A New Functional Screening System for Identification of Regulators for the Generation of Amyloid β-Protein*

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Presenilin (PS) is essential for γ-cleavage, which is required for the generation of amyloid β-protein (Aβ) from the β-amyloid precursor protein. However, it remains to be clarified how γ-cleavage is regulated. To elucidate the regulation of PS-mediated γ-cleavage, we developed a new functional screening method for identifying cDNA that enhances γ-cleavage. This screening system utilizes our own developed cell line, where the expression of cDNA that enhances γ-cleavage confers puromycin resistance. The cDNA library is retrovirally delivered to the above-mentioned cell line, allowing the identification of our target cDNAs by a combination of puromycin resistance selection and Aβ assay screening. With this screening method, we isolated several cDNAs enhancing γ-cleavage, including the previously reported Herp. Here we also demonstrate that Rab1A, identified with this screening, can be a regulator of Aβ generation. Thus, our established screening method is a powerful tool for identifying multiple regulators involved in γ-cleavage in the Aβ generation pathway, including modulators of γ-secretase activity or the intracellular trafficking of factors necessary for γ-cleavage.

Aβ,1 which is the major component of senile plaques in the brain of patients with Alzheimer’s disease (AD), is generated from APP through its sequential proteolytic cleavage catalyzed by β- and γ-secretase (reviewed in Ref. 1). Although β-secretase was identified as a membrane-tethered aspartyl protease (2), the molecule responsible for γ-secretase activity remains to be clarified. Mutations in the presenilin (PS) genes, PS1 and PS2, cause early-onset familial AD (reviewed in Ref. 1). Accumulating evidence showed that PS is required for the proteolytic cleavage catalyzed by γ-secretase, which occurs in the transmembrane domain of APP (γ-cleavage) (Refs. 3–5; reviewed in Ref. 6). Interestingly, although the γ-cleavage is a critical step toward Aβ production, the major intramembranous cleavage site of APP is distinct from the γ-cleavage (named as ε-cleavage site) (7, 8). In addition, recent studies revealed that PS mediates several intramembranous cleavages including those of APP, Notch (3–5), ErbB4 (9, 10), and E-cadherin (11), indicating that the PS-mediated intramembranous cleavage plays a critical role in biological functions. The PS complex appears to be responsible for inducing γ-secretase activity (12, 13); however, it is still controversial whether PS itself is γ-secretase (Refs. 14–16, reviewed in Ref. 17). The discrepancy between the intracellular major distribution of the PS complex and the intracellular site of γ-cleavage also remains to be clarified (reviewed in Ref. 17). Therefore, the understanding of the mechanism underlying γ-cleavage will require clarification of multiple factors involved in γ-cleavage, including the components of the γ-secretase complex and modulators of the γ-secretase activity or the trafficking of factors necessary for γ-cleavage. To elucidate how PS-mediated γ-cleavage is regulated, we developed a new functional screening method for identifying cDNA that enhances γ-cleavage using a combination of puromycin resistance assay and Aβ quantitation. To date, a number of PS-interacting proteins have been identified by the yeast two-hybrid screening method that utilizes the binding affinity with PS; however, no natural interactors with PS have been found to modulate Aβ generation except for nicastrin (13, 18). Therefore, we employed the functional screening assay to measure the Aβ level, instead of the binding assay of PS. In addition, prior to the screening step using the Aβ assay, we selected cDNAs that confer puromycin resistance on the cells resulting from an increase in the degree of intramembranous proteolytic cleavage. This selection step facilitates the identification of our target cDNAs. Thus, the screening system that we developed is a powerful tool for identifying multiple factors involved in γ-cleavage, including modulators of γ-secretase activity or the trafficking of factors necessary for γ-cleavage. Previously, we reported that the ER stress-inducible protein, Herp (19), which was identified by this method, increased Aβ generation (20). Here we present the details of our newly developed functional screening method for the identification of γ-cleavage-enhancing factors, and also demonstrate that Rab1A, which is implicated in protein trafficking from the ER to the Golgi apparatus (reviewed in Ref. 21), can be another regulator of γ-cleavage in the Aβ generation pathway.

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†† The abbreviations used are: Aβ, amyloid β-protein; PS, presenilin; AD, Alzheimer’s disease; APP, β-amyloid precursor protein; sAPP, soluble APP; ER, endoplasmic reticulum; ELISA, enzyme-linked immunosorbent assay.
Identification of Regulators of γ-Secretase Cleavage

EXPERIMENTAL PROCEDURES

Antibodies and Cell Lines—The monoclonal antibody 6E10 specific to human Aβ1–17 was purchased from Senetek (St. Louis, MO). The other Aβ antibodies were all characterized previously (22). The antibody against Rab1A was purchased from Santa Cruz Biotechnology, Inc. The anti-APP N-terminal antibody was purchased from Sigma. The affinity-purified rabbit antibody, B12/4, was raised against 20 C-terminal amino acid residues of APP695 (23). Ba/F3 cells of the murine pro-B cell line were maintained as previously described (24). F(ab)2-knockout and wild-type murine fibroblasts immortalized with large T antigen were maintained as previously described (20).

Plasmids—By the PCR method, we generated a chimeric cDNA encoding C53NICD, which consists of the first Met plus Aβ9–42 fragment of APP695 and the NICD (from Ser644 to the C terminus) of mouse Notch-1 (25). The primer sequences used for the PCR are as follows: primers for C53, 5′-ATCTGGTACCCTCACCAGTGAGGATGAGAATTCCGACATGAC-3′ and 5′-GCTTCTAGACAGACATCAACAAAGGTAGTGACGAT-3′; primers for NICD, 5′-CTGCTTAGGAAGCGCCTGGCCG-CAGCTATGGGAGGGCCGGCCTAGT-3′ and 5′-ATGGTTCACCCTGGCCGCGGCGCCCAGT-3′. This chimeric cDNA was inserted into pcxN (26) (designated as pCxN-C53NICD). The resulting C53NICD does not contain a signal peptide similar to C100 construct without a signal peptide, which has been well characterized (27–31). A plasmid named pHESE1-pac, which carries a puromycin resistance gene (pac), driven by the HES-1 promoter (25, 32), was constructed as follows. (i) The HES-1 promoter isolated by the PCR method (primer sequences: 5′-GGGGTACCCCTCAG-GGCGCGCGCCATTGGCC-3′ and 5′-AGAGAAGAAGCTTCCTTGTTTACATCAAGCTGTATCCATG-3′) from the mouse genomic DNA was inserted into KpnI and HindIII sites of a vector, PGV-B (TOYO B-Net Co., Ltd., Japan). The resulting plasmid was designated as PGV-B-HES-1, which carries the luciferase-coding fragment under the control of the HES-1 promoter. (ii) A HindIII-BamHI luciferase-coding fragment in PGV-B-HES-1 was replaced with a HindIII-BamHI fragment of pPUR (CLONTECH) containing pac (designated as pHESE1-pac). pMX-Rab1A is a 1.4 kb BamHI-SalI fragment carrying the Rab1A-coding region inserted into the BamHI and SalI sites of a retrovirus vector, pMX (24). pMX-APP695 is APP695-coding region inserted into the HindIII-Nool sites of pMX. pcDNA-C100 is pcDNA-3 (Invitrogen) carrying a HindIII-Nool fragment encoding the first Met plus C99 as previously described (30). cDNA encoding APP695 lacking the cytoplasmic tail (APPΔC) (33), which is truncated at Trp623 of APP695 (it contains the four membrane-flanking amino acid residues, KKQK), was inserted into the HindIII-Nool sites of pMX (designated as pMX-APPΔC). pMX-APPΔC was made by replacing the EcoRI-Nool fragment of pMX-APP695 with an EcoRI-Nool fragment of the PCR fragment generated from pMX-APP695 using the following primers: 5′-TGCAGA-ATCATCATGACATGACG-3′ as a sense primer, and 5′-TATAGCGGCCCTACTGTCTTGTTTACATCAAGCTGTATCCAG-3′ as an antisense primer. All resulting constructs were verified by DNA sequence.

Constructions of Human Hippocampus-derived cDNA Library—Human hippocampus cDNAs were synthesized from human hippocampus mRNA (CLONTECH) using the SuperScriptTM Choice system (Invitrogen) according to the instructions from the manufacturer. The cDNAs were inserted into the BstXI site of pMX, using the BstXI adaptor (Invitrogen). The resulting cDNA library contains 4 x 10⁶ independent colonies, and the average size of the insert DNA is 1.6 kb.

Retrovirus-mediated Gene Expression and Functional Screening Procedure—Retrovirus-mediated gene expression in cells was carried out as previously reported (24, 34). Briefly, we transfected cDNA library or pMX carrying the identified cDNA into Phoenix-Eco cells. The viral supernatant obtained after 24 h of culture at 48 h after transfection was used to infect cells. For the screening, we used Ba/F3 cells (24, 36) stably transfected with pcxN-C53NICD and pHESE1-pac (designated as A or A5-9) or A9-5 cells stably expressing human PS1 (designated as A5-9-PS1) as the target cells. The target cells (4 x 10⁶) were retrovirally expressed with human hippocampus cDNA library and grown on 96-well plates (the initial cell number and density were 10⁵ cells/well and 10⁵ cells/ml, respectively). At 48 h after retroviral infection, the cells were subjected to puromycin resistance selection using the minimum lethal dose of puromycin (9 μg/ml puromycin for A5-9-PS1 cells; 20 μg/ml puromycin for A5-9 cells). The functional screening procedure was described in the text. The cDNAs transduced into the cells were extracted and amplified by PCR using vector primers (primer sequences: 5′-GGTGGAACATCATCTGACTGCT-3′ and 5′-GTTTACTTGACGATCTCCTTGC-3′) and sequenced.

Detection of Aβ and Other Immunoblotting Techniques—The secreted Aβ was immunoprecipitated with 6E10 and detected using a highly sensitive immunoblotting technique with BA27 (for Aβ40) or BC05 (for Aβ42) as previously described (30, 35). ELISA for Aβ was performed as previously described (22). ELISA data were statistically analyzed by analysis of variance using StatView-J.4.11. Intracellular C99 was immunodetected as previously described (30).

Luciferase Assay—The cells cotransfected with PGV-B-HES-1 and pRL-tk were analyzed using a luciferase assay system (Promega). The Renilla luciferase expression plasmid, pRL-tk (Promega), was used as an internal control for transfection.

RESULTS AND DISCUSSION

A Chimeric Protein of C53NICD Generates Aβ and Activates HES-1 Promoter-mediated Transcription in Cells—Our functional screening system utilizes the transcriptional activity of the Notch-1 intracellular domain (NICD) released from the membrane following transmembranous cleavage of Notch-1 (25). We generated a chimeric gene encoding C53NICD by replacing the C-terminal intracellular domain of C99 (β-secretase-cleaved APP fragment; reviewed in Ref. 6) with the murine NICD (Fig. 1A). We also generated a puromycin resistance gene (pac) driven by the HES-1 promoter (a DNA element responsible for Notch-dependent gene expression) (25, 32) (Fig. 1B). Using the two DNAs, we designed a functional screening system based on the following idea. (i) In the cells stably expressing C53NICD and pac driven by the HES-1 promoter, an increase in the degree of γ-cleavage should confer an increase in puromycin resistance and Aβ generation (Fig. 1B). (ii) Therefore, when the above-mentioned stable cells were transfected with a cDNA library, the cells harboring a cDNA that enhances γ-cleavage should be first selected as puromycin-resistant clones, and then identified by the screening for cells generating a higher Aβ level.

Prior to carrying out the above functional screening, we first investigated whether the expression of C53NICD in cells leads to Aβ generation and activates the HES-1 promoter. As shown in Fig. 2A, Neuro 2a cells transiently expressing C53NICD generated Aβ40 and Aβ42 as well as the cells expressing C99, indicating that C53NICD undergoes γ-cleavage in the cells as APP does. We next addressed whether C53NICD activates the
C53NICD generates Aβ and activates the HES-1 promoter in the cell. A, Aβ secreted from N2a cells transiently expressing C53NICD after a 48-h culture was immunoprecipitated with 6E10 and detected using a highly sensitive immunoblotting technique. Cells were transiently transfected with pCxn-N-C53NICD or pcDNA-C100 (harboring the cdna encoding the first methionine plus C99 or pCxn2 (mock transfection). B, N2a cells were co-transfected with PGV-B-HES-1 (see “Experimental Procedures”) and a pRL-tk plus pCxn-C53NICD or pcDNA-C100 or an empty vector. At 1 day after transfection, luciferase assay was performed. Renilla luciferase expression plasmid, pRL-tk, was used as an internal control for transfection. The results are presented as -fold induction, which is the relative luciferase activity (ratio of HES-1 promoter-driven luciferase/Renilla luciferase) of the cells over that of control cells (the cells transfected with PGV-B-HES-1 and a pRL-TK plus an empty vector). C, PS1/PS2 double-deficient fibroblasts (PS−/−) or wild-type fibroblasts (wt) were transiently co-transfected with PGV-B-HES-1 and a pRL-tk plus pCxn-C53NICD or an empty vector (mock). At 2 days after transfection, luciferase assay was performed. Renilla luciferase expression plasmid, pRL-tk, was used as an internal control for transfection. The results are presented as luciferase activity (arbitrary unit), which is the ratio of HES-1 promoter-driven luciferase/Renilla luciferase.

expression of the gene under the control of the HES-1 promoter. For this purpose, we constructed a plasmid carrying the luciferase-coding gene driven by the HES-1 promoter, and performed luciferase assay. As shown in Fig. 2B, the expression of the luciferase gene was induced by the HES-1 promoter when it was cotransfected with a plasmid encoding C53NICD, but not with a plasmid encoding C99. This result indicates that the expression of C53NICD causes NICD-dependent HES-1 promoter-mediated gene activation in the cells. Furthermore, to exclude the possibility of the activation of the HES-1-driven gene by nonspecific generation of NICD-like fragments from C53NICD, we next determined using PS-deficient cells whether the activation of the luciferase gene depends on PS. As shown in Fig. 2C, the activation of the luciferase gene completely depended on PS, indicating that the release of NICD from C53NICD, as well as that from Notch-1, depends on PS. This result strongly supports the reliability of the puromycin selection step in the above-described screening system. Thus, we decided to develop our designed functional screening method, because we ascertained that γ-cleavage of C53NICD generates Aβ, accompanied by PS-dependent activation of HES-1 promoter-mediated transcription. For a functional screening assay, a stable expression of cDNA in cells is preferable. Therefore, we employed the retrovirus-mediated gene expression method (24, 34, 36). As parental cells of the target cells for the transduction of a cdna library, we chose BaF/3 cells of the murine pro-B cell line because high efficiency retrovirus infection is attained in BaF/3 cells (24, 36) (Note that BaF/3 cells do not express endogenous APP; however, the transfection of human APP into BaF/3 cells resulted in the generation of Aβ, indicating that BaF/3 cells have endogenous factors necessary for γ-cleavage (data not shown).)

Thus, as target cells for stable transduction of a cdna library, we established BaF/3 cells stably transfected with pCxn-N-C53NICD (see “Experimental Procedures”) and pHES-1-
pac (see “Experimental Procedures”) (designated as A5-9). We also established A5-9 cells stably expressing human PS1 (designated as A5-9-PS1), because the use of the cells overexpressing PS1 might help to identify the activator of γ-secretase, as PS is essential for the γ-secretase activity.

Identification of cDNA Encoding γ-Cleavage-enhancing Factor—The scheme of the screening procedure used is shown in Fig. 3. To initiate screening, a human hippocampus-derived cDNA library was retrovirally transduced into A5-9 or A5-9-PS1 cells. At 48 h after retroviral infection, the cells were subjected to puromycin resistance selection. The cells were treated with a lethal dose of puromycin to select the cells expressing cDNA encoding a γ-cleavage-enhancing factor. After 2 weeks of growth, surviving clones were picked up and again treated with the same dosage of puromycin to eliminate clones that were transiently resistant to puromycin. To identify the clones exhibiting an increased Aβ level, Aβ secreted from puromycin-resistant clones was detected using a highly sensitive immunoblotting method. We found that some of the puromycin-resistant clones clearly exhibited an increased Aβ level (Fig. 4). Table I shows the number of puromycin-resistant clones and the number of the clones with an increased Aβ (Aβ40) level. Out of 4 × 10^6 cells transduced with cDNA library, approximately 30 (target cells, A5-9-PS1) or 10 (target cells, A5-9) clones exhibited the puromycin-resistant phenotype (Table I). Among them, approximately 10 clones exhibited an increase in the Aβ level (Table I). Next, the cDNAs from the puromycin-resistant clones with an increased Aβ40 level were amplified by PCR using vector primers. One of these DNAs encoded Herp (19), which was previously reported to enhance Aβ generation from APP (20). From another puromycin-resistant clone with an increased Aβ level (clone 28 in Fig. 5a), we obtained 1.4-kb PCR product containing the open reading frame encoding the entire Rab1A amino acid sequence. Rab1A is implicated in protein trafficking from the ER to the Golgi apparatus (reviewed in Ref. 21). We further determined whether Rab1A is responsible for conferring puromycin resist-

### Table I

| Target cell | No. of puromycin-resistant clones | No. of clones with an increased Aβ40 level |
|-------------|----------------------------------|------------------------------------------|
| A5-9-PS1    | 32 (5)                           | 12                                       |
| A5-9-PS1*   | 45 (14)                          | 13                                       |
| A5–9        | 18 (2)                           | 5                                        |

* Data are the results of two independent screening.

Fig. 5. Expression of Rab1A in the parental cells resulted in an increase in Aβ generation from C53NICD. Clone 28 was isolated as a puromycin-resistant clone with increased Aβ level by our screening method using A5-9-PS1 cells. PCR product from clone 28 encoded Rab1A. A. Aβ secreted from clone 28 was detected as described in Fig. 3 legend. B. Puromycin-resistant cells were obtained following the retroviral expression of Rab1A in A5-9-PS1 cells as described in Table II. The levels of Aβ secreted from the puromycin-resistant cells and A5-9-PS1 cells were detected. The cell lysates (25 μg) were immunoblotted with the anti-Rab1A antibody.

Fig. 6. Effects of a high expression level of Rab1A on the generation of Aβ and sAPP from full-length APP. A, murine fibroblasts (2 × 10^4) were plated on a 100-mm tissue culture dish. After human Rab1A (or mock) and APP695 were retrovirally expressed, Aβ40 and Aβ42 secreted from the cells during 72 h of culture were detected using a highly sensitive immunoblotting technique. The cell lysates (25 μg) were immunoblotted with anti-Rab1A. B, Aβ secreted from the cells during 72-h culture was quantified by ELISA. Values are means ± S.D. of three independent dishes (n = 3). *, p < 0.01; **, p < 0.01 (Mann-Whitney U test). Similar results were obtained in three independent experiments. Figures in parentheses indicate relative Aβ levels determined by calculating the ratio of the amount of Aβ from the fibroblasts expressing Rab1A to the mean amount of Aβ from the mock fibroblasts. C, upper panel indicates the levels of sAPP. The medium used for 72-h culture was immunoblotted with the anti-APP N-terminal antibody. (→), medium prepared from the fibroblasts not expressing human APP695; 1/2 Rab1A and 1/3 Rab1A, 2- and 3-fold diluted conditioned medium of the fibroblasts expressing Rab1A, respectively. Lower panel indicates intracellular APP level. The radioimmune precipitation assay buffer-solubilized lysates (25 μg) were immunoblotted with the anti-Rab1A antibody. D, the intracellular C99 level in radioimmune precipitation assay buffer-solubilized lysates (1.5 mg) was immunoprecipitated and immunoblotted as previously described (20). The lysate prepared from one culture dish were each loaded in one lane. The lysate prepared from fibroblasts retrovirally expressing C99 was also immunodetected with B12/4 (left lane).
ance and an increased ability of Aβ generation on A5-9-PS1 cells. As shown in Table II, the expression of Rab1A conferred puromycin resistance on A5-9-PS1 cells. We also confirmed that the puromycin-resistant cells obtained after the retroviral transduction of Rab1A cDNA secreted higher levels of both Aβ40 and Aβ42 than A5-9-PS1 cells (Fig. 5B). These results clearly indicated that the Rab1A cDNA is a gene responsible for exhibiting puromycin resistance and enhanced Aβ generation.

Expression of Rab1A Increases Aβ Generation from Full-length APP—We next investigated whether Rab1A increases Aβ generation from full-length APP using murine fibroblasts. As shown in Fig. 6A, the expression of Rab1A clearly increased secreted Aβ40 and Aβ42 levels. Quantification by ELISA revealed that the expression of Rab1A increased the levels of Aβ40 and Aβ42 3- and 2-fold, respectively (Fig. 6B). The difference in the degrees of increase in Aβ40 and Aβ42 levels suggested distinct molecular mechanisms underlying the generation between the two Aβ variants. The level of soluble APP (sAPP; reviewed in Ref. 1), which is generated through γ- or β-cleavage, was also slightly increased by Rab1A expression, whereas no significant change in the intracellular APP level was observed (Fig. 6C). The degree of an increase in sAPP level was less than 2-fold, because the band intensity of one-half the amount of sAPP secreted from the fibroblasts expressing Rab1A was lower than that of total sAPP secreted from the mock fibroblasts (Fig. 6C). These results indicated that Rab1A expression increased the degree of γ-cleavage greater than that of α- or β-cleavage. In addition, the intracellular level of C99, which is a substrate of γ-secretase, was significantly reduced by a high expression level of Rab1A (Fig. 6D), providing strong evidence that Rab1A expression enhances γ-cleavage, resulting in an increase in Aβ generation.

The cytoplasmic tail of APP contains important sorting determinants (37) and sequence elements required for interaction with proteins including X11, which influences APP processing.

### Table II

Effect of the expression of Rab1A cDNA on puromycin resistance

| Retroviral expression | No. of surviving cells |
|----------------------|------------------------|
| Mock                 | <1                     |
| Rab1A                | 60 ± 20                |

![Fig. 7](image)

**Fig. 7. Effects of a high expression level of Rab1A on the generation of Aβ and sAPP from APPΔC.** A, Aβ secreted from fibroblasts (2 × 10⁵) retrovirally expressing APP695 or APPΔC during a 72-h culture was quantified by ELISA. Figures in parentheses indicate relative Aβ levels determined by calculating the ratio of the amount of Aβ from the fibroblasts expressing Rab1A to the mean amount of Aβ from the mock fibroblasts. Values are means ± S.D. of three independent dishes (n = 3). Similar results were obtained in four independent experiments. B, the medium for 72-h culture in panel A was immunoblotted with the anti-APP N-terminal antibody (sAPP) or 6E10 (sAPPα). Bottom panel, the cell lysates (25 μg) were immunoblotted with anti-Rab1A antibody. C, the medium of the 72-h culture in panel A at various amounts was immunoblotted with the anti-APP N-terminal antibody (sAPP) or 6E10 (sAPPα). The intensities of the bands corresponding to sAPP (left panel) and sAPPα (right panel) in the medium were quantified densitometrically using NIH Image software (PDI, Inc.). Closed circle, mock fibroblasts; open circle, Rab1A-expressing fibroblasts.
and Aβ generation (38–40). Therefore, to know whether the effect of Rab1A on Aβ generation depends on the cytoplasmic tail of APP, we next addressed whether the expression of Rab1A increases the Aβ generation from APP lacking the cytoplasmic tail (APPΔC). As shown in Fig. 7A, the expression of Rab1A increased Aβ generation even from APPΔC, although the degree of increase was slightly small. Therefore, the cytoplasmic domain of APP or the intracellular trafficking of APP mediated by the cytoplasmic domain is not essential for an increase in Aβ generation by the expression of Rab1A. The deletion of the cytoplasmic tail of APP drastically enhanced sAPP generation as previously reported (33, 38) (Fig. 7B). It is also noted that the expression of Rab1A did not result in an enhanced sAPP generation from APPΔC (Fig. 7B; for the quantitative analysis of sAPP production, see Fig. 7C). An increase in Aβ generation from APPΔC caused by Rab1A expression, which was accompanied by no increase in sAPP generation, clearly indicates that the expression of Rab1A increases γ-cleavage, which is not the result of the simple stimulation of the trafficking of the APPΔC from the ER to the Golgi apparatus. We also found that a high expression level of Rab1A did not alter the steady-state levels of full-length PS and its en-docleaved product, N-terminal fragment (data not shown), strongly suggesting that a high expression level of Rab1A did not affect the trafficking of PS from the ER to the Golgi apparatus, because the endoproteolysis of PS is likely to occur in the ER-cis-Golgi intermediate compartment (reviewed in Ref. 17).

Thus far, the involvement of several Rab proteins in the pathway for Aβ or soluble APP generation has been addressed (41–43). However, the role of Rab proteins in the γ-cleavage pathway is not yet clarified. Our study provided strong evidence that Rab1A, which is known to be involved in protein trafficking from the ER to the Golgi apparatus, because the endoproteolysis of PS is likely to occur in the ER-cis-Golgi intermediate compartment (reviewed in Ref. 17), plays a role in the γ-cleavage pathway. Previously, the ER-cis-Golgi intermediate compartment has been suggested to be a site for Aβ generation (44, 45). In this regard, ER localization of Rab1A well agrees with the ER being the intracellular site of Aβ generation. In addition, in PS1-deficient cells, C99 is accumulated in the ER without undergoing γ-cleavage (46, 47). One possible explanation for this is that C99 in PS1-deficient cells is retrogradely trafficked to the ER after it is generated through the β-secretase cleavage of APP in a late Golgi compartment or trans-Golgi network. Therefore, Rab1A could enhance γ-cleavage of ER-localized C99, which is retrogradely trafficked back to the ER. However, accumulating evidence showed that the major intracellular site of γ-cleavage is not the ER, but, most likely, the trans-Golgi network (30, 31, 48, 49). In this regard, it is noteworthy that Rab1A not only exists in the ER, but is also associated with the transcytotic vesicle (50). This indicates that the function of Rab1A is limited to protein trafficking from the ER to the Golgi apparatus. Rab1A could be involved in γ-cleavage in its other destinations or during its travel to the transcytotic vesicle or an as yet identified compartment. At present, we do not know whether Rab1A activates γ-secretase or modulates the trafficking of the factors involved in γ-cleavage, including PS and nicastrin. Further study on the mechanism underlying the enhancement of γ-cleavage caused by a high expression level of Rab1A is necessary.

In this study, we showed a new functional screening method for identifying factors involved in γ-cleavage and also identified Rab1A by this method. Previously, we reported that the ER stress-inducible protein, Herp, which was identified by this method, enhanced γ-cleavage and bound to PS (20). These results strongly suggest that Herp is an inducible regulator of γ-secretase. In contrast, Rab1A appears to constitutively regul-
Identification of Regulators of γ-Secretase Cleavage

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