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Specific transcellular binding between membrane proteins crucial to Alzheimer disease

(transfected cells/cell aggregation/β-amyloid precursor protein/presenilins)

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ABSTRACT **Molecular genetic studies of families suffering from genetic forms of early onset Alzheimer disease (AD) have identified three genes and their protein products as being crucially involved in the etiology of AD. The three proteins are all integral membrane proteins. One of them is β-APP, the precursor of the β-amyloid found in the characteristic neuritic plaques present in the brains of AD patients. The other two, S182 and STM2, are homologous in amino acid sequence to one another but are unrelated to β-APP. It is shown here, using cultured cells transfected for each of these proteins, that β-APP binds specifically and transcellularly to either S182 or STM2. We propose that this transcellular binding may not only be important in normal neuronal physiology and development but may be directly involved in the process of formation of β-amyloid from β-APP.**

Important information about the molecular and cellular basis of Alzheimer disease (AD) has been obtained in recent years by the study of families having genetic forms of AD that result in the relatively early onset of the disease. All known cases of such familial AD (FAD) are accounted for by mutations in any one of only three genes. The first of these discovered was a gene on chromosome 21 that encodes the β-amyloid precursor protein (β-APP) (for review, see ref. 1). β-APP has long been thought to be involved in the disease because a set of ≈4-kDa oligopeptides called β-amyloid, clearly derived from β-APP by proteolytic cleavages, had been found (2) to be a key constituent of the neuritic plaques whose accumulation in the brains of AD patients is one of the hallmarks of the disease. However, mutations in the gene for β-APP account for only a small fraction of the cases of early onset FAD. The remaining cases are associated with the other two genes, one on chromosome 14 that encodes a protein designated S182 (3) and one on chromosome 1 that encodes a protein designated STM2 (4). All three proteins are integral membrane proteins. β-APP is actually a family of Type I proteins with large and somewhat different single N-terminal extracellular domains, the same single-membrane-spanning region, and a cytoplasmic domain (1). Members of the family are found throughout the body, but their normal physiological functions are still largely unknown. S182 and STM2 are seven-transmembrane-spanning proteins that are 67% homologous to one another in amino acid sequence (4). Little is as yet known about the normal functions of these two proteins.

These genetic findings with early onset FAD imply that all three of these proteins in their nonmutant forms are crucially involved in the normal genesis of AD and, further, that appropriate mutations in any one of these three genes can accelerate the processes of β-amyloid formation and accumulation that are widely thought to be normally responsible for AD. If this is so, then what is the connection among these three proteins? How might S182 and STM2 participate in the processes that produce β-amyloid from β-APP?

We recently proposed a mechanism that suggests answers to these questions (5). The mechanism is based on an analogy with developmentally important molecular and cellular interactions that have been studied in *Drosophila* and *Caenorhabditis elegans*. We postulated that one or more forms of β-APP can specifically bind either to S182 or to its close homologue STM2 through the extracellular domains that protrude from their respective cell membranes. It was suggested that this binding *in vivo* induces an intercellular signaling event of significance to normal neural physiology or development. It was further proposed that as a by-product of this transcellular molecular binding, processes of vesicle formation, cellular internalization, and proteolytic degradation are set in motion that result in the formation and cellular release of β-amyloid and its slow accumulation in regions of the brain (see below).

We now report the results of a first critical test of this proposal, seeking for evidence that β-APP and either S182 or STM2 can bind to one another specifically and transcellularly. To this end, we have prepared suitable cDNA constructs of the 695 isoform of β-APP (1), of S182, and of STM2. Each of these constructs was used to transfect cultured DAMI (human megakaryoblast) cells (6). Appropriate cell mixtures and controls were then prepared, their cell aggregation behaviors were observed, and double immunofluorescent microscopy of the cells was carried out to identify their surface components. These experiments have demonstrated that, as proposed (5), β-APP interacts specifically and transcellularly with either S182 or STM2.

MATERIALS AND METHODS

Cloning of S182 and STM2. Full-length S182 and STM2 cDNAs were obtained by PCR, using the following primers. S182: 5′ primer, 5′-CAGTGGTACCAATGACAGAGTTA-3′ synthesized to bases 246–260 (numbering kept identical to GenBank sequence) and including an additional *Kpn*I site (underlined); 3′ primer, 5′-GTGGATCCATATAAAATT-GATGGAATGC-3′) synthesized to bases 1629–1648 and including an additional *Bam*HI site (underlined). STM2: 5′ primer, 5′-GAGGTACCGCTATGCTCACATTCATG-3′ synthesized to bases 365–382 an including an additional *Kpn*I site (underlined); 3′ primer, 5′-GGTGGATCCCGGATGTA-GAGCTGATGGGAG-3′ synthesized to bases 1693–1711 and including an additional *Bam*HI site (underlined). Reactions were carried out in a Perkin–Elmer thermocycler for 30 cycles. Each cycle consisted of denaturation at 94°C for 45 sec, annealing at 50°C for 30 sec, and extension at 72°C for 2 min, with a hot-start at 94°C for 5 min using 0.5 ng of cDNA from whole human brain (CLONTECH) and started with 2.5 units of *Taq* polymerase. The identity of the cDNAs was confirmed

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Abbreviations: AD, Alzheimer disease; FAD, familial AD.

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by sequencing the PCR products using SEQUENASE version 2.0 according to standard protocols.

A full-length cDNA for the 695 isoform of β-APP (1) was kindly furnished by E. R. Shelton (Syntex Research, Palo Alto, CA).

Antibodies. Polyclonal anti-peptide antibodies specific for S182 or STM2 were raised in rabbits to the following peptide sequences: S182, DSHLGPHRST [residues 422–431 (3)], raised to sequences in the exoplasmic loop between transmembrane domains 6 and 7; STM2, ESPTPRSCQEGR [residues 24–35 (4)], raised to sequences in the extracellular N-terminal region. The antibodies to β-APP that were used for immunofluorescent labeling were mouse monoclonal antibodies purchased from Boehringer Mannheim (item no. 1285262).

Cell Culture and Transfections. DAMI cells were purchased from ATCC (CRL 9792) and grown in Iscove's modified Dulbecco's medium containing 10% horse serum, penicillin (100 units/ml), and streptomycin (100 units/ml). Cells were plated at a density of about 5×10^5 in 75-mm flasks and allowed to attach for 4–5 hr at 37°C in Iscove's modified Dulbecco's medium containing 10% horse serum and antibiotics. Cells were transiently transfected with 15 μg of supercoiled DNA using the lipofectamine (BRL) method. In all of the transfection and mixing experiments, success was critically dependent upon the growth state of DAMI cells. Only attached cells were used for transfections. DAMI cells generally grow loosely attached, and for these experiments, it was important to use cells that when dislodged, were in suspension as single cells, not clusters. This was generally achieved by plating cells at low densities and by replacing the culture medium every day. If allowed to overgrow or to remain in the same culture medium for longer times, the cells spontaneously detached and grew in suspension in clusters.

Cell Mixing. The transfected cell medium was replaced by fresh culture medium, and cells were detached by tapping the flask a few times. Equal numbers of β-APP-transfected DAMI cells and S182- or STM2-transfected DAMI cells (0.5×10^6/ml) were mixed gently in suspension for 30 min at room temperature. The cells were then centrifuged and, without further treatment, resuspended in 3% BSA in PBS (100 μl). An aliquot was immediately placed between a slide and coverslip and viewed under the light microscope. (Prolonged incubation of the live cells in BSA at this concentration at room temperature resulted in nonspecific aggregation of the cells.)

Immunolabeling. The mixed cells were precipitated by centrifugation at $100 \times g$ for 3 min, and the medium was removed. After washing once with PBS, cells were fixed by suspending them in 1 ml of 4% formaldehyde in PBS for 5 min. After rinsing once with PBS, the antibody labeling reactions were carried out. Cells were resuspended in PBS containing 3% BSA, and the following primary antibodies were added: mouse monoclonal antibodies (50 μg/ml) to the β-APP (at 1:100 dilution) and rabbit polyclonal antisera to either STM2 or S182 (at 1:50 dilution). These antibodies were allowed to react for 30 min at room temperature with cell-surface proteins on the fixed but not permeabilized transfected cells. After washing with PBS three times by centrifugation, the cells were resuspended in 3% BSA in PBS, and the following secondary antibodies were added: rhodamine (tetramethylrhodamine B isothiocyanate)-conjugated affinity-purified goat anti-mouse IgG (Jackson ImmunoResearch; 1.5 mg/ml, 1:100) and fluorescein (dichlorotriazinylamino fluorescein)-conjugated affinity purified donkey anti-rabbit IgG (Jackson ImmunoResearch Laboratories; 1.5 mg/ml; 1:100). Incubation was carried out at room temperature for 20 min, after which the cells were washed with PBS as described and resuspended in a mixture of 50% glycerol/culture medium.

Fluorescence Microscopy. Immunofluorescent microscopy was performed using oil immersion with a ×60 objective lens. The slides were viewed using a green fluorescent protein/tetramethylrhodamine B isothiocyanate dual filter set (Chroma Technology, Brattleboro, VT) and a Zeiss Photoscope III instrument, or with Nomarski optics. The resultant colors were then enhanced by laser scanning.

Growth and Infection of Sf9 Cells. Sf9 cells (ATCC CRL 1711) were grown in TMN-FH complete medium (PharMingen) in suspension and infected at a multiplicity of infection of 2 with a titred stock of recombinant virus. Conditioned media and/or cells were harvested 6 days after infection. Cells and debris were removed by centrifugation (at $1000 \times g$ for 15 min).

Construction of Recombinant Baculovirus for Expression of β-APP 695. Recombinant baculoviruses were produced according to published protocols (7). A fragment containing full-length β-APP 695 (kindly furnished by E. R. Shelton) was subcloned into the baculovirus transfer vector pBlueBac3 (Invitrogen). The resulting recombinant vector was cotransfected with baculovirus DNA into Sf9 cells. Recombinant β-APP expression was verified by Western blot hybridization using antibodies to β-APP.

Immunoprecipitation of Soluble Fragments of β-APP. Conditioned TMN-FH medium (10 ml) from Sf9 cells infected with recombinant baculovirus harboring full-length β-APP was taken 6 days after infection and was treated with 200 μl of a mixture of polyclonal rabbit antibodies to the extracellular domain of β-APP. This mixture included antibodies to full-length β-APP 695 and anti-peptide antibodies raised to residues 597-607, 556-576, and 52-72 of β-APP 695 (8). After 5 hr at room temperature, protein A-Sepharose was added to the mixture and allowed to bind overnight. The antigen–antibody–Sepharose complex was removed by centrifugation, and the resulting treated medium was used in the cell mixing experiments.

RESULTS

Expression of β-APP, S182, and STM2 on Untransfected and Transfected DAMI Cells. Separate batches of DAMI cells in culture were transiently transfected with full-length cDNA for β-APP, S182, or STM2 using the lipofectamine method. Transfection efficiency was 60–80%, as judged either by immunofluorescence labeling experiments (see Fig. 2) or by parallel transfection with β-galactosidase expression vector PCMV-β-gal. Detection of the expression of these proteins was carried out using specific antibodies, with whole cell extracts by Western blotting or with intact cells by immunofluorescence microscopy. The anti-peptide antibodies to S182 and STM2 each recognized only one protein band by Western blotting with a mobility corresponding to 57 kDa for the former and 55 kDa for the latter (not shown), close to the predicted values for these proteins of 53 and 50 kDa, respectively, and corresponding to the single bands we observed after the *in vitro* translation of the mRNAs for the two proteins. Small amounts of S182 and STM2 were detected in extracts of untransfected DAMI cells by Western blotting, as well as by indirect immunofluorescent labeling of intact cells. However, a large fraction of the cells transfected with either S182 or STM2 pcDNA3 constructs showed much more robust surface immunofluorescent labeling for the respective proteins (see Fig. 2*A*) than did the untransfected cells.

β-APP was not detected by indirect immunofluorescent labeling of untransfected DAMI cells (not shown), consistent with a previous report that DAMI cells produce little or no β-APP (9). Cells transfected with a pcDNA3 construct of β-APP 695, however, showed substantial cell surface immunofluorescent labeling for β-APP (see Fig. 2).

Heterotypic Aggregation of Cells Expressing β-APP with Cells Expressing S182 or STM2. To test whether β-APP can interact transcellularly with either S182 or STM2, appropriately transfected DAMI cells were mixed, and their aggregation properties were observed. DAMI cells were selected for

these studies because, as indicated above, they do not express β-APP, and although we found that they made S182 and STM2, the amounts produced by untransfected cells were too small to influence our results. DAMI cells also have the advantage of growing either in suspension or on loosely attached monolayers; in the latter case, the cells do not require trypsinization to dislodge them, but they can be freed by a few sharp taps to the flask.

Transiently transfected cells that expressed β-APP 695 (at 0.5×10^6 cells/ml) were mixed 1:1 in suspension at room temperature with transfected cells expressing either S182 or STM2, and the mixtures were sampled at 30 min or 3 hr. The samples were then examined in the light microscope (Fig. 1 *A–D* and *K–N*). (In this figure, two fields from two independent experiments are shown for each cell mixture.) As controls, transfected cells expressing either S182 (Fig. 1 *A* and *B*) or STM2 (Fig. 1 *K* and *L*) were mixed with cells transfected with just the vector pcDNA3. As seen in these panels, no significant aggregation of these cells was observed either 30 min after mixing or at 3 hr (not shown). Mixtures of S182-transfected cells with STM2-transfected cells likewise did not exhibit any aggregation (not shown). However, when cells expressing either S182 (Fig. 1 *C* and *D*) or STM2 (Fig. 1 *M* and *N*) were mixed with cells expressing β-APP 695, cell aggregates were clearly observed. At 30 min, these mixtures exhibited, along with some single cells, a significant number of cell couples together with some larger aggregates. At later times, large aggregates predominated. In addition, a few disintegrated cells were found. No significant differences in aggregation behavior were observed between cell mixtures containing S182-transfected cells as compared with STM2-transfected cells. (The remaining panels in Fig. 1 deal with aggregation-inhibition experiments described below.)

To determine the nature of the individual cells within the aggregates observed in Fig. 1 *C*, *D*, *M*, and *N*, appropriate cell mixtures and their controls were incubated for 30 min at room temperature, then fixed but left impermeable, and then double immunofluorescently labeled for β-APP (rhodamine label) and for either S182 or STM2 (fluorescein label). The immunolabeling of these impermeable cells was, therefore, confined to the cell surfaces. The cell surface labeling of these proteins was confirmed with live as well as fixed cells (not shown, but

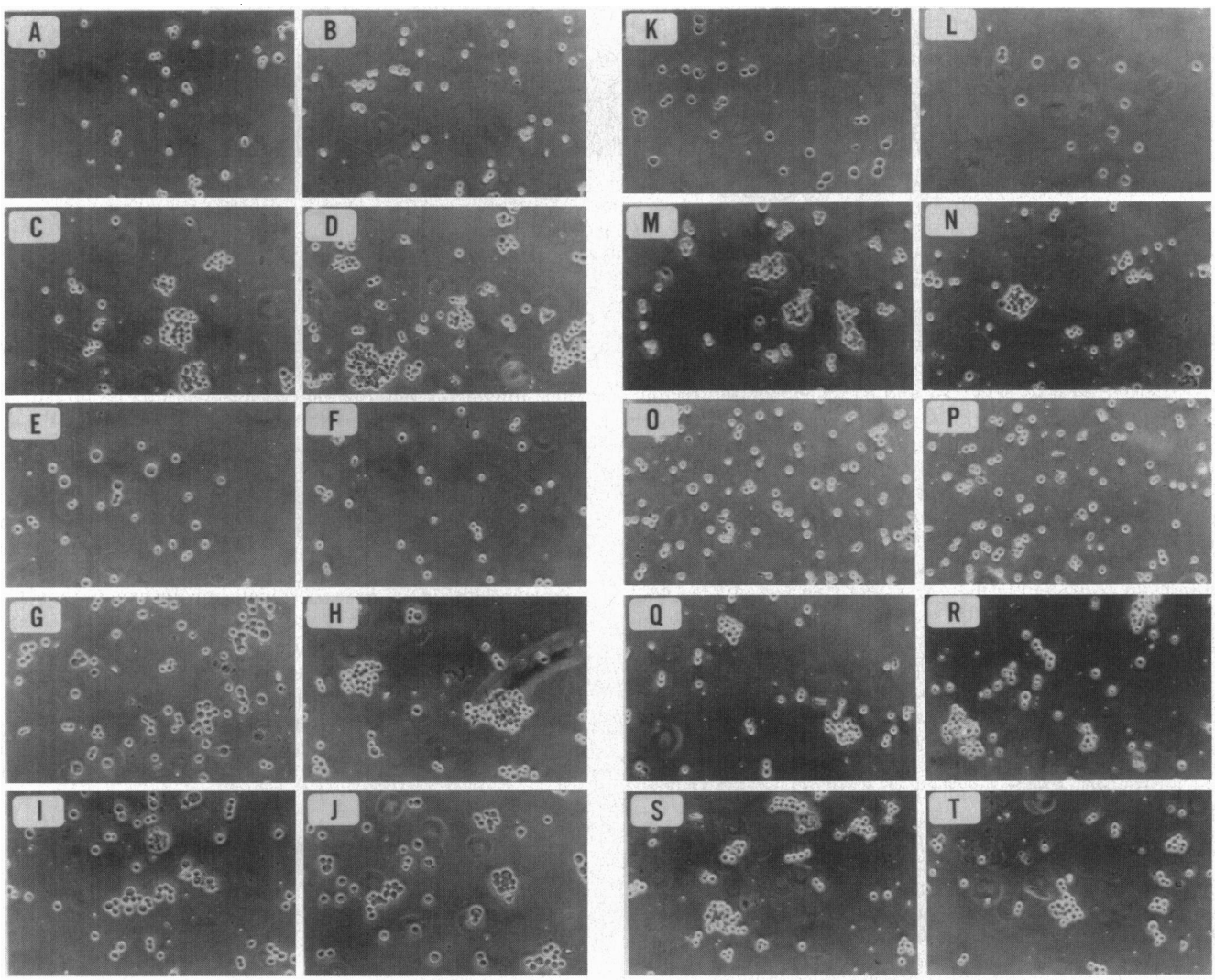

FIG. 1. Light micrographs of the aggregation of transfected DAMI cells and its inhibition by soluble fragments of β-APP. As controls, DAMI cells transfected with pcDNA3 vector alone were mixed with cells expressing S182 (*A* and *B*) or STM2 (*K* and *L*) in Iscove's modified Dulbecco's medium containing 10% horse serum. Aggregates were formed when S182-expressing (*C* and *D*) and STM2-expressing (*M* and *N*) DAMI cells were mixed with β-APP-expressing DAMI cells in Iscove's modified Dulbecco's medium or in conditioned TMN-FH medium (*I* and *J* for S182, and *T* and *S* for STM2) from uninfected Sf9 cells. The conditioned medium from Sf9 cells infected with a β-APP 695–baculovirus construct inhibits the aggregation of β-APP-expressing DAMI cells with S182-expressing (*E* and *F*) and STM2-expressing (*O* and *P*) DAMI cells. This inhibition was eliminated when soluble β-APP was first removed from this conditioned medium by immunoprecipitation, and β-APP-expressing cells were then mixed in the resulting medium with S182-expressing (*G* and *H*) and STM2-expressing (*Q* and *R*) cells.

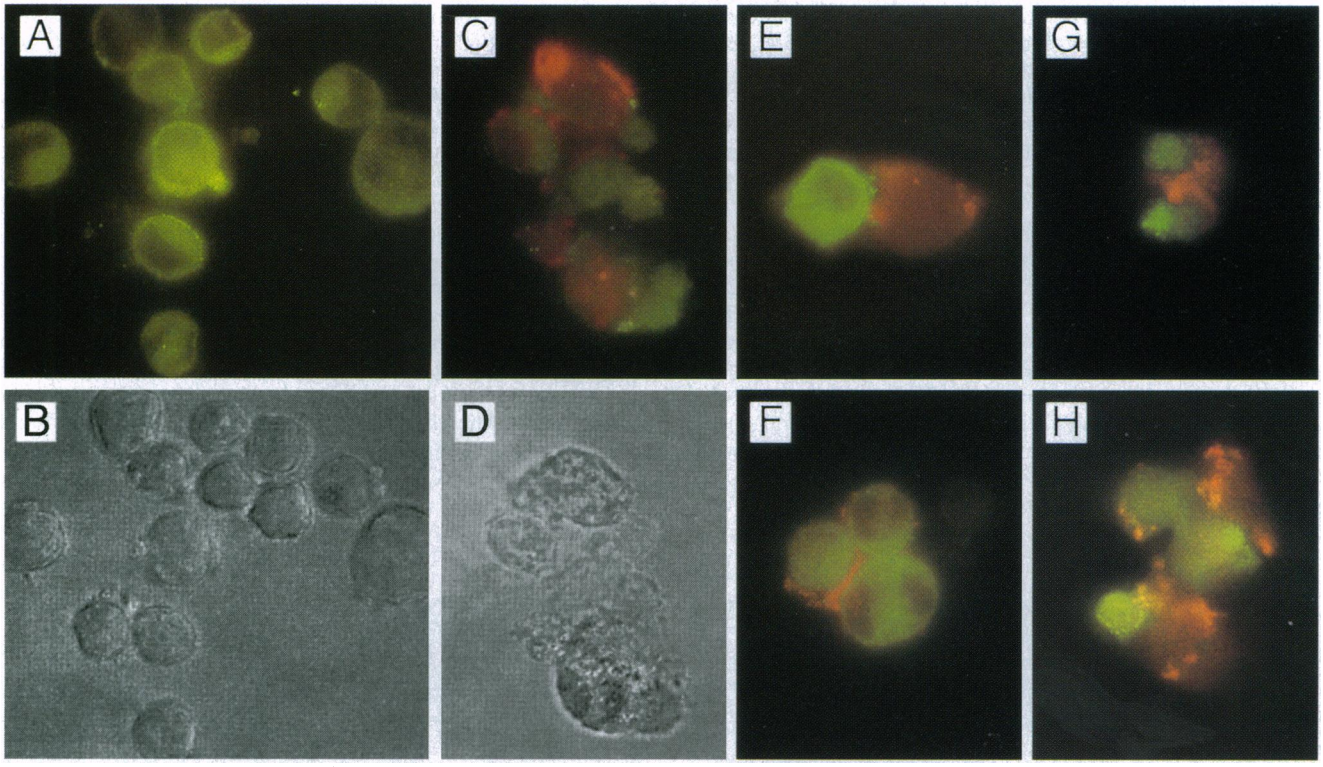

FIG. 2. Immunofluorescent detection of cell-surface β-APP, and S182 or STM2, after fixation, but without permeabilization, of cell aggregates. No aggregates were formed and no labeling for β-APP was observed when control cells transfected with vector pcDNA3 alone were mixed with equal numbers of cells expressing S182 (*A* and *B*) and were then double immunofluorescently labeled for β-APP (rhodamine label) and for S182 (fluorescein label). Red (β-APP) and green (S182) aggregates of various sizes were formed when cells transfected with S182 (*C–H*) were mixed for 30 min at room temperature with cells transfected with β-APP. *B* and *D* show Nomarski images of fields *A* and *C*, respectively.

used in Fig. 3 below). In Fig. 2, experiments with only S182-transfected cells are shown; the results with STM2-transfected cells were entirely parallel. Control mixtures of S182-transfected cells (Fig. 2 *A* and *B*) with cells transfected with the vector pcDNA3 alone, showed no significant aggregate formation nor any labeling for β-APP. Cells transfected with S182 (Fig. 2 *C–H*) mixed with cells transfected with β-APP, exhibited aggregates of various sizes, as in Fig. 1. Further, however, the immunolabeling showed that all aggregates contained some cells stained for one, and some cells stained for the other, of the two proteins. Cells that did not express either protein in these transient transfectants did not enter the cell aggregates. These results confirm that the cell aggregation observed in Fig. 1 involves the heterotypic interaction of cells expressing β-APP with cells expressing either S182 or, in parallel experiments, STM2.

Individual heterotypic cell couples among the live cell aggregates formed at 30 min (corresponding to the fixed red-green pair in Fig. 2*E*) were counted, and their numbers were compared with homotypic (green-green or red-red) pairs whose cells appeared to be in contact. As shown in Fig. 3, there were more than twice as many heterotypic couples as the sum of the two kinds of homotypic couples, providing another demonstration of the significance of the heterotypic interaction. Furthermore, among the heterotypic couples (as in Fig. 2*E*), as well as within the larger aggregates, the bound cells often showed extensive areas of contact of their adjoining cell surfaces, whereas in the homotypic couples the cells barely touched one another. Such extensive cell surface contacts are a feature to be expected of a specific transcellular molecular binding and cell adhesion process (10).

Specific Inhibition of Heterotypic Cell Aggregation by Soluble Fragments of β-APP. β-APP, as a type I integral membrane protein, would be expected to use its N-terminal extracellular domain for a transcellular interaction with the membrane proteins S182 or STM2. This extracellular domain of β-APP and its fragments, present in excess over cell-surface β-APP, might then compete for binding to S182 or STM2 and, thus, inhibit the heterotypic cell aggregation. To investigate this possibility, cell mixing experiments were carried out in the presence of long-term conditioned medium from Sf9 insect cells infected with a baculovirus construct of β-APP 695, and overexpressing β-APP 695. In such Sf9 cells, β-APP has been shown to undergo α-secretase-like cleavage from the membrane, much as with human cells (11), releasing substantial amounts of the extracellular domain of β-APP and its fragments into the medium. Accordingly, mixing experiments like those of Fig. 1 *A–D* and *K–N*, of either S182- or STM2-transfected DAMI cells with equal numbers of β-APP-transfected DAMI cells, were carried out in the presence of conditioned medium from a culture of Sf9 cells 6 days after the baculovirus infection, and overexpressing β-APP 695. No significant cell aggregation occurred (Fig. 1 *E* and *F* compared

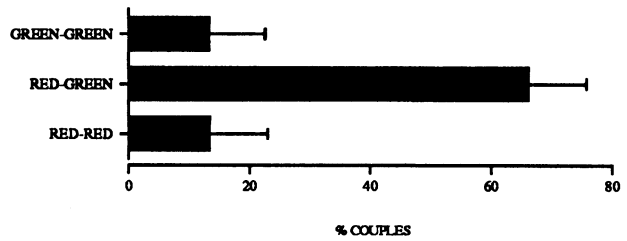

FIG. 3. Quantitative analysis of heterotypic versus homotypic cell couples formed after mixing DAMI cells transfected with S182 and β-APP 695. DAMI cells were transfected, mixed, and double immunolabeled live without prior fixation. Individual cell couples of red (β-APP) and green (S182), or green-green or red-red cells (as in Fig. 2) were counted from three independent samples of 80 or more couples.

with *C* and *D*, for S182 cells, and Fig. 1 *O* and *P* compared with *M* and *N*, for STM2 cells). As a control, conditioned medium from cultures of uninfected Sf9 cells did not inhibit the cell aggregation (Fig. 1 *I* and *J* for S182 cells, and *S* and *T* for STM2 cells). Furthermore, if the conditioned medium from β-APP-baculovirus-infected Sf9 cells were first treated with specific anti-β-APP antibodies to remove the solubilized β-APP products, and the residual medium was then used in comparable cell mixing experiments, the inhibition of cell aggregation observed with the whole conditioned medium (Fig. 1 *E* and *F*, and *O* and *P*) was eliminated (Fig. 1 *G* and *H*, and *Q* and *R*, respectively). These experiments demonstrate that the aggregation of β-APP-transfected DAMI cells with either S182- or STM2-transfected DAMI cells is mediated by the extracellular domain of the β-APP membrane protein.

DISCUSSION

We have shown that appropriately transfected cultured cells bear β-APP, S182, or STM2 on their surfaces, as demonstrated by the labeling of live cells as well as of fixed but impermeable cells. This is in contrast to results obtained with other types of cells transfected with S182 and STM2 (12), in which expression was reported to be confined intracellularly. Our results further show that β-APP-expressing cells bind to, and aggregate with, transfected cells expressing either S182 or STM2 exposed on their surfaces. This transcellular binding is specific for the transfected proteins; soluble fragments of β-APP, released into the culture medium by proteolysis of β-APP from β-APP-transfected cells, and identified by specific antibodies, when added to the transfected cell mixtures, inhibit the binding and cell aggregation. We believe that these findings provide the key to understanding the mechanism by which these three proteins, β-APP, S182, and STM2, identified genetically as the primary agents involved in early onset FAD, might together play essential roles in the genesis of AD.

The formation and slow accumulation of β-amyloid in neuritic plaques in the hippocampus and adjoining regions of the brain is widely believed to be in some way responsible for AD. This is strongly supported by the fact that several mutational changes close to or within the β-amyloid portion of the amino acid sequence of β-APP result in early onset FAD (1). In our original paper (5), we proposed that the formation of β-amyloid from β-APP was a by-product of the postulated transcellular binding of β-APP on a neuronal cell with either S182 or STM2 expressed on some auxiliary cell in the brain. This was proposed by analogy to the mechanisms that had been shown to operate in the interaction of a pre-R7 cell with an R8 cell in the development of the *Drosophila* eye (13). In this system, the Type I single transmembrane spanning SEV protein on the pre-R7 cell surface binds specifically to the seven-transmembrane-spanning BOSS protein on the R8 cell surface (a binding similar to that of β-APP to either S182 or STM2). Following this transcellular binding, the SEV and BOSS proteins in their entirety are incorporated into intracellular vesicles within the pre-R7, but not the R8, cell (14). These vesicles then fuse with multivesicular bodies (or secondary lysosomes), organelles of the pre-R7 cells that contain a variety of degradative enzymes. Once inside the multivesicular bodies, the intact SEV and BOSS proteins are likely broken down into proteolytic fragments. We, therefore, suggested by analogy that as a by-product of the transcellular interaction of one or more isoforms of β-APP on the surfaces of appropriate neurons in the hippocampus and adjoining cortex of the brain, with S182 (or alternatively, STM2) on the surfaces of neighboring auxiliary cells, vesicles would be pinched off the cell surfaces and incorporated into the interior of the neuronal cell. These vesicles would then fuse with multivesicular bodies inside the neuronal cell, where the β-APP would be broken down by proteases resident in the multivesicular bodies, β-amyloid being one product of this proteolysis. The usual intracellular traffic between the lysosomal compartment and the plasma membrane would then gradually release the β-amyloid from the neuronal cell, where it might slowly accumulate within extracellular neuritic plaques. We have, however, as yet no direct evidence to support this proposal that β-amyloid is a by-product of the transcellular interaction of β-APP with either S182 or STM2. Further study of the intracellular events following the β-APP:S182 and β-APP:STM2 transcellular binding is needed to investigate this possibility.

If indeed β-amyloid formation is only a by-product of the transcellular binding of β-APP with either S182 or STM2, what might be the normal physiological role of these interactions? A clue may be provided by studies of a β-APP-like gene (*appl*) present in *Drosophila* (15). The protein product (APPL) of this gene is homologous to human β-APP, but the two are not alike in the β-amyloid region of the amino acid sequence. The APPL protein is expressed exclusively in differentiated neurons in the fly. Generation of *Drosophila* deletion mutants lacking *appl* (*appl*$^-$) produces viable flies which, however, show distinct behavioral abnormalities. These abnormalities can be rescued by transgenes expressing the wild-type but not mutant APPL protein (16). Of special interest is the fact that *appl*$^-$ flies can also be rescued by a transgene encoding a normal isoform of the human β-APP protein, suggesting a conserved function of these fly and human protein homologues in their respective nervous systems (16). The fact that the β-amyloid region of *Drosophila* APPL is not closely conserved in the human β-APP suggests that this region may not be important for the normal physiological functions of β-APP, which accords with the suggestion that the formation of β-amyloid is only a by-product of those normal functions in the human. Whether there are *Drosophila* genes encoding homologues of S182 or STM2 is not yet known.

As already mentioned, the SEV:BOSS transcellular binding involved in *Drosphila* eye development shows strong similarities to the β-APP:S182 and β-APP:STM2 interactions. The SEV:BOSS interaction transmits a developmentally important signal from the R8 cell into the pre-R7 cell by activating the latent tyrosine kinase activity in the cytoplasmic domain of the SEV protein (17). β-APP does not have a tyrosine kinase domain, and if the β-APP:S182 or β-APP:STM2 interaction causes an analogous signal to be transmitted into the neuron which expresses β-APP on its surface, then some other direct or indirect enzyme activity of the cytoplasmic domain of β-APP would be involved. Further support for the proposal that the β-APP:S182 or β-APP:STM2 transcellular interaction may be important to normal human development is provided by a related system in *C. elegans*. In this organism, the Type I single-membrane-spanning protein product of the *lin* gene and the seven-membrane-spanning protein product of the *sel-12* gene are genetically interactive components that are critical to vulval development (18). Of special interest is the fact that the SEL-12 protein is homologous in amino acid sequence to S182 and STM2, suggesting that the worm and human proteins may be related functionally.

Finally, a few words about nomenclature. The proteins S182 and STM2 have been called presenilins (19). In view of our results, however, a more meaningful designation for these proteins is the β-APP receptor (β-APPR), with S182 and STM2 referred to as β-APPR1 and β-APPR2, respectively.

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