We show here that alternative splicing influences the polarized secretion of amyloid precursor protein (APP) as well as the release of its proteolytic 3-4-kDa fragments βA4 and p3. In Madin-Darby canine kidney II cells stably transfected with various APP isoforms and APP mutants, APPsec was consistently secreted basolaterally. In contrast, Madin-Darby canine kidney II cells transfected with L-APP677, which occurs naturally by alternative splicing of exon 15, secreted this isoform both apically and basolaterally, while maintaining the basolateral sorting of endogenous APPsec. This suggests that the alternative splicing of APP exon 15 modulates the polarized sorting of secretory APP. The same alternative splicing event also decreased the production of βA4 relative to p3. This is the first example of alternative splicing regulating polarized trafficking of a secretory protein.

The Alzheimer βA4-amyloid precursor protein (APP) is a ubiquitously expressed transmembrane glycoprotein. It occurs in eight different isoforms that are generated by alternative splicing of the APP exons 7, 8, and 15 (4). APP lacking the residues encoded by exon 15 is denoted L-APP (5). L-APP isoforms have been found to be the major isoform in several different tissues (4). While exon 15 is part of the divergent region of the APP family, a similar splice pattern exists for another member of the APP family, the APLP2 gene (6, 7). In the APP region of the APP family, a similar splice pattern exists for another member of the APP family, the APLP2 gene (6, 7). In both proteins, alternative splicing may result in the addition of a chondroitin sulfate side chain (8–10). All APP isoforms including L-APP are secreted from secretory forms by as yet unidentified proteases termed APP secretases. Secretase activities are involved in release of APPsec as well as in the production of a 4-kDa (βA4) and 3-kDa (p3) peptides (for a recent review, see Refs. 11 and 12). βA4 is the principal component of the amyloid plaques found in Alzheimer’s disease brains. Point mutations in the APP gene increasing the amount or the length of βA4 released segregate with familial disease and lead to Alzheimer’s disease at a certain age of the affected person, demonstrating an association between βA4 production and Alzheimer’s disease pathogenesis.

Here, we have analyzed the influence of splicing on APP sorting and βA4 release. For this purpose we used MDCK cells, which are a widely used model for investigations of polarized secretion of proteins. Two cellular domains can be distinguished: the basolateral side, which in the kidney would be exposed to the basal membrane and is engaged in cell-matrix interactions, and the apical side, which would be exposed to the kidney tubular lumen. Sorting of transmembrane proteins, including APP (13–15) to the basolateral domain generally appears to be dominantly determined by short cytoplasmic signals. However, identification of the signals required for basolateral targeting of soluble proteins or for apical sorting in general, with the exception of glycosylphosphatidylinositol-anchored proteins, has been a long and unsuccessful process (for a recent review, see Refs. 16 and 17).

The APP cytoplasmic signal including the QYTSI motif has been shown to result in efficient sorting of transmembrane APP to the basolateral side (14). Removal of this signal causes the transmembrane APP to become distributed equally on both sides and concomitantly increases the release of βA4 to the apical rather than the basolateral side, to which it is usually restricted. However, this mutation does not affect the polarized secretion of APPsec to the basolateral domain, suggesting that an additional determinant of basolateral sorting is present in the luminal domain and that the majority of APPsec is generated intracellularly (14, 15, 18).

In this study, we investigated the polarized secretion of an alternatively spliced form of APP, L-APP. Surprisingly, we found that L-APPsec is sorted differently than is APPsec. Furthermore, we show that this alternative splicing also results in a decrease in the generation of βA4 relative to p3.

MATERIALS AND METHODS

DNA—Human APP cDNAs APP695, APP751, APP770, L-APP677, L-APP677A, L-APPex16ASTOP, APP695ΔCHO, APP695y, and SPA4CT (3) were cloned into the vector pHD (19) for MDCK studies. Human APP cDNAs APP695, APP751, and L-APP677 were also cloned into the vector pCEP4 (Invitrogen) for COS cell studies. L-APP677A and L-APPex16ASTOP, containing a Ser to Ala, respectively, Ser to Leu substitution at position 563 (L-APP677 numbering), was generated by polymerase chain reaction-mediated mutagenesis. APPex16ASTOP contains an artificial stop codon at position 565 (L-APP677 numbering). The sequence of the polymerase chain reaction-amplified regions was

1 The abbreviation used are: APP, amyloid precursor protein (for convenience, all APP isoforms containing the residues encoded by exon 15 will be referred to as APP, those devoid of these residues as L-APP); MDCK, Madin-Darby canine kidney; APLP2, amyloid precursor like protein; GAG, glycosaminoglycan.

* This work was supported by SFB 317, the Bundesministerium für Bildung und Forschung of Germany through Grant 030666A, Fonds der Chemischen Industrie, the Forschungsschwerpunkt Baden-Württemberg, and the National Health Medical Research Council of Australia. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Zentrum für Molekulare Biologie University of Heidelberg, D-69120 Heidelberg, INF282, Tel.: 49-6221-546847; Fax: 49-6221-545891; E-mail: iy9@ix.urz.uni-heidelberg.de.

‡ Both authors were funded by the Deutsche Forschungsgemeinschaft (Graduiertenkolleg Neurobiologie Universität Heidelberg).

§ Both authors were funded by the Deutsche Forschungsgemeinschaft (Graduiertenkolleg Neurobiologie Universität Heidelberg).

¶ To whom correspondence should be addressed: Zentrum für Molekulare Biologie University of Heidelberg, D-69120 Heidelberg, INF 282, Tel.: 49-6221-546847; Fax: 49-6221-545891; E-mail: iy9@ix.urz.uni-heidelberg.de.
confirmed by sequencing both DNA strands of the subcloned polymerase chain reaction product. APP695 was generated by EcoRI and CiaI digestion of pBluescript (Life Technologies, Inc.). Stable transfectants were selected in 300 μg/ml g4000 into MDCK II cells using Lipofectin (Life Technologies, Inc.) according to the manufacturer’s instructions.

FIG. 1. Schematic diagram of the protein structure of APP and constructs used. Absence of the exon 15 domain resulted in apical as well as basolateral secretion of APPsec. Further deletion removing also the neighboring regions of exon 15 led to recovery of the polarized secretion to the basolateral side. SP = signal peptide; M = membrane domain. Δ, β, α, γ = APP secretes cleavage sites. S → A, serine to alanine mutation; S → L, serine to leucine mutation, abolishing GAG modification of L-APP. Thin lines indicate alternatively spliced exons of APP. Broken lines indicate deleted regions.

Cell Culture and Transfections—All cell culture media were obtained from Sigma. MDCK II cells were obtained from Kai Simons (EMBL, Heidelberg, Germany) and cultivated as described (23). The pHd vector containing APP cDNA was cotransfected with the selectable vector pSV2neo at a ratio of 1:20 into MDCK II cells using Lipofectin (Life Technologies, Inc.). Stable transfectants were selected in 300 μg/ml gentamycin (Life Technologies, Inc.) and subcloned. A minimum of three independent clones expressing the transfected protein in roughly equal amounts were endogenous APP were selected for further analysis, except for L-APPex16A1STOP (two clones). COS 7 cells were cultivated in MEM/F-12 medium containing 10% fetal calf serum. The pCEP4 vector containing APP cDNA was transfected into COS 7 cells using Lipofectin (Life Technologies, Inc.) according to the manufacturer’s instructions. Stable transfectants were selected in 300 μg/ml G418 (Boehringer Mannheim). Polarity Assay—Only experiments in which endogenous (Kunitz-type protease inhibitor containing) APP was sorted to the basolateral chamber with an efficiency >90% were selected for further analysis. In addition, all established MDCK II cell clones were analyzed for polarity using the [35S]methionine uptake assay (24). Uptake was set as 1% apical. Sorting Assay—Stably transfected MDCK cells were plated on 24-mm collagen transwell chambers (Costar) with a porous size of 0.4 μm and analyzed after 4–6 days. Sodium butyrate was not used to enhance expression. Cells were used for up to 12 passages (total including transfection). Sorting of endogenous Kunitz-type protease inhibitor containing APP was used as an internal control to assay the polarity of the cells.

Antibodies—The following antibodies were used: 692 polyclonal rabbit antisemurum raised against synthetic βA4 peptide 1–40, G210 monoclonal antibody specific for βA4 ending with amino acid 40 and polyclonal anti-FdAPP (2) as well as monoclonal antibody 22C11 (25) (both against APP ectodomain).

Immunoprecipitation and Western Blot Detection—APP from overexpression constructs was precipitated using 692 (1:50) or G210 (20 μg/ml, 1:500) as described (26). The samples were separated on 11% polyacrylamide gels (28). Band intensity was determined using a Fuji phosphorimager BAS 1000.

RESULTS

Sorting of APP and L-APP in Polarized MDCK II Cells—Recent evidence suggested that APP contains two independent basolateral sorting signals. One is located in the cytoplasmic domain and controls the sorting of transmembrane APP; the other, used for basolateral sorting of APPsec, is thought to reside in the luminal domain of APP (14, 15, 18) and does not influence the distribution of transmembrane APP. In order to identify the luminal sorting signal, we expressed different APP constructs (Fig. 1) in stably transfected MDCK II cells and analyzed the amount of secreted APP in both the apical and basolateral chambers. As expected, APP695, APP751 (data not shown), APP695γ, and endogenous APP containing the Kunitz-type protease inhibitor exon (APP751 and/or APP770) were sorted basolaterally (mean value 90%basolateral) (Fig. 2 and Fig. 4). In contrast, L-APP677 tended to be secreted apically (65% apical), although the absolute values were variable between individual experiments (absolute variation ± 25 apical, standard deviation ± 15.3) (Fig. 3 and Fig. 4). This variation did not depend on the expression level or on the individual clone analyzed (data not shown). The observation that L-APP is secreted differently than is APP suggests that L-APP contains a different sorting signal, probably generated by the alternative splicing of exon 15. Several mechanisms might be postulated: (a) exon 15 itself might contain a dominant luminal basolateral sorting signal or an essential part of this; (b) the fusion of exon 14 to exon 16 might give rise to a new...
cells. This deletion extends from exon 12 to the beginning of exon 16. If the GAG or any other modification at this site functioned as an apical sorting signal, the mutant protein should revert to basolateral sorting. Expression of L-APP677A, in contrast to L-APP677, did not generate high molecular weight APP, demonstrating that the L-APP677A does not contain GAG (Fig. 6). However, expression of L-APP677A in MDCK II cells resulted in apical sorting of L-APP677Asec equal to or only slightly less than that of L-APPsec, indicating that the GAG has little or no enhancing effect on apical sorting of L-APP (Fig. 4). Also, a soluble L-APP mutant (L-APPex16ASTOP), which is not a substrate to APP secretases (α, β, γ, δ), nor does it contain their cleavage sites, was secreted apically as well as basolaterally, similar to the other L-APP constructs (Figs. 3B and 4).

βA4 and p3 Generation—Upon observing that L-APP is sorted differently than APP, we decided to investigate whether it also differs with regard to βA4/p3 production. We therefore analyzed the polarized secretion of βA4 and p3 for APP695 and L-APP677 as well as for the truncated construct SPA4CT lacking the complete luminal domain of APP except for the extramembranous part of βA4. In all cases, we found the secretion of the peptides βA4 and p3 to be restricted to the basolateral chamber and thus parallels the secretion of APP and confirms earlier reports of βA4 and p3 secretion for non-L-APP isoforms of APP (data not shown; Refs. 13–15). Since βA4 production in this MDCK II strain is low, we conducted further investigations using African green monkey kidney epithelial cells (COS 7), stably transfected with L-APP677, APP695, or APP751.

The observation, that L-APP is sorted differently than is APP, raised the question whether L-APP is also processed differently by APP secretases. Among the secreted low molecular weight fragments, p3 is generated by the action of α-secretase while βA4 is generated by β-secretase. βA4 is not degraded into p3 but both peptides seem to be formed via parallel mechanisms from different APP pools or degradative intermediates (30). If APP and L-APP isoforms differ with respect to βA4 or p3 generation, APP695 and APP751 should be processed similarly, while L-APP677 should differ. COS 7 cells were thus stably transfected with L-APP677, APP695, and APP751, metabolically labeled with [35S]methionine, and the p3 and βA4 peptides immunoprecipitated with specific antibodies (Fig. 7, A and B).

In order to verify that our polyclonal antibody 692 is equally sensitive to βA4 and p3, we used the monoclonal antibody G210, which is specific for βA4 sequences ending with amino acid 40 (βA4 numbering). Such an antibody should detect βA4, and p3 with equal efficiency. We found that the βA4p3 ratio did not differ from the results obtained with antibody 692 (data not shown).

Whereas the βA4p3 ratio was 0.21 for APP695 and APP751, the ratio decreased to 0.14 for L-APP (p < 10−7; Student’s t test). These ratios were highly reproducible in different experiments and were independent of the absolute levels of (L)-APP, βA4 and p3 being produced (Fig. 7B). Also, donor variation seemed not to influence the βA4p3 ratios, since stably expressing cells were used from three different transfections for APP695 and L-APP677 (two for APP751) and found to secrete βA4p3 in the same ratio for each construct independent of the transfection. In addition to p3 and βA4, we observed one or two bands migrating between the major βA4 band and p3 (reviewed proteins, carbohydrates could function as an apical sorting signal (16, 17). This could be the case for L-APP, because a chondroitin sulfate glucosaminoglycan (GAG) may be added to at least some of the L-APP molecules (9, 10, 29). This addition can be inhibited by mutation of the serine residue 563 (APP677 numbering) at the beginning of exon 16 (9). If the GAG or any other modification at this site functioned as an apical sorting signal, the mutant protein should revert to basolateral sorting.

The possibility that the fusion of exon 14 to exon 16 generates a dominant apical signal was analyzed using a point mutation (L-APP677A). It has been postulated that in some
in Ref. 12), which we refer to as p3 related peptides. These peptides presumably contain longer p3 species that were cleaved more N-terminally from the major \( \alpha \)-secretase site at Lys16/Leu17, since they were not detected by monoclonal antibodies specific for \( \beta \)A4 1–8 (data not shown). The intensity of these two bands was correlated with p3 rather than with \( \beta \)A4, as both p3 and p3 related peptides were increased with L-APP expressing cells compared with APP-expressing cells. However, the upper band could not always be observed.

Pulse-chase experiments showed overall parallel kinetics of the transfected secretory proteins and the 3–4 kDa peptides (Fig. 8, A and B).

**DISCUSSION**

APP is synthesized in cells as a transmembrane precursor protein that is cleaved to generate soluble APP (APPsec), which is subsequently secreted. This secretion has been shown to be polarized in MDCK cells and to depend on unknown luminal signals. In contrast, the sorting of transmembrane APP and other transmembrane proteins has been found to depend on short signals on the cytoplasmic tail of these proteins. The mutant protein APP695\( _{\alpha} \) unlike APP and L-APP will not integrate into cellular membranes and lacks cytoplasmic sequences responsible for the polarized sorting of transmembrane APP. However, APP695\( _{\gamma} \) was secreted basolaterally. It therefore shows the influence of APP-luminal sequences on polarized sorting of soluble APP. Similar results have been obtained for APP695\( _{\beta} \) and APP695\( _{\alpha} \), mutants that end at \( \alpha \)-secretase and \( \beta \)-secretase sites, respectively (14, 15). These observations make it very likely that APP contains at least one luminal sorting signal for soluble APP.

In this study, we show that the sorting of soluble APP is regulated by alternative splicing. The very same splicing event also influences the release of \( \beta \)A4 and p3 and thus might be of concern in Alzheimer’s disease pathology. Expression of L-APP resulted in increased apical secretion of the soluble derivative L-APPsec as compared with expression of APP in MDCK II cells (Figs. 3A and 4). L-APP is distinguished from APP by the absence of the residues encoded by the alternatively spliced exon 15. It has recently been reported that the absence of exon 15 residues results in the generation of a GAG attachment signal (9, 10, 29). Glycosylation has been speculated to be a possible sorting signal (16, 17).

We thus analyzed the polarized sorting of L-APP677A. This mutant lacks the serine residue in the GAG attachment site and hence is not modified by GAG. The sorting of L-APP677A was similar to the sorting of L-APP677 (Fig. 4) and it is concluded that the reported carbohydrate addition specific to L-APP does not play a major role in APP sorting in MDCK II cells. The observation that L-APP is secreted differently as compared with APP must therefore depend on a different mechanism generated by the alternative splicing of exon 15. Several mecha-
anisms might be postulated. First, exon 15 itself might contain a dominant luminal basolateral sorting signal or an essential part of this signal. The basolateral secretion, observed with the deletion clone APP<sup>D</sup>CHO, provides strong evidence that exon 15 itself does not contain the basolateral sorting signal for APPsec. If it were, the deletion of exon 15 as well as the neighboring regions would remove the dominant basolateral sorting signal for APPsec and would cause the mutant protein, like L-APP, to be sorted randomly. However, APP<sup>ΔCHO</sup>sec was stringently sorted basolaterally (Figs. 4 and 5).

Thus exon 15 alone cannot be responsible for basolateral sorting. Furthermore the results obtained with APP<sup>ΔCHO</sup> suggest, that if basolateral sorting for soluble proteins requires the presence of a specific signal, such a signal must be present on the first half of the luminal domain of APP or that more than one basolateral signal coexists on APPsec. APP<sup>ΔCHO</sup>sec does contain in addition to the first N-terminal half of the APP luminal domain also the luminal domain of β<sub>A4</sub>. We cannot exclude that the latter domain influences sorting of the transmembrane APP/APP<sup>D</sup>CHO. However, it seems unlikely that they are important for the sorting of APPsec since APP<sub>α</sub> as well as APP<sub>β</sub> (which is devoid of the β<sub>A4</sub> domain) have been reported to be secreted basolaterally (14, 15).

A second possibility is that in L-APP a more distant signal is inactivated due to a change in conformation. Since neither the GAG modification of L-APP is necessary for apical sorting nor exon 15 itself contains a basolateral signal, it might be postulated that the effect observed in L-APP would be conferred by a more complex structural phenomenon. A structural difference between L-APP and APP is further supported by the finding that L-APP expressed in E. coli, without carbohydrate modification migrates at an aberrantly high molecular weight in SDS gel electrophoresis, as compared with APP<sup>3</sup> APP and L-APP sorting could be controlled by a more complex signal such as a structural domain, as previously suggested for luminal signals.

<sup>3</sup> L. Hesse and G. Multhaup, unpublished observation.
Such a structure has been described as the luminal sorting signal of chromogranin B, a protein normally sorted to secretory granules in the regulated secretory pathway (32). A structural domain important for sorting could be either disrupted or created by alternative splicing out exon 15, i.e. removal of exon 15 could either interfere with the binding of L-APP to proteins involved in basolateral sorting or generate a new signal that leads to apical sorting. In principle, a third explanation for differences in polarized sorting could be an unequal distribution of APP secretases. However, the results obtained here and by others (14, 15) show that soluble APP mutants (APPβ, APPα, APPγ, and L-APPex16ASTOP), that are not substrates for APP secretase are still sorted according to the presence or absence of exon 15. This conclusion is further supported by Haass et al. (14), which showed that transmembrane APP, which has been targeted by mutation of the cytoplasmic signal QYTSI to 50% to the apical membrane, still was released stringently basolaterally after cleavage by the APP secretases. A possible accumulation of intracellular L-APPsec after secretase cleavage in a compartment incapable of sorting seems unlikely, since L-APPex16ASTOP, which does not undergo secretase cleavage, was also secreted similarly to L-APP. In addition, L-APPex16ASTOP contains none of the binding sites identified for APP secretases.

Since APP is a transmembrane molecule with secretory derivatives, a differential basolateral versus apical localization of APP α-secretase could be used as a mechanism for polarized sorting. However, this mechanism seems unlikely to play a role in the sorting differences observed for the truncated constructs L-APPex16ASTOP and APPγ, which are not substrates for APP-secretases, but are sorted according to the presence or absence of exon 15-encoded residues. Since the APP secretases are not known, we cannot completely rule out the possibility of differences at the level of secretases, but in light of the results with the above soluble mutants, this possibility seems less likely.

The conditions for polarized sorting of soluble proteins are not known. It has been speculated that either basolateral or apical sorting does not require a specialized sorting signal or that ubiquitous signals are being used for a default pathway (16, 17). If basolateral sorting was used as a default pathway, the results obtained with APPsec, L-APPsec, APP695γ, and APP1CHO could be explained by the generation of a new (dominant) apical sorting signal in L-APP due to the fusion of exon 14 to exon 16. If apical sorting was used as the default pathway, a basolateral signal would be disrupted or interfered in L-APP, resulting in constitutive apical sorting. Sorting of L-APPsec is more variable than sorting of APPsec. This could reflect true variability or may indicate that L-APPsec sorting is additionally modulated by factors that do not influence the sorting of APP.

While alternate splicing of exon 15 can lead to alterations in polarized sorting, the actual identity of a putative luminal (apical) sorting signal remains unclear.

Amyloid Production—The difference found in polarized sorting of APP and L-APP raised the possibility that βA4 production or sorting is also affected by alternative splicing. βA4 was sorted basolaterally by APP as well as by L-APP expressing cells. While transmembrane APP was not studied here, this is interesting as it shows that the transmembrane products of the APP-secretases α and β (A4CT and p3CT) are sorted according to the cytoplasmic signal and independently of the L-APPsec sorting signal.

While βA4 was sorted basolaterally when produced from APP as well as from L-APP, a decrease of βA4 relative to p3 was observed in L-APP expressing cells, which was not due to differences in the overall secretory kinetics of either βA4, p3, APPsec, or L-APPsec in the transfected cells.

This difference in p3 and βA4 generation might be of importance in the generation of plaques, since N-terminally shorted βA4 peptides (including p3) should contribute differently to the aggregation process of βA4 to amyloid. Altered production of βA4 could be either directly or indirectly linked to the altered polarized sorting of L-APP. This could be due to a difference in subcellular compartmentalization of APP and L-APP. Alternatively, a difference in primary or secondary structure in L-APP could also inhibit the β-secretase. The relative increase of p3-related peptides possibly suggests that these peptides are derived by a mechanism related to α-secretase and not β-secretase.

In summary, the results presented here show that L-APP is both sorted and processed differently than APP. The difference in polarized sorting shown here, combined with the different tissue distributions (4), suggests that L-APP differs in function from APP and that the GAG attachment may be involved in this function.

Very recently, a downstream exon has been identified in the nonhomologous region of APLP2 that is alternatively spliced to generate a chondroitin sulfate attachment site (8). However, this modification does not influence the polarized secretion in MDCK cells (33), which is in line with our findings, that this GAG modification alone does not influence polarized secretion of APP. The general homology of APP and APLP2 splicing does seem to make it plausible that L-APP and L-APLP2 might be sorted similarly, but this is not the case. At the protein level, however, the sequences at and around exon 15 of APP show no homology to APLP2. Thus a prediction for the sorting of L-APP and L-APLP2 based on the homology might be difficult.

Several lines of evidence suggest that the amount of βA4 produced is an important factor in the development of Alzheimer’s disease (31). In light of the differences in βA4 generation between APP and L-APP shown in this report, one could speculate that L-APP might contribute differently than APP to the development of amyloid plaques or even have a protective effect. It is interesting that the brain is the only organ with established βA4 amyloid deposition in Alzheimer’s disease and that neurons are the only cell type expressing high amounts of APP but only low amounts of L-APP (4).

Acknowledgment—We are grateful to K. Simons for the gift of MDCK II cells.

REFERENCES
1. Kang, J., Lemaire, H. G., Unterbeck, A., Salbaum, J. M., Masters, C. L., Grzeschik, K. H., Mutthaup, G., Beyreuther, K., and Muller, H. B. (1987) Nature 325, 733–736.
2. Weidemann, A., König, G., Bunke, D., Fischer, P., Salbaum, J. M., and Beyreuther, K. (1989) Cell 57, 115–126.
3. Dyrrs, T., Weidemann, A., Mutthaup, G., Salbaum, J. M., Lemaire, H. G., Kang, J., Muller-Hill, B., Masters, C. L., and Beyreuther, K. (1988) EMBO J. 7, 949–957.
4. Sandbrink, R., Masters, C. L., and Beyreuther, K. (1994) J. Biol. Chem. 269, 1510–1517.
5. König, G., Mönning, U., Czech, C., Prior, R., Banati, R., Schreiter Gasser, U., Bauer, J., Masters, C. L., and Beyreuther, K. (1992) J. Biol. Chem. 267, 10804–10809.
6. Sandbrink, R., Masters, C. L., and Beyreuther, K. (1994) J. Biol. Chem. 269, 10427–10434.
7. Wada, A., Nakahara, H., Arai, H., and Suzuki, S. (1987) Nature 325, 733–736.
8. Weidemann, A., König, G., Bunke, D., Fischer, P., Salbaum, J. M., and Beyreuther, K. (1989) Cell 57, 115–126.
9. Thirukkan, G., and Söldna, M. S. (1994) J. Biol. Chem. 269, 22099–22100.
10. Poulou, M. N., Ethimosikou, S., Shid, J. P., and Robakis, N. K. (1995) J. Biol. Chem. 270, 10388–10391.
11. Sandbrink, R., Masters, C. L., and Beyreuther, K. (1996) Ann. N. Y. Acad. Sci. 777, 281–287.
12. Evins, G., Beyreuther, K., and Masters, C. L. (1994) Amyloid: Int. J. Exp. Clin. Invest. 1, 263–280.
13214 Alzheimer's Disease βA4 Protein Release and APP Sorting

Van Leuven, F., and Van Den Berghe, H. (1995) J. Biol. Chem. 270, 4058–4065
16. Matter, K., Yamamoto, E. M., and Mellman, I. (1994) J. Cell Biol. 126, 991–1004
17. Fiedler, K., and Simons, K. (1995) Cell 81, 309–312
18. Lo, A. C., Haass, C., Wagner, S. L., Teplow, D. B., and Sisodia, S. S. (1994) J. Biol. Chem. 269, 30966–30973
19. Muller, G., Ruppert, S., Schmid, E., and Schutz, G. (1988) EMBO J. 7, 2723–2730
20. Dyrks, T., Dyrks, E., Hartmann, T., Masters, C., and Beyreuther, K. (1992) J. Biol. Chem. 267, 16210–16217
21. Hartmann, T., Dyrks, T., Weidemann, A., Masters, C. L., and Beyreuther, K. (1994) Neurobiol. Aging 15, 551
22. Maruyama, K., Kawamura, Y., Asada, H., Ishiura, S., and Obata, K. (1994) Biochim. Biophys. Res. Commun. 202, 1517–1523
23. Simons, K., and Virta, H. (1994) in Cell Biology-A Laboratory Handbook (Celis, J. E., ed) Vol. 1, pp. 225–231, Academic Press, San Diego
24. Hunziker, W., and Mellman, I. (1989) J. Cell Biol. 109, 3291–3302
25. Hillich, C., Monning, U., Grund, C., Masters, C. L., and Beyreuther, K. (1993) J. Biol. Chem. 268, 26571–26577
26. Dyrks, T., Dyrks, E., Monning, U., Urmoniok, B., Turner, J., and Beyreuther, K. (1993) FEBS Lett. 335, 89–93
27. Laemmli, U. K. (1970) Nature 227, 680–685
28. Schägger, H., and Von Jagow, G. (1987) Anal. Biochem. 166, 368–379
29. Shio, J., Pangalos, M. N., Ripellino, J. A., Vassilacopoulos, D., Mytilineou, C., Margolis, R. U., and Robakis, N. K. (1995) J. Biol. Chem. 270, 11839–11844
30. Haass, C., Hung, A. Y., Schlossmacher, M. G., Teplow, D. B., and Selkoe, D. J. (1993) J. Biol. Chem. 268, 3021–3024
31. Selkoe, D. J. (1994) J. Neuropathol. Exp. Neurol. 53, 438–447
32. Chanat, E., Weiss, U., Huttner, W. B., and Tooze, S. A. (1993) EMBO J. 12, 2159–2168
33. Loo, A. C. Y., Thinakaran, G., Slunt, H. H., and Sisodia, S. S. (1995) J. Biol. Chem. 270, 12641–12645
Alzheimer's Disease βA4 Protein Release and Amyloid Precursor Protein Sorting Are Regulated by Alternative Splicing

Tobias Hartmann, Christian Bergsdorf, Rupert Sandbrink, Pentti J. Tienari, Gerd Multhaup, Nobuo Ida, Sophie Bieger, Thomas Dyrks, Andreas Weidemann, Colin L. Masters and Konrad Beyreuther

*J. Biol. Chem.* 1996, 271:13208-13214.
doi: 10.1074/jbc.271.22.13208

Access the most updated version of this article at [http://www.jbc.org/content/271/22/13208](http://www.jbc.org/content/271/22/13208)

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 33 references, 16 of which can be accessed free at [http://www.jbc.org/content/271/22/13208.full.html#ref-list-1](http://www.jbc.org/content/271/22/13208.full.html#ref-list-1)