were originally proposed to define restricted areas in the developing fly called compartments (*1*). Although this idea of selector genes is now embedded in the concept of developmental hierarchies of genes, it still has a special utility when the evolution of developmental processes is considered, a point emphasized by new results on the selector gene *Ultrabithorax* (*Ubx*) from Gibson and Hogness (*2*).

Selector genes activate "realizator genes" (*1*), which eventually build the anatomical structures of the adult body. Ectopic expression of a selector gene can completely reprogram a compartment, sometimes leading to a fully developed morphological structure in the wrong place on the body (*3*). The most famous examples of this phenomenon are the *Drosophila* homeotic mutants, with legs instead of antennae or with four wings instead of two. Such mutants have sometimes been considered the perfect raw material for evolution, because single mutational events could bring about large and sudden changes and might create "hopeful monsters."

A population geneticist would instead see these animals as "hopeless monsters." Such a large morphological change is not likely to be adaptive. Although it might confer new features, it would also disrupt other adaptations and would make the individual less viable within its population. Only very strong selective advantages (which are unlikely to occur) could compensate for this. Thus, population geneticists tend to believe that changes relevant for adaptation and speciation are found in the realizator genes, not the selector genes, and that these changes generate only small steps, not large, dramatic ones. Any macroevolutionary change would have to be achieved by microevolutionary steps. Of course, over large evolutionary distances, selector genes should somehow be subject to change as well, particularly with respect to their regulatory interactions (*4*). However, a population geneticist would not normally expect to find a polymorphism with phenotypic consequences in a selector gene.

Now, Gibson and Hogness show that such a polymorphism can in fact be identified (*2*). They have analyzed a well-known phenomenon—that phenocopies of bithorax (a partial transformation of the third thoracic segment) can be produced in *Drosophila* by exposing embryos to ether vapor. Flies with a higher sensitivity to ether can be generated by repeatedly inbreeding those flies that show the strongest effects in a given generation (*5*), proving that genetic variation exists for ether sensitivity. The results of this breeding experiment had already suggested that the variation in the sensitivity to ether is likely to occur in selector, not realizator, genes, because the ether-induced bithorax phenocopies resemble known mutations in the selector gene *Ubx*. However, Gibson and Hogness have now shown that *Ubx* itself is polymorphic. They repeated the selection experiment and analyzed randomly chosen DNA polymorphisms in the region of the *Ubx* gene. They show that such polymorphisms

The author is in the Zoologisches Institut der Universität München, Luisenstrasse 14, D-80333 München, Germany. E-mail: tautz@zi.biologie.uni-muenchen.de