Generation and Characterization of Mutant Cell Lines Defective in γ-Secretase Processing of Notch and Amyloid Precursor Protein*

Annie Oly, Patricia Chastagner, Alain Israël, and Christel Brou‡

From the Unité de Signalisation Moléculaire et Activation Cellulaire, URA 2582, CNRS, Institut Pasteur, 25 Rue du Dr. Roux, 75724 Paris Cedex 15, France

Several type I integral membrane proteins, such as the Notch receptor or the amyloid precursor protein, are cleaved in their intramembrane domain by a γ-secretase enzyme, which is carried within a multiprotein complex. These cleavages generate molecules that are involved in intracellular or extracellular signaling. At least four transmembrane proteins belong to the γ-secretase complex: presenilin, nicastrin, Aph-1, and Pen-2. It is still unclear whether these proteins are the only components of the complex and whether a unique complex is involved in the different γ-secretase cleavage events. We have set up a genetic screen based on the permanent acquisition or loss of an antibiotic resistance depending on the presence of an active γ-secretase able to cleave a Notch-derived substrate. We selected clones deficient in γ-secretase activity using this screen on mammalian cells after random mutagenesis. We further analyzed two of these clones and identified previously undescribed mutations in the nicastrin gene. The first mutation abolishes nicastrin production, and the second mutation, a point mutation in the ectodomain, abolishes nicastrin maturation. In both cases, γ-secretase activity on Notch and APP is impaired.

The Notch receptor is a transmembrane (TM) molecule that is expressed at the cell surface as a heterodimer. Notch signaling occurs after binding of a TM ligand. In mammals, these ligands belong to the Delta or Jagged family. The binding of the ligand causes a series of events that lead to the release of the intracellular domain of the Notch receptor (ICV). This intracellular domain then translocates to the nucleus, where it participates in transcriptional activation of target genes. The complete mechanism allowing signal transmission through Notch receptors remains unclear. However, it is now widely accepted that two sequential proteolysis events are required after ligand binding. In mammals, the Notch receptor is first cleaved in its ectodomain by the TNF-α converting enzyme (TACE) (1) or possibly by another metalloprotease such as ADAM10 (2). The Notch receptor is then cleaved in its TM domain by γ-secretase.

Notch belongs to a family of proteins, such as APP, Delta, ErbB-4, and N-cadherin, which undergo TM cleavage (3). At least four proteins, presenilin (PS1 or PS2), nicastrin (Nct), Pen-2, and Aph-1, make up γ-secretase, forming a high molecular weight complex. The catalytic subunit of the complex is probably PS, but all four proteins are necessary for the maturation and the overall catalytic activity of γ-secretase (4).

APP, the other canonical γ-secretase substrate, can be cleaved at various sites within its TM domain (respectively called γ40, γ42, and e), giving rise to Aβ40, Aβ42, or and APP intracellular domain. The balance between these different cleavage products appears to be crucial, since changes in this equilibrium can contribute to the development of Alzheimer’s disease. These cleavages depend on a PS-containing complex, and mutations in PS can change the ratio between the different products. However, the parameters regulating the ratio between these events remains unknown (5). Other γ-secretase substrates, such as Notch, APLP1, or APLP2, also undergo multiple cleavages (6). Furthermore, some mutations affecting Nct strongly modulate γ-secretase activity on APP, but not on Notch (7), and a number of drugs have been described as differentially inhibiting γ-secretase activity on Notch and APP (8). Therefore, it is possible that several enzymes able to discriminate between various substrates and/or various cleavage sites may exist, grouped under the generic name of γ-secretase. The core complex of these enzymes may contain the four identified proteins, but other minor factors or specific modifications of these proteins could generate the diversity of γ-secretase activity.

Although coimmunoprecipitation experiments allowed the purification and identification of Nct (9), the biochemical isolation of minor components is always difficult for TM substrates and multisubunit membrane-anchored enzymatic complexes. Therefore, we have used a genetic system allowing the identification of any event or component needed for γ-secretase activity, including proteins not part of the complex but regulating its activity. Here we describe the basis of this screening method. Following mutagenesis, we selected cells that no longer cleave a constitutively active form of Notch at the γ-secretase site. We characterized two of these γ-secretase-deficient cell lines.

EXPERIMENTAL PROCEDURES

Materials

HEK 293T and Rat-1 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum and 1% penicillin/streptomycin (Invitrogen). Supplementary reagents were blasticidin (10 µg/ml; Invitrogen), G418 (4 mg/ml; Invitrogen), hygromycin (1 mg/ml; Invitrogen), and tamoxifen (10⁻⁶ M; Sigma).

Antibodies used were V1744 (Cell Signaling), anti-Nct antibodies (Abcam), anti-β-actin antibodies (Sigma, 1:1000), and anti-HA antibodies (HA.11, Covance). NOS-1 (Sigma, 1:1000), and anti-Pan cadherin antibodies (Sigma), Alexa Fluor® 488 conjugates (Molecular Probes,
Sequence of PS1, Nct, Aph-1, and Pen-2 cDNA

Total RNA was extracted from 10^6 AD6, clone 1, and clone 2 cells using the High Pure RNA isolation kit (Roche Applied Science). We used the Superscript II first strand synthesis system for RT-PCR to generate the cDNA (Invitrogen). PCR products were sequenced using the following primers: PS1-5'-GAC GAG GTA CCT GCA CC-3', and 5'-GAG AGG GAA CAG ACA C-3', Nct-5'-ATG ACG CTC GCT AGG G-3', 5'-CCA GAG CAC CTT CAG C-3', and 5'-CGA CTT ACG TTG TCG AG-3', Aph-1 (5'-ATT ATC ATG CTC CAT ACC-3') and Pen-2 (5'-CTA AGT TAG AGC GGG-3'). Chromatogram profiles were manually checked to determine whether a wild type and mutant allele were coexpressed.

Sequence of Genomic Nct Gene Fragment

Genomic DNA was extracted from 10^6 cells with the QiAamp DNA miqikit (Qiagen). The two mutated regions were genotyped by PCR using primers for clone 1 (5'-CCA AGA CAG AGC GGC and 3'-CTA CGT GGG CCG AGA GC) and clone 2 (5'-CAG TGG AGA GGA AGA TC and 3'-GAT TTG TGT TTG TCG GC). Chromatogram profiles were manually checked to detect any allelic polymorphism.

RESULTS

Total RNA was isolated from 10^6 AD6, clone 1, and clone 2 cells using TRizol reagent (Invitrogen) according to the manufacturer’s protocol. We separated 8 μg of total RNAs on 1% formaldehyde-agarose gels, transferred them to Hybond-N membrane (Ambion), and hybridized them in ULTRAhyb buffer (Ambion) with a 32P-labeled DNA probe (Nct cDNA from nucleotide +341 to nucleotide +768) by incubation at 42 °C overnight. The membrane was washed twice with 0.1% SDS, 0.1 M NaCl buffer by incubation at 42 °C for 5 min, twice with 0.1% SDS, 0.1× SSC buffer by incubation at 42 °C for 5 min, and visualized by autoradiography. We used the random primed DNA labeling kit (Roche Molecular Biochemicals) for 32P-labeling.

Cell Extracts and Immunoblots

AD6, clone 1, and clone 2 cells grown on 100-mm dishes were transiently transfected with 10 μg of total DNA. The cells were harvested 24 h after transfection, washed in phosphate-buffered saline buffer, and lysed in lysis buffer (50 mM Tris-HCl, pH 8.0, 1% Nonidet P-40, 400 mM NaCl) supplemented with 1× protease inhibitor mixture (Roche Applied Science). After 20 min on ice, the cell lysates were cleared by centrifugation at 14,000 rpm for 20 min at 4 °C. Samples were resuspended in Laemmli buffer for SDS-PAGE, and immunoblots were carried out as described earlier (1).
between TNF-α converting enzyme cleavage and γ-secretase cleavage, such as ubiquitination, endocytosis, or other as yet unknown events). Therefore, such a molecule should allow us to identify mutations that affect any step occurring before γ-secretase cleavage.

As we planned to complement the mutants cells with a retroviral library, we chose rodent diploid cells, Rat-1, as a host (15). These cells have been successfully used for this type of approach (16).

We established a cell line expressing a chimeric molecule containing a ΔE-derived fragment of Notch1 (signal peptide plus 21 amino acids N-terminal to the plasma membrane plus TM domain plus 60 N-terminal amino acids of the intracellular part of Notch1; see Fig. 1), fused to a vesicular stomatitis virus epitope, the Cre recombinase, and a modified hormone binding domain of the estrogen receptor (ER-T2; mutated to respond only to 4-hydroxytamoxifen) (11, 17). The delivery of Cre recombinase depends on any event, including γ-secretase processing, that releases ICv from the membrane, and then the nuclear activity of the Cre-ER-T2 chimera depends on the presence of tamoxifen.

The reporter construct for the recombinase activity consists of an antibiotic resistance gene (hygromycin) driven by a strong promoter. Its transcription and translation are interrupted by the introduction, between the promoter and the gene, of two loxp sites separated by a transcription/translation STOP sequence and an SV40 enhancer-driven neomycin resistance gene in the reverse orientation (see Fig. 1B). Non-tamoxifen-treated wild-type cells do not display recombination between the two loxp sites and are resistant to neomycin and sensitive to hygromycin. After tamoxifen treatment, the activated Cre recombinase allows excision of the STOP sequence and the neomycin resistance gene, allowing the hygromycin resistance gene to be expressed.

If γ-secretase is inactivated following mutagenesis, the cells will no longer be sensitive to neomycin and resistant to hygromycin, even in the presence of tamoxifen. Complementation of the defect will restore tamoxifen-dependent sensitivity to neomycin and resistance to hygromycin. Rat-1 cells stably expressing our effector and reporter constructs described above are called AD6.

ICR191 Treatment Generates Mutant AD6 Cells Affected in γ-Secretase Activity—We exposed AD6 cells to several cycles of mutagenesis with the drug ICR191. This is a frameshift mutagen that preferentially causes single bp additions in stretches of G nucleotides (18). We carried out six rounds of mutagenesis. In these conditions, mutants can be isolated at a much higher frequency (more than 30 times higher) than after one cycle of mutagenesis (19). We first selected clones growing on neomycin and tamoxifen-containing medium and then transiently transfected them with the ΔE expression vector. Immunofluorescence analysis using anti-Myc and V1744 antibodies allowed us to identify cells deficient in γ-secretase activity. We used anti-Myc antibodies as a control, since they recognize all the forms derived from ΔE, irrespective of their cleavage status or subcellular location. The V1744 antibodies specifically recognized the γ-secretase cleavage product, ICv (14). Therefore, V1744 nuclear staining is a specific and sensitive assay that allows us to visualize γ-secretase activity acting on Notch. Some clones (e.g. clone 1 and clone 2 in Fig. 2) displayed a strictly non-nuclear localization of ΔE-derived products, as detected with anti-Myc antibody, and no labeling with the V1744 antibody. The other G418-resistant clones exhibited a nuclear staining with both antibodies (data not shown). Other components of the system, such as the Cre recombinase, the ER-T2 fragment, or the loxp sequences, were probably affected in these γ-secretase-positive clones. Of the 186 clones obtained after mutagenesis and selection, only 26 were γ-secretase-deficient. We chose two of them, clone 1 and clone 2, for further analysis. We investigated whether γ-secretase processing of APP was also impaired. Before TM cleavage, APP can be alternatively cleaved in its ectodomain at two different sites. The first cleavage, which involves β-secretase, generates a transmembrane β-stub fragment, whereas the second cleavage involves α-secretase and generates an α-stub fragment. These products are then direct targets of γ-secretase. We transiently expressed a β-stub fragment fused to six Myc epitopes (called C100) in AD6 cells and in clone 1 and clone 2 (Fig. 2B). Western blot analysis of AD6 cell lysates (Fig. 2B, lane 1) showed the C100 protein, a faster migrating fragment resulting from α-secretase processing of the C100 protein, and a third fragment corresponding to the intracellular domain of the C100 protein (indicated as AICD). In clone 1 and clone 2 (Fig. 2B, lanes 2 and 3), we did not detect APP intracellular domain but detected the accumulation of the α-stub fragment. These data suggested that γ-secretase processing of APP was also impaired in these clones.

Two Independent Mutants Are Complemented by Nct—The mutant phenotype of clone 1 and clone 2 may be due to loss of activity of one or more of the four known components of the PS complex or of an unknown protein necessary for γ-secretase activity. We transiently cotransfected Nct, Aph-1, PS1, or Pen-2 with a ΔE expression vector and assayed the γ-secretase activity by immunofluorescence. When Aph-1, PS1, or Pen-2 expression vectors were cotransfected, the ΔE-derived forms displayed no nuclear localization by anti-Myc antibodies and no staining with V1744 antibodies (see Fig. 2A, panels C for PS1; data not shown). However, after the co-expression of Nct in clones 1 and 2, ΔE-derived forms displayed nuclear immunostaining by V1744 antibodies in most transfected cells (Fig. 2A, panels B). Thus, Nct expression restored ΔE cleavage at the γ-secretase site, suggesting either a mutation in the Nct gene itself or a mutation in a gene encoding a protein acting upstream of Nct and necessary for Nct activity.

Western blot analysis of AD6 cell lysates using an antibody raised against the N terminus of Nct revealed two bands with apparent molecular masses of about 130 and about 110 kDa (Fig. 3A, lane 1). In clone 1, we detected no immunoreactive band (Fig. 3A, lane 2), whereas we detected a single product of about 110 kDa in clone 2 (Fig. 3A, lane 3). Furthermore, no soluble form was detected in the medium of the three cell lines (data not shown). The 110-kDa migrating form seen in clone 2-derived extracts was possibly an immature, not fully glycosylated Nct or a shorter Nct. Therefore, we examined the susceptibility of these Nct proteins to deglycosidase treatment.

**Fig. 1. Constructs used for the selection.** A, effector construct. The chimeric molecule containing a ΔE-derived fragment, vesicular stomatitis virus (VSV) epitope, CRE recombinase, and ERT2 is expressed under the control of a cytomegalovirus (CMV) promoter. B, reporter construct. The hygromycin resistance gene is driven by a cytomegalovirus promoter but is expressed only if the CRE recombinase mediates excision of the STOP sequence and neomycin resistance gene. Otherwise, the neomycin resistance gene is expressed under the control of an SV40 promoter.
After endoglycosidase H digestion of AD6 Nct immunoprecipitates, Western blot analysis showed that two bands were replaced by two faster migrating ones, indicating that both forms contained high mannose N-linked oligosaccharides (Fig. 3B, lane 1). After removal of all N-linked oligosaccharide chains by N-glycosidase F digestion, Western blot analysis showed only one single species (Fig. 3B, lane 2, indicated as deglyc). The endoglycosidase H treatment only partially deglycosylates the mature Nct (upper band in Fig. 3B, lane 1), indicating that the mature Nct is modified by both endoglycosidase H-sensitive and endoglycosidase H-resistant N-linked oligosaccharides. The immature and partially glycosylated Nct contains only endoglycosidase H-sensitive N-linked oligosaccharides, confirming that it is only modified by high mannose oligosaccharides in the ER and does not reach the Golgi apparatus, where subsequent complex glycosylations occur. Endoglycosidase H or N-glycosidase F digestion of clone 2 Nct-immunoprecipitates similarly reduced their apparent molecular mass (Fig. 3B, lanes 3 and 4). Therefore, the Nct protein present in clone 2, being endoglycosidase H-sensitive and only modified by high mannose oligosaccharides in the ER, is similar to the immature Nct. The apparent molecular mass of deglycosylated AD6 and clone 2 Nct were identical. This indicated that clone 2 does not express a truncated Nct protein.
We detected Nct mRNA in AD6 and clone 2 by Northern blot analysis (Fig. 3C, lanes 1 and 3, respectively). However, we did not detect Nct mRNA in clone 1 (Fig. 3C, lane 2), although it could be amplified by RT-PCR in the three cell lines (data not shown). Quantitative RT-PCR analysis showed that the amount of Nct mRNA was 5 times lower in clone 1 than in AD6 cells (data not shown). Thus, the two clones exhibited different characteristics, clone 1 lacking the Nct protein and clone 2 having a defect in Nct protein maturation.

**Clones 1 and 2 Carry Different Mutations**—The defects in Nct expression and maturation observed in clones 1 and 2 may be due to mutations in the gene encoding Nct itself and/or other γ-secretase complex proteins acting upstream of Nct in the formation of the complex. Therefore, we sequenced the PS1, PS2, Aph-1, Pen-2, and Nct cDNAs from RT-PCR of AD6, clone 1, and clone 2. We found Nct was mutated in both clones, whereas the other cDNAs were not. We identified an insertion of a C at position 1891 in the Nct cDNA in clone 1, leading to a frameshift (Fig. 4) and a new STOP codon 10 nucleotides downstream from the mutation. Nonsense-mediated mRNA decay rules (20) state that a truncated protein would be produced only if a new STOP codon was located downstream from the last exon-exon junction or within 50–55 nucleotides upstream from this junction. If a new STOP codon is located more than 50–55 nucleotides upstream from the last exon-exon junction, as in this case, mRNA is mostly eliminated and not translated. Therefore, the Nct mRNA in clone 1 is likely to be transcribed but rapidly degraded.

We identified a missense mutation at position 178 in the Nct cDNA in clone 2 that led to the replacement of a glycine by a leucine at position 60 (GGC to CTC, as indicated in Fig. 4). We inserted the cDNA encoding this mutant protein (named G60L) and its wild-type counterpart (G60L-HA) expression (Fig. 5). Cells were fixed, Triton-permeabilized, and incubated with anti-NIC, anti-HA, and the appropriate dye-labeled secondary antibodies. Preparations were analyzed by confocal microscopy (0.4-μm sections). As expected, expression of Nct-HA restored ICv production in mutant clones (Fig. 5B, lanes 5 and 6). Thus, G60L-HA expression was not sufficient to restore γ-secretase activity in these clones, indicating that the G60L mutation most probably ac-
countants for the mutant phenotype. The G60L protein did not behave as a dominant negative molecule, since its overexpression did not affect γ-secretase activity in AD6 cells (Fig. 5, A (C1) and B (lane 7)).

In the mutant clones, we expected inactivation of the two Nct alleles because of the loss of function phenotype. RT-PCR of Nct in clones 1 and 2 only amplified the mutant allele. However, analysis of genomic DNA around nucleotide 178 in clone 2 indicated the presence of a mutant allele because of the loss of function phenotype. RT-PCR of Nct alleles revealed that they carry a mutation in Nct but not in the second allele in the two clones was probably not transcribed because of other mutations.

The 24 other γ-secretase-deficient clones were also rescued by Nct overexpression. Among these, 23 carry the same mutation in the Nct gene as clone 2 (data not shown). The remaining clone appeared different, and we are in the process of characterizing it.

Biochemical Characterization of γ-Secretase Complexes in Wild-type Versus Mutant Cells—DNA analysis of clones 1 and 2 revealed that they carry a mutation in Nct but not in the other members of the γ-secretase complex. We examined whether these γ-secretase complex proteins were affected by the Nct mutations. We observed, as expected from previous studies (21), that the lack of detectable Nct in clone 1 led to a lack of detectable PS1, Aph-1, and Pen-2 proteins (Fig. 6A, lane 2 versus lane 1). In clone 2, levels of PS1 and Pen-2 were reduced, whereas the level of Aph-1 remained the same (Fig. 6A, lane 3). The doublet observed for Aph-1 in Fig. 6A, lane 3, may correspond to an intermediate glycosylation of the protein (22). Thus, the presence of G60L Nct led to decreased levels of PS1 and Pen-2. Although the effect was less severe than that observed in the absence of Nct, γ-secretase activity was abolished in both cases.

Properties of G60L Nct—We further characterized the G60L Nct to understand how a single substitution in the extracellular domain of the protein could lead to such a strong effect. This point mutation replaces a polar residue with a hydrophobic residue and occurs in a region with no known functional or structural importance. We suggested that this substitution interferes with the correct folding of the protein, preventing its correct maturation. Therefore, the location of Nct and/or its interaction with the other complex proteins may be affected.

When overexpressed in AD6 cells, Nct-HA and G60L-HA showed an apparently similar punctate staining (Fig. 5A, B1–D1). We could not detect endogenous Nct in AD6 cells and clone 2 by immunofluorescence, and therefore, we could not directly determine whether the lack of G60L Nct maturation affected the localization of this protein. However, deglycosylation experiments (Fig. 3B) strongly suggested that G60L Nct is retained in the ER, whereas wild type Nct is further modified in the Golgi apparatus.

Since Nct interacts with the substrates of γ-secretase (7, 23), we tested the interaction of Nct and G60L with the Notch-derived substrate ΔE. We coexpressed ΔE and Nct-HA or G60L-HA in HEK 293T cells. Nct coimmunoprecipitation revealed that Nct and G60L Nct proteins both interacted with ΔE (Fig. 6B, lanes 2 and 3). Thus, the G60L mutation did not affect the interaction between Nct and ΔE, at least when Nct-HA or G60L-HA was overexpressed.

DISCUSSION

We set up a genetic screen in mammalian cells to identify new components of the γ-secretase complex and to characterize potential differences between γ-secretase substrates or between different cleavage sites within a given substrate. As well as the genetic studies that discovered Nct, Aph-1, and Pen-2 in Caenorhabditis elegans (24–26), several screens for neurogenic genes and for modifiers of Notch signaling have already been carried out in flies. However, these did not identify any γ-secretase components. When compared with other organisms, additional subunits or regulatory components of the γ-secretase complex may be present in mammals. We wanted to establish a system that allowed easy monitoring of γ-secretase activity acting on the Notch receptor. However, activation of the Notch receptor under most “physiological” conditions (i.e., mixing Notch-expressing cells with ligand-expressing cells) is inherently inefficient and transient. Therefore, we designed a tamoxifen-dependent system that made activation of the Notch receptor an easily detectable, inducible, and stable event. The system relies on the permanent acquisition or loss of antibiotic resistance after γ-secretase cleavage of Notch.

Although the diploidy of the mammalian genome is a major hurdle to such an approach, recessive mutants have provided useful information in other cases (18, 27, 28). Indeed, complementation cloning in mammals is often difficult for autosomal genes. For example, S2P, which is encoded by a single copy gene on the X chromosome, was cloned by complementation after mutagenesis of Chinese hamster ovary cells and selecting for cholesterol auxotrophy (29). A similar approach was unsuccessful for S1P cloning, until multiple copies of S2P were introduced into the cells before mutagenesis. This allowed the selection of cells mutated in the two S1P alleles (30, 31). We avoided this problem by carrying out multiple rounds of mu-
tagenesis, with the aim of mutagenizing the two alleles of any given gene (19).

With this approach, we isolated 26 mutants. We characterized two of them (clone 1 and clone 2), both affected in an autosomal gene (the Nct gene is on chromosome 13 in rats). We found that all but one of the remaining mutant cell lines had the same mutation as clone 2. We also identified one other mutant, which is currently being characterized. Nct cDNA sequencing in clones 1 and 2 indicated that only one mutant allele was expressed, whereas genomic sequencing of the regions surrounding the mutations showed an allelic dimorphism. We did not sequence the entire Nct gene in our clones, and Rat-1 cells have been described as not functionally haploid (32). Therefore, it is very possible that the second allele was mutated somewhere else in such a way that it was not transcribed at all. The direct consequences of these mutations in the Nct gene were either the absence of the protein (clone 1) or the production of a non-fully glycosylated protein (clone 2). In these two mutant cell lines, γ-secretase activity operating on Notch and on APP was largely abolished. The G60L Nct protein has a phenotype similar to that of other recently described point mutants affecting various regions of the molecule (Cys248Glu333, and Gly339) (33). However, for our mutant, reduction in PS1 and Pen-2 protein levels was much less, consistent with being able to detect some residual γ-secretase activity. These data suggest that this second cell line (clone 2) may be useful for studying the first steps of γ-secretase complex assembly, without needing to rely on RNA interference and overexpression of the mutant form of Nct (21, 23).

Clone 2 carries a single point mutation in the ectodomain of Nct. The resultant Gly to Leu substitution at position 60 occurs without needing to rely on RNA interference and overexpression of the mutant α-mannosidase) undergoes the conformational change, interacts with PS1-CTF, localizes at the cell surface, and is associated with γ-secretase activity (38, 39). Thus, the conformational change in the Nct ectodomain seems to be more important than the late glycosylation step of the protein. Our data suggest that the immature Nct-Aph-1 subcomplex forms in the ER, with a conformational change of Nct then being essential for the transport to the Golgi compartment, where subsequent glycosylation of Nct and incorporation of PS1 and Pen-2 into the complex then occurs.

In conclusion, the generation of these nicastrin-deficient cells, as well as providing tools for studying Nct itself, validates our genetic approach. The identification of different types of mutations indicates that our screen is potentially able to identify new components of the γ-secretase complex.

Acknowledgments—We thank R. Kopan for the Notch ΔE construct; D. Metzger for CRE-R2T2 plasmid and advice; and G. Thnakanur, B. De Strooper, G. Yu, H. Xu, and L. Pradier for generous gifts of materials. We thank Pedro Baussero for quantitative RT-PCR analysis, and we thank Frédérique Logeat and Neetu Gupta-Rossi for helpful discussions.

REFERENCES

1. Brou, C., Logeat, F., Gupta, N., Bessia, C., LeBail, O., Doedens, J. R., Cunnamo, A., Roux, P., Black, R. A., and Israel, A. (2000) Mol. Cell 5, 207–216
2. Hartmann, D., de Strooper, B., Serneels, L., Craessaerts, K., Herreman, A., Aouni, W., Umano, E., Lebail, O., T. Lena Iurlin, S., von Figure, K., and Safi, P. (2002) Hum. Mol. Genet. 11, 2615–2624
3. Landman, N., and Kim, T.-W. (2004) Cytokine Growth Factor Rev. 15, 357–351
4. De Strooper, B. (2003) Neuron 38, 21–22
5. Okochi, M., Steiner, H., Fukumori, A., Tanii, H., Tomita, T., Tanaka, T., Iwatsubo, T., Kudo, T., Takeda, M., and Haass, C. (2002) EMBO J. 21, 5408–5416
6. Eggert, S., Paliga, K., Soba, P., Evin, G., Masters, C. L., Weidemann, A., and Bayreuther, K. (2004) J. Biol. Chem. 279, 18146–18156
7. Chen, F., Yu, G., Arawaka, S., Nishimura, M., Kawarai, T., Yu, H., Tandon, A., Supala, A., Song, Y. Q., Rogaeva, E., Milman, P., Sato, C., Yu, H., Janus, J. Lee, J., Song, L., Zhang, L., Fraser, P. E., and St George-Hyslop, P. H. (2001) Nat. Cell Biol. 3, 751–754
8. Peitel, A., Biehl, F., da Costa, C. A., Pourquie, O., Checher, F., and Kraus, J. L. (2001) Nat. Cell Biol. 3, 507–511
9. Yu, G., Nishimura, M., Arawaka, S., Levitan, D., Zhang, L., Tandon, A., Song, Y. Q., Rogaeva, E., Chen, F., Kawarai, T., Supala, A., Levesque, L., Yu, H., Yang, D. S., Holmes, E., Milman, P., Li, S. Zhang, D. M., Xu, H., Sato, C., Rogaeva, E., Smith, M., Janus, J. C., Aebersold, R., Faller, L. S., Sorbi, S., Bruni, A., Fraser, P., and St George-Hyslop, P. (2000) Nature 407, 48–54
10. Logeat, F., Bessia, C., Brou, C., Lebail, O., Jarriault, S., Seidah, N. G., and Bayreuther, K. (2000) J. Biol. Chem. 275, 752–757
11. Feil, R., Wagner, J., Metzger, D., and Chambon, P. (1997) Biochem. Biophys. Res. Commun. 237, 759–764
12. Six, E., Ndiaye, D., Laabi, Y., Brou, C., Gupta-Rossi, N., Israel, A., and Logeat, F. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 7638–7643
13. Poon, J. C., O’Neill, J. P., Peck, R. M., and Stark, G. R. (1989) Virology 173, 8108–8112
14. Gupta-Rossi, N., Six, E., LeBail, O., Logeat, F., Chastagner, P., Olry, A., Israel, A., and Brou, C. (2004) J. Cell Biol. 166, 73–83
15. Topp, W. C. (1981) Virology 113, 498
16. Chinnanwainai, N., Miura, H., Yamamoto, N., and Yamaoka, S. (2002) FEBS Lett. 531, 553–560
17. Infra, A. K., Worot, X., Brocard, J., Bornert, J. M., Xiao, J. H., Chambon, P., and Metzger, D. (1999) Nuclease Acids Res. 27, 4324–4327
18. Pellegrini, S., John, J., Shearer, M., Kerr, I. M., and Stark, G. R. (1989) Mol. Cell. Biol. 9, 4605–4611
19. McIndoe, R. J., Flavell, R. D., Muller, M., Kerr, I., and Stark, G. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 11455–11459
20. Nagy, E., and Maquat, L. E. (1998) Trends Biochem. Sci. 23, 198–199
21. Shiratori, K., Edshauer, D., Ibsen, A., Schmitz, J., Steiner, H., and Haass, C. (2003) J. Biol. Chem. 278, 16474–16477
22. Fortina, R. P., Crystal, A. S., Morais, V. A., Pijak, D. S., Lee, V. M., and Dobrowsky, R. W. (2004) J. Biol. Chem. 279, 3685–3691
23. Capell, A., Kaether, C., Edshauer, D., Shiratori, K., Merkl, S., Steiner, H., and Haass, C. (2003) J. Biol. Chem. 278, 52519–52523
24. Goutte, C., Hepler, W., McMichan, K. M., and Press, J. R. (2000) Development 127, 2483–2492
25. Goutte, C., Tsouknicou, M., Hale, V. A., and Press, J. R. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 775–777
26. Francis, R., McGrath, G., Zhang, J., Rudly, D. A., Sym, M., Apfeld, J., Nicoll, M., Maxwell, M., Hai, E., Ellis, M. C., Parks, A. L., Xu, W., Li, J., Gurney, M., Myers, R. L., Hines, C. S., Hiebsch, R., Ruble, C., Nye, J. S., and Curtis, D. (2002) Dev. Cell 3, 85–97
27. Velazquez, L., Fellou, M., Stark, G. R., and Pellegrini, S. (1992) Cell 70, 313–322
28. Yamaoka, S., Courtous, G., Bessia, C., Whiteside, S. T., Weil, R., Agou, F., Kirk, H. E., Kay, J. R., and Israel, A. (1998) Cell 93, 1231–1240
29. Rawson, R. B., Zelenski, N. G., Nijhawan, D., Ye, J., Sakai, J., Hasan, M. T., Chang, T. Y., Brown, M. S., and Goldstein, J. L. (1997) Mol. Cell 1, 47–57
30. Rawson, R. B., Cheng, D., Brown, M. S., and Goldstein, J. L. (1998) J. Biol. Chem. 273, 28261–28269
31. Sakai, J., Rawson, R. B., Espenshade, P. J., Cheng, D., Seegmiller, A. C., Goldstein, J. L., and Brown, M. S. (1998) Mol. Cell 2, 505–514
32. Shichiri, M., Hanson, K. D., and Sedivy, J. M. (1993) Cell Growth Differ. 4, 93–104
33. Shirotani, K., Edbauer, D., Kostka, M., Steiner, H., and Haass, C. (2004) J. Neurochem. 89, 1520–1527
34. Yang, D.-S., Tandon, A., Chen, F., Yu, G., Yu, H., Arawaka, S., Hasegawa, H., Duthie, M., Schmidt, S. D., Ramabhadran, T. V., Nixon, R. A., Mathews, P. M., Gandy, S. E., Mount, H. T. J., St George-Hyslop, P., and Fraser, P. E. (2002) J. Biol. Chem. 277, 28135–28142
35. Kimberly, W. T., LaVoie, M. J., Ostanzewski, B. L., Yu, W., Wolfe, M. S., and Selkoe, D. J. (2002) J. Biol. Chem. 277, 35113–35117
36. LaVoie, M. J., Fraering, P. C., Ostanzewski, B. L., Ye, W., Kimberly, W. T., Wolfe, M. S., and Selkoe, D. J. (2003) J. Biol. Chem. 278, 37213–37222
37. Morais, V. A., Crystal, A. S., Pijak, D. S., Carlin, D., Costa, J., Lee, V. M.-Y., and Doms, R. W. (2003) J. Biol. Chem. 278, 43284–43291
38. Herreman, A., Van Gassen, G., Bentahir, M., Nyahi, O., Craessaerts, K., Mueller, U., Annaert, W., and De Strooper, B. (2003) J. Cell Sci. 116, 1127–1136
39. Shirotani, K., Edbauer, D., Prokop, S., Haass, C., and Steiner, H. (2004) J. Biol. Chem. 279, 41340–41345
Generation and Characterization of Mutant Cell Lines Defective in γ-Secretase Processing of Notch and Amyloid Precursor Protein
Annie Oly, Patricia Chastagner, Alain Israël and Christel Brou

J. Biol. Chem. 2005, 280:28564-28571.
doi: 10.1074/jbc.M502199200 originally published online June 15, 2005

Access the most updated version of this article at doi: 10.1074/jbc.M502199200

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 39 references, 21 of which can be accessed free at http://www.jbc.org/content/280/31/28564.full.html#ref-list-1