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COPI-mediated retrograde transport is required for efficient γ-secretase cleavage of the amyloid precursor protein ☆

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Abstract

Sequential cleavage of the amyloid precursor protein (APP) by β- and γ-secretases results in the production of β-amyloid peptide, which is a key determinant in Alzheimer's disease. Since several putative locations for γ-secretase cleavage have been identified along the secretory pathway, trafficking of APP may be of importance for β-amyloid peptide production. Here we have studied the role of retrograde transport in APP processing. We found that APP interacts with the β subunit of the coatomer protein I (COPI) complex, which is involved in retrograde transport. In line with a role of retrograde trafficking in APP transport, inhibition of COPI-dependent transport altered APP trafficking, decreased APP cell surface expression, and coincided with a profound reduction in γ-secretase cleavage. These results suggest that COPI-dependent retrograde transport is important for APP processing and influences production of β-amyloid peptide.

Keywords: β-Amyloid; Alzheimer's disease; APP; Retrograde transport; COPI; Neurodegeneration

Alzheimer's disease (AD) is characterized by the accumulation of β-amyloid (Aβ) peptide in the brain [1]. Although the molecular mechanism for Aβ peptide toxicity is not clear, a large body of evidence supports the notion that Aβ peptide plays a key role in the pathophysiology of AD [2]. Aβ peptide is derived from the amyloid precursor protein (APP), which is a type I transmembrane protein that travels to the cell surface through the secretory pathway. Sequential cleavage of APP by β- and γ-secretases gives rise to the Aβ peptide [3].

The cleavage by the γ-secretase complex is most interesting as a therapeutic target since it can give rise to two different Aβ fragments, $A\beta_{1-40}$ and $A\beta_{1-42}$, of which the longer fragment is more aggregation-prone and notoriously linked to AD [4]. Hence it is important to understand γ-secretase-mediated proteolytic cleavage of APP and to identify external and internal factors that influence APP processing. An important conundrum is the precise location of γ-secretase cleavage [5]. The γ-secretase complex is assembled in the endoplasmic reticulum (ER) and the Golgi compartments. This implies that APP is exposed to the γ-secretase complex throughout the secretory pathway. Accordingly, it has been shown that Aβ peptide generation

☆ *Abbreviations:* AICD, β-APP intracellular domain; APP, amyloid precursor protein; Aβ, β-amyloid; AD, Alzheimer's disease; Chapso, 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1propanesulfonate; COPI, coatomer protein I; APP CTFs, APP C-terminal fragments; DAPT, (*N*-[*N*-(3,5-difluorophenacetyl)-l-alanyl]-*S*-phenylglycine-*t*-butyl-ester); ER, endoplasmic reticulum; GVP, Gal4/VP16 fusion; L-685, 458, [1s-benzyl-4*R*-[1-(1s-carbonyl-2-phenylethylcarbomoyl)-1s-3-methylbutylcarbomoyl]-2*R*-hydroxy-5-phenylpentyl]carbamic acid *tert*-butyl ester; PBS, phosphate-buffered saline; PBS–GS/BSA, PBS containing 10% goat serum and 5% BSA.

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can take place in the ER, in the Golgi complex, and at the cell surface but the individual contributions of these locations to amyloidogenesis are unclear [6–8].

The ER has a sophisticated protein quality control machinery that keeps immature proteins from reaching the Golgi complex [9]. A number of studies suggest that immature proteins that have escaped the ER protein quality control are subject to retrograde transport from the Golgi complex back to the ER [10]. Once back in the ER, their maturation can be completed [11,12], or alternatively they can be disposed of by ER associated degradation [13]. Golgi to ER transport is mediated by at least two independent mechanisms, namely canonical retrograde transport dependent on the coatomer protein I (COPI) [14], and at least one additional transport pathway that does not require COPI [15]. Interestingly, it has recently been demonstrated that presenilin 1, which is part of the γ-secretase complex, is transported in COPI-coated vesicles [16,17]. Moreover, another retrograde protein transport pathway, which is dependent on the GTPase Rab6 but does not require COPI, seems to influence APP processing [18]. In addition, Rab6 membrane association is presenilin-dependent [19]. Together these data suggest that APP and the γ-secretase complex are both undergoing retrograde transport. Importantly, it is not known whether retrograde transport in any respect influences APP processing.

In the present investigation, we studied the role of COPI-mediated retrograde transport in trafficking and processing of APP. We found that inhibition of COPI has a profound effect on APP localization and strongly reduces cleavage of APP by the γ-secretase complex. Thus, retrograde transport of APP may be an important determinant for APP processing.

Materials and methods

Cell culture. The Chinese hamster ovary (CHO)-derived ldlF-2 cell line (hereafter ldlF-2 cells) and control CHO cells (hereafter control cells) were kindly provided by Dr. Monty Krieger (MIT, Cambridge, MD). LdlF-2 cells express a temperature-sensitive ε-COPI subunit [20]. Cells were grown at permissive (34 °C) or restrictive (39 °C) temperature in Ham's F-12 medium with 5 mM L-glutamine containing 5% fetal bovine serum, 100 μg/ml penicillin, and 100 μg/ml streptomycin (Invitrogen, Carlsbad, CA). Human embryonic kidney 293 (HEK293) cells were grown in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% fetal calf serum (Invitrogen), 10 U/ml penicillin, and 100 μg/ml streptomycin. All transfections were performed using Lipofectamine Plus reagent according to manufacturer's instructions (Invitrogen). Stable APP-expressing CHO, ldlF-2, and HEK293 cell lines were generated by transfection with pcDNA3.1 APPwt695 expression vector and subsequent selection with 150 μg/ml G418 (Invitrogen) for three weeks. Surviving clones were expanded and APP expression was analyzed by Western blot.

Immunoblotting. Cells were lysed in RIPA buffer (50 mM Tris, pH 8, 150 mM NaCl, 0.5% deoxycholic acid, 0.1% SDS, 1% NP-40, and protease inhibitor cocktail (Roche, Basel, Switzerland)) and equal amounts of protein were loaded on 10–20% Tricine gel (Invitrogen). The samples were blotted onto PVDF membrane (Bio-Rad, Hercules, CA) and analyzed using the following antibodies: mouse monoclonal 6E10 (1:2000 dilution; 1–17 aa of Aβ, Chemicon, Temecula, CA), mouse monoclonal C1/6.1 (1:1000 dilution; C-terminal APP, gift from Dr. Paul Mathews, Nathan Cline Institute, Orangeburg, NY), rabbit polyclonal 369 (1:1000 dilution; C-terminal APP, gift from Dr. Sam Gandy, Thomas Jefferson University, Philadelphia, PA), rabbit polyclonal anti-β-COPI (1:1000 dilution; Abcam, Cambridge, UK), and rabbit polyclonal anti-ε-COPI (1:2000 dilution; Santa Cruz, Santa Cruz, CA).

Immunoprecipitation. Control CHO, ldlF-2 cells, and HEK 293 cells stably transfected with APP were grown in 100 mm plates. 24 h posttransfection, the cells were harvested and solubilized in 500 μl CHAPSO buffer (50 mM Hepes, pH 7.4, 150 mM NaCl, 2 mM EDTA, 1% 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1propanesulfonate (CHAPSO), and protease inhibitor cocktail (Roche)), followed by centrifugation at 14,000 rpm for 30 min at 4 °C to remove insoluble material. The lysates were pre-cleared with 50 μl Protein G–Sepharose (GE Healthcare, Amersham, Little Chalfont, UK) for 1 h at 4 °C. Supernatants were incubated for 16 h at 4 °C with monoclonal anti-APP antibody 6E10 (1:200 dilution). Next 50 μl of protein G Sepharose was added and the sample was incubated for 1 h at 4 °C. The beads were washed three times with 1 ml of 1% CHAPSO buffer, eluted by adding 30 μl of 2× Laemmli buffer (Sigma, St. Louis, MO), and incubated for 20 min at 50 °C. Samples were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, transferred to PVDF membrane (Millipore), and probed with a polyclonal anti-β-COPI antibody (Abcam, Cambridge, UK).

Immunostaining. Control and ldlF-2 cells stably expressing APP were grown on sterile coverslips for 15 h at permissive or restrictive temperature. Cells were fixed with 2% paraformaldehyde and 0.2% glutaraldehyde for 20 min at 4 °C. For cell surface staining, cells were blocked in phosphate-buffered saline (PBS) containing 10% goat serum and 5% BSA (PBS–GS/BSA). The primary antibody used was anti-N-terminal APP (dilution 1:250; OMA1, Affinity Bioreagents, Rockville, MD) diluted in PBS–GS/BSA. Cells were incubated with primary antibodies for 3 h at 4 °C. After washing three times with PBS, the cells were incubated for 1 h with the appropriate secondary antibody diluted in PBS–GS/BSA. The secondary antibody used was Alexa Fluor 488 goat anti-mouse (dilution 1:300; Molecular Probes Europe, Leiden, The Netherlands). Cells were then rinsed three times with PBS and mounted using Vecta-shield. The immunostainings were analyzed using confocal laser scanning microscope (Carl Zeiss) and software (LSM 5 Image Browser).

Cell surface biotinylation. Control and ldlF-2 cells stably expressing APP were grown in 75 cm^2 flasks. Subsequently cells were treated with 100 μM chloroquine (Sigma) for 15 h at permissive and restrictive temperatures. Cells were then washed three times with ice-cold PBS and incubated in 5 ml of 1 mg/ml EZ-link Sulfo-NHS-LC-Biotin (Pierce, Rockford, IL) in PBS for 30 min at 4 °C. Biotin solution was then removed and cells were washed three times with ice-cold PBS. Cells were scraped off into ice-cold PBS and spun down by centrifugation at 3,000 rpm for 5 min at 4 °C. The pellet was lysed in 350 μl PBS containing 1% NP-40 and protease inhibitor cocktail (Roche), followed by brief centrifugation to remove insoluble material. Lysates were then added to 80 μl streptavidin–agarose beads from Pierce (Rockford), which had been pre-washed three times in PBS with 1% NP-40 and protease inhibitor. Samples were incubated with streptavidin beads for 16 h at 4 °C, spun down by centrifugation at 5,000 rpm for 3 min at 4 °C, and washed three times with ice-cold PBS with 1% NP-40 and once with ice-cold 0.9% NaCl. The beads were eluted by addition of 30 μl of 2× Laemmli buffer and boiling for 5 min. Samples were then analyzed by immunoblotting using antibody 369 against APP.

γ-Secretase cleavage assay. The cleavage assay has been previously described [21]. In brief, control and ldlF-2 cells stably expressing APP were transfected with 100 ng MH100 (Gal4/VP16 fusion (GVP)-regulated luciferase expression vector), and 50 ng CMV-β-galactosidase plasmid (to control for transfection efficiency) combined with either 100 ng control pcDNA3 plasmid or 100 ng C99-GVP plasmid (γ-secretase reporter). MH100 and CMV-β-galactosidase plasmid have been described previously [22]. Following transfection, cells were kept at permissive or restrictive temperature. Twenty-four hours after transfection, cells were harvested and analyzed for reporter gene activity using a bioluminescence reader. γ-Secretase inhibitors L-685,458 (10 μM), or *N*-[*N*-(3,5-difluorophenacetyl)-l-alanyl]-*S*-phenylglycine *t*-butyl ester (DAPT) (2.5 μM) were included in the medium during the recovery phase.

Results and discussion

APP interacts with the COPI complex

A recent histological study suggests that APP can be found in COPI-coated vesicles [16]. We wanted to biochemically address this issue by investigating whether APP interacts with the COPI complex in cells. We argued that, even though retrograde transported proteins do not necessarily have to interact with the COPI complex, a direct or indirect interaction between APP and COPI would strongly support the idea that APP is indeed subject to retrograde transport. We addressed this issue by immunoprecipitating under mild conditions APP from HEK293 cells, which express low levels of endogenous APP, and HEK293/APP cells, which overexpress ectopic human APP, and analyzing the precipitates for the presence of the β-COPI subunit. Notably, HEK293/APP cells express much higher levels of APP than wild-type HEK293 cells (Fig. 1A) We found that β-COPI was present in APP immunoprecipitates (Fig. 1B). The recovery was dose dependent with higher levels of β-COPI in the immunoprecipitates of the APP-overexpressing HEK293/APP cells and more moderate levels in wild-type HEK293 cells. These data support the notion that APP interacts with COPI, which strongly suggests that APP is subject to COPI-dependent retrograde transport.

β-COPI-APP interaction depends on integrity of the COPI complex

Next we investigated whether the interaction between APP–COPI interaction was dependent on the integrity of the COPI complex. For this purpose, we used the well-established ldlF-2 cells [20]. LdlF-2 cells are Chinese hamster ovary (CHO) cells that express a temperature-sensitive ε-COPI subunit. As a consequence, the COPI complex assembly is perturbed when ldlF-2 cells are grown at the restrictive temperature (39 °C) whereas COPI assembly and retrograde transport are functional at the permissive temperature (34 °C) [20]. For this study, we generated stable CHO and ldlF-2 cell lines that express equal levels of human APP (Fig. 2A).

First we analyzed in these stable cell lines the integrity of the COPI complex in ldlF-2 cells at permissive and restrictive temperatures. Culturing the cells at restrictive temperature caused a striking reduction in the levels of ε-COPI in ldlF-2 cells but not in control CHO cells (Fig. 2B, upper panel). This effect was specific since the levels of the β-COPI subunit were not affected (Fig. 2B, middle panel).

Next we studied the importance of the COPI integrity for the COPI–APP interaction. At permissive temperature, β-COPI was readily co-immunoprecipitated with APP from CHO and ldlF-2 lysates (Fig. 2C), similar as found

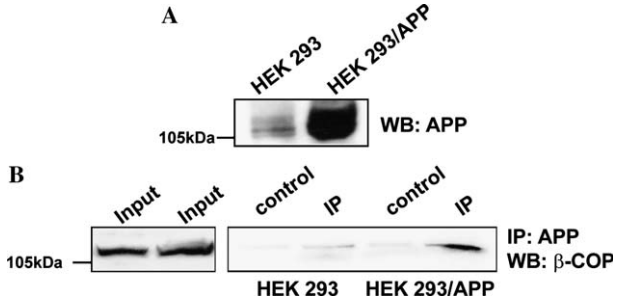

Fig. 1. APP interacts with the COPI complex. (A) Expression levels of APP in wild-type HEK293 and HEK293/APP cells. (B) Wild-type HEK293 and HEK293/APP cells were lysed and APP was immunoprecipitated under mild conditions. The lysates were incubated with Protein G Sepharose only (control) or anti-APP antibody with Protein G Sepharose. Total lysates (10%) and the immunoprecipitated sample were analyzed by Western blotting with a β-COPI-specific antibody. All samples were run, transferred, probed, and stained in parallel. Identical exposure times are shown.

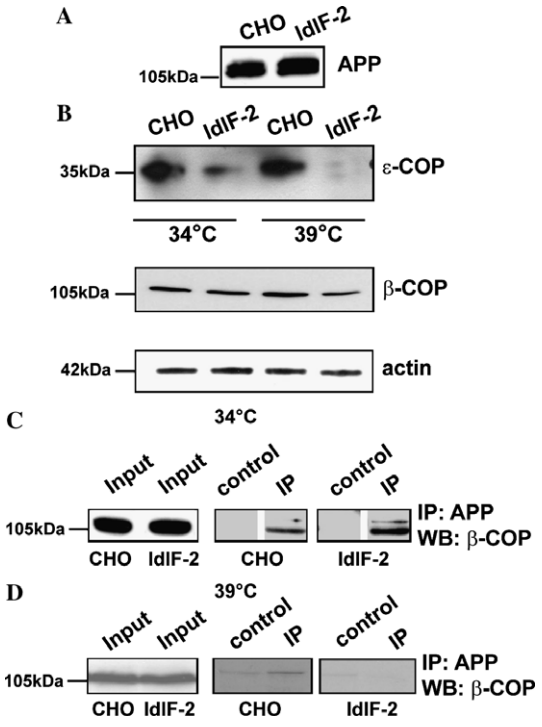

Fig. 2. β-COPI–APP interaction depends on integrity of the COPI complex. (A) Expression levels of human APP in control CHO and ldlF-2 cells stably transfected with an APP expression plasmid. (B) Expression levels of ε-COPI (upper panel), β-COPI (middle panel and loading control β-actin (lower panel) in control CHO and ldlF-2 cells at permissive (34 °C) and restrictive (39 °C) temperatures were analyzed by Western blot. ε-COPI could not be detected in lysates from ldlF-2 cells grown at the restrictive temperature, while the levels of β-COPI were unchanged in both wild-type and ldlF-2 cells at 34 °C and 39 °C. (C) APP was immunoprecipitated from control CHO and ldlF-2 lysates under mild conditions and probed with a β-COPI-specific antibody. APP was found to interact with β-COPI in both control CHO and ldlF-2 cells at permissive (34 °C) and restrictive (39 °C) temperatures. APP was no longer associated with β-COPI in ldlF-2 cells at the restrictive temperature. Total lysates (10%) and the immunoprecipitated sample were analyzed by Western blotting with a β-COPI-specific antibody. All samples were run, transferred, probed, and stained in parallel. Identical exposure times are shown.

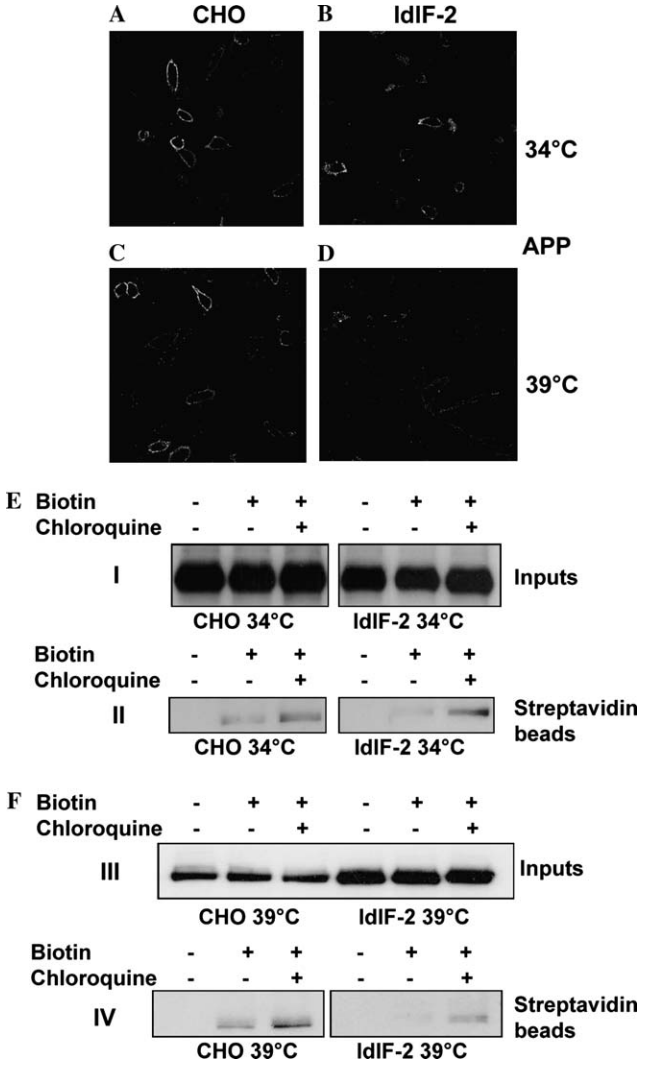

Fig. 3. APP cell surface expression is decreased in the absence of functional COPI complex. Cell surface APP staining in non-permeabilized cells in (A) control CHO cells, permissive temperature and (B) ldlF-2 cells, permissive temperature, (C) control CHO cells, restrictive temperature, and (D) ldlF-2 cells, restrictive temperature. Control CHO and ldlF-2 cells were cultured at (E) permissive and (F) restrictive temperatures in the absence or presence of 100 μM chloroquine for 16 h. The cells were biotinylated and biotinylated material were precipitated with streptavidin beads. Total lysate (input) and precipitated biotinylated material was probed in Western blotting with an APP antibody. Total lysates (10%) and the immunoprecipitated sample were analyzed by Western blotting with an APP specific antibody. All samples were run, transferred, probed, and stained in parallel. Identical exposure times are shown.

for HEK293 cells. Thus, this interaction was found in two cell lines obtained from different species (human and Chinese hamster) and of different origins (ovary and kidney) for both endogenous and ectopically expressed human APP. Importantly, the interaction between β-COPI and APP was abrogated in ldlF-2 cells, but not in control CHO cells, at the restrictive temperature (Fig. 2D). Since CHO and ldlF-2 cells express equal levels of β-COPI at permissive and restrictive temperatures, this strongly suggests that in the absence of a functional COPI complex APP does not interact anymore with the COPI complex.

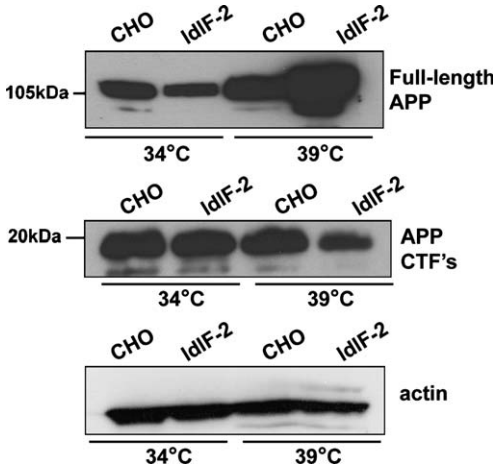

Fig. 4. APP processing is reduced in the absence of functional COPI complex. Cell lysates from control CHO and ldlF-2 cells grown at permissive and restrictive temperature were probed in immunoblotting with an antibody that is specific for the C terminus of APP. The bands representing full length APP and APP CTFs are shown. As a loading control the blot was reprobed with a β-actin-specific antibody.

APP cell surface expression is decreased in the absence of functional COPI complex

A number of studies indicate that retrograde transport is important for maturation of cell surface proteins [12]. We argued that if retrograde transport would be important for APP maturation, inhibition of retrograde transport was likely to cause a decrease in APP maturation and hence a downregulation in APP cell surface expression. However, if retrograde transport is irrelevant for APP maturation, one would expect rather an increase in cell surface expression since the APP that escaped from retrograde transport would now be transported to cell surface. We investigated the levels of APP at the cell surface in the presence or absence of functional COPI complex by immunostaining non-permeabilized control CHO and ldlF-2 cells with an APP antibody. The cell surface staining of CHO cells was not changed at 39 °C (Fig. 3A and C) whereas cell surface APP of ldlF-2 cells was strongly reduced at the restrictive temperature (Fig. 3B and D).

To obtain more quantitative data on the effect of COPI functionality, we performed cell surface biotinylation experiments. Control CHO and ldlF-2 cells were grown at permissive and restrictive temperatures, subjected to cell surface biotinylation and the levels of biotinylated APP were related to the total levels of APP. Since it has been described that endocytosis may also be compromised in the absence of COPI complex [23], we used this assay also to evaluate the status of APP endocytosis in COPI deficient cells by performing the experiments in the presence or absence of chloroquine, which inhibits endocytosis. At the permissive temperature, low levels of APP were found at the cell surface of CHO and ldlF-2 cells (Fig. 3E). As anticipated the levels were increased in both cell lines when endocytosis was inhibited by chloroquine confirming that

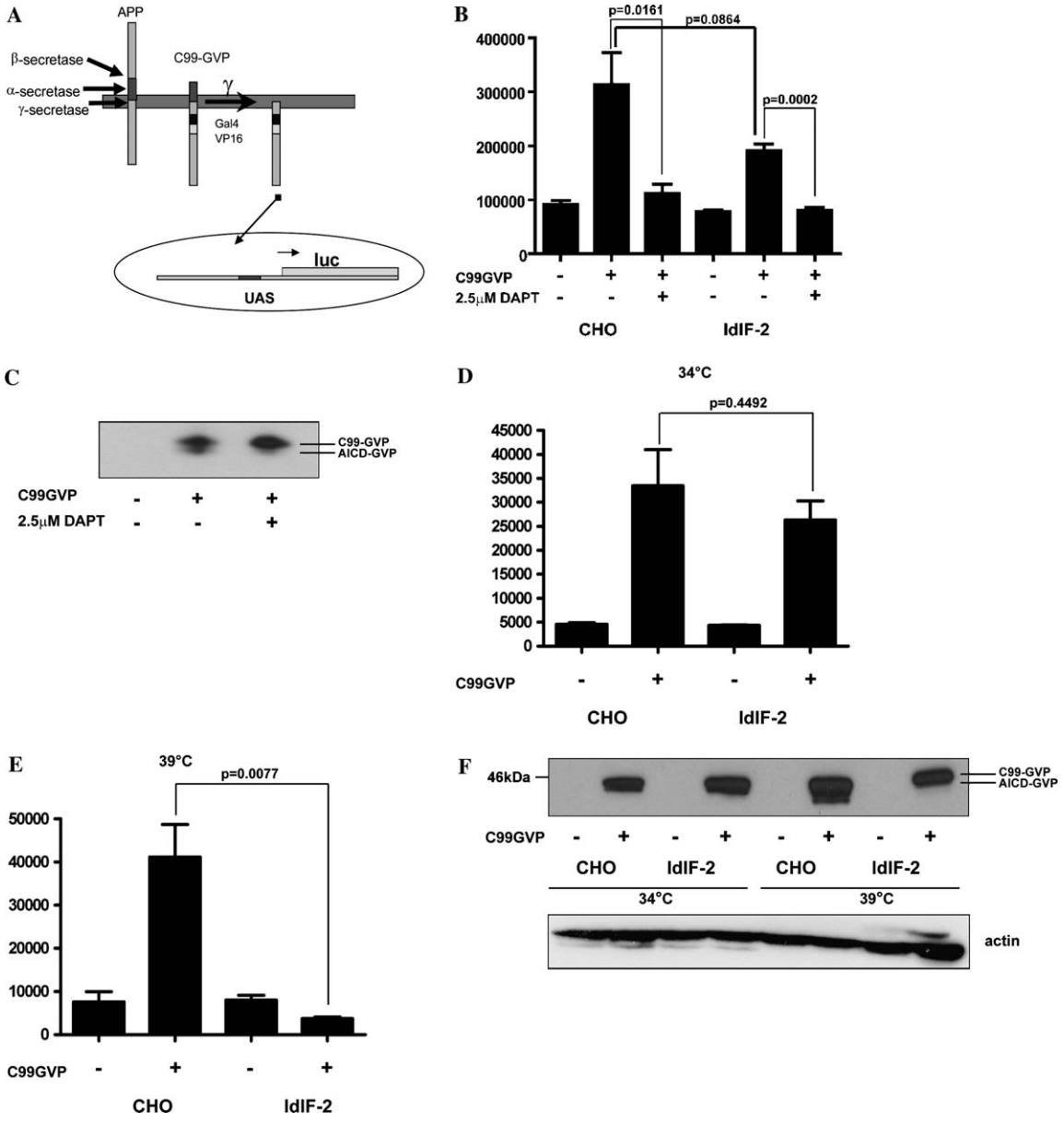

Fig. 5. Cleavage by γ-secretase is reduced in the absence of functional COPI complex. (A) γ-secretase reporter assay. Cells are transfected with a plasmid encoding a fusion of the APP fragment C99 fused to GVP, which is composed of the Gal4 DNA binding domain and VP16 transactivation domain, and a plasmid in which luciferase expression is driven by GVP. The C99–GVP fusion is membrane bound but upon cleavage by γ-secretase at the natural cleavage site in the APP fragment a soluble AICD–GVP fragment is released that translocates to the nucleus and activates luciferase expression. (B) Cleavage of C99–GVP was inhibited in both control CHO and ldlF-2 cells grown at 34 °C, following treatment with 2,5 μM γ-secretase inhibitor DAPT. Student's *t*-test was used to analyze the results of this experiment. $^{*}P < 0.05$, $^{***}P < 0.05$. There was no significant difference in luciferase activity between control CHO and ldlF-2 cells not treated with DAPT. (C) Western blot analysis of lysates from CHO cells transfected with C99–GVP using an APP-specific monoclonal antibody (C1/6.1). Amounts of AICD are reduced in the presence of γ-secretase inhibitor DAPT (2,5 μM). (D) Luciferase acitivity was recorded in control CHO and ldlF-2 cells transfected with C99–GVP and grown at the permissive temperature (34 °C). A representative experiment of three independent experiments is shown. Error bars represent standard error of the mean of triplicate samples in the experiment. Student's *t*-test was used to analyze the data. No significant difference was found in luciferase activity between control CHO and ldlF-2 cells. Luciferase activity recorded in (D) control CHO, and (E) ldlF-2 cells transfected with C99–GVP and kept at the restrictive temperature (39 °C) overnight. The chart is representative of three independent experiments and error bars show standard error of the mean of triplicate samples in this experiment. Cleavage of C99–GVP is significantly decreased in ldlF-2 cells. Student's *t*-test was used to analyze these results. $^{**}P < 0.05$. (F) Western blot analysis of lysates from control CHO and ldlF-2 cells transfected with C99–GVP and grown at the permissive or restrictive temperature. An APP-specific monoclonal antibody (C1/6.1) was used. The AICD band could not be detected in ldlF-2 cells grown at the restrictive temperature. The blot was probed with a β-actin-specific antibody to check for equal loading.

APP is internalized by endocytosis. The levels at the cell surface were reduced in ldlF-2 but not in control CHO cells at the restrictive temperature (Fig. 3F), confirming the model that retrograde transport stimulates APP maturation and cell surface expression. It is noteworthy that even though the total levels of APP were substantially higher in

ldlF-2 cells at the restrictive temperature as compared to control CHO cells (see also Section APP processing is reduced in the absence of functional COPI complex), we nevertheless retrieved much less biotinylated material from the ldlF-2 cells underscoring the effect of COPI retrograde transport on APP cell surface expression. Importantly, also at the restrictive temperature administration of chloroquine enhanced cell surface expression showing that endocytosis of APP is not affected in ldlF-2 cells at the restrictive temperature under our experimental conditions. Together these data show that a functional COPI complex is important for proper cell surface expression and suggests that retrograde transport contributes to APP maturation.

APP processing is reduced in the absence of functional COPI complex

Next we examined whether the absence or presence of a functional COPI complex affected APP processing. To this end, we detected by immunoblotting full length APP and APP C terminal fragments (CFTs) in control CHO and ldlF-2 cells at permissive and restrictive temperature. We found that the levels of full length APP were strongly increased in ldlF-2 cells when grown at the restrictive temperatures while a very modest increase was found in the control cells (Fig. 4). The increase in full length APP coincided with a decrease in APP CTFs (Fig. 4). This suggests that absence of COPI-mediated retrograde transport induces accumulation of full length APP and reduces APP processing.

Cleavage by γ-secretase is reduced in the absence of functional COPI complex

Although the fact that full length APP accumulated in the absence of functional COPI complex suggested that cleavage by both β- and γ-secretases is impaired, we decided to focus in the next set of experiments on γ-secretase cleavage since this particular cleavage event is most relevant for AD. For this purpose, we used a γ-secretase reporter assay that gives highly quantitative data on the rate of γ-secretase cleavage in living cells [21]. This assay is based on the expression of a fusion of C99, the APP domain containing the natural γ-secretase cleavage site, to the Gal4/VP19 (GVP) chimeric protein that drives expression of a luciferase reporter gene. Cleavage by γ-secretase releases from the membrane bound C99–GVP fusion AICD–GVP, which can translocate to the nucleus and induce luciferase expression (Fig. 5A).

As anticipated, high levels of luciferase were detected in control CHO and ldlF-2 cells that expressed the C99–VCP fusion and luciferase reporter plasmid. Luciferase activity was completely inhibited by administration of the specific γ-secretase inhibitor DAPT confirming that the assay detects specifically γ-secretase activity (Fig. 5B). Immunoblotting also showed that C99-GVP was cleaved to the slightly smaller fragment AICD-GVP and that this cleavage was sensitive to DAPT (Fig. 5C). Next we assessed the effect of COPI on γ-secretase cleavage of C99-VCP. No significant differences were found in γ-secretase cleavage of the reporter in control CHO and ldlF-2 cells at the permissive temperature (Fig. 5D). Strikingly, at the restrictive temperature, cleavage of the reporter was completely blocked in ldlF-2 cells while at the same temperature cleavage was not affected in control CHO cells (Fig. 5E). Immunoblotting also showed that at the restrictive temperature the generation of AICD–GVP was blocked (Fig. 5F). Together these data show that inhibition of retrograde transport has a profound effect on γ-secretase cleavage of APP.

In summary, we have demonstrated that inhibition of COPI-dependent pathways in cells expressing APP leads to: (i) decreased cell surface expression of APP, (ii) accumulation of full-length APP, (iii) decreased processing of C99 by γ-secretase, and (iv) decreased levels of AICD. Our results link together COPI-dependent retrograde transport and amyloidogenic processing of APP, gaining further insight into the molecular mechanisms involved in trafficking and processing of APP. Understanding of these mechanisms is essential for the identification of additional factors responsible for the cell's preference for amyloidogenic or non-amyloidogenic pathway.

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