Neurogenin 2 Mediates Amyloid-β Precursor Protein-stimulated Neurogenesis*

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Background: Amyloid-β precursor protein is implicated in neural stem cell development.

Results: Neurogenin 2 expression and neuronal differentiation correlated with amyloid-β precursor protein expression in neural stem/progenitor cells.

Conclusion: Amyloid-β precursor protein regulates neuronal differentiation by altering neurogenin 2 expression.

Significance: The study provides a new mechanism to explain the effects of amyloid-β precursor protein on neural stem cell differentiation.

Amyloid-β precursor protein (APP) is well studied for its role in Alzheimer disease, although its normal function remains uncertain. It has been reported that APP stimulates the proliferation and neuronal differentiation of neural stem/progenitor cells (NSPCs). In this study we examined the role of APP in NSPC differentiation. To identify proteins that may mediate the effect of APP on NSPC differentiation, we used a gene array approach to find genes whose expression correlated with APP-induced neurogenesis. We found that the expression of neurogenin 2 (Ngn2), a basic helix-loop-helix transcription factor, was significantly down-regulated in NSPCs from APP knock-out mice (APPKO) and increased in APP transgenic (Tg2576) mice. Ngn2 overexpression in APPKO NSPCs promoted neuronal differentiation, whereas siRNA knockdown of Ngn2 expression in wild-type NSPCs decreased neuronal differentiation. The results demonstrate that APP-stimulated neuronal differentiation of NSPCs is mediated by Ngn2.

Amyloid-β precursor protein (APP)2 is an integral type I transmembrane protein that is the precursor of amyloid-β protein (Aβ) of Alzheimer disease. Although APP has been well studied for its role in Alzheimer disease, little is known about the normal function of APP. A number of physiological functions have been attributed to APP including synapse formation (1) and stabilization (2, 3), neurite outgrowth promotion (4–8), stimulation of neuronal migration (9), intracellular signaling (10, 11), promotion of cell growth (12–14), neural repair (15–17), and neurogenesis (18–23). However, the mechanism by which APP may regulate these functions is still unknown.

**EXPERIMENTAL PROCEDURES**

**Animals**—APPKO mice and their corresponding C57Bl/6 wild-type (WT) control mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Human APP-overexpressing, APPSW Tg2576 mice, and the corresponding background strain controls, C57Bl/6_SIL, were purchased from Taconic Farms (Hudson, NY). Mice were housed in the animal facility at the University of Tasmania. All experiments were approved by the University of Tasmania Animal Ethics Committee.

**Materials and Antibodies**—Dulbecco’s modified Eagle’s medium (DMEM), B27 supplement, and poly-1-lysine were from Invitrogen. Penicillin, streptomycin, and human re-
APP, Neurogenin 2, and Neurogenesis

combinant EGF were all obtained from Sigma. Human recombinant bovine FGF was from PeproTech (Rocky Hill, NJ). Poly-L-lysine was from Sigma. Human recombinant sAPPα was from Sigma. Aβ 1–40 and Aβ 1–42 were from Keck Foundation Biotechnology (New Haven, CT). Anti-rabbit neurogenin2 was from Sapphine Bioscience (Waterloo, Australia), anti-mouse β-actin was from Sigma, anti-mouse βIII-tubulin mAb was from Promega (Alexandria, Australia), anti-mouse 6E10 mAb was from Covance (Sydney, Australia), and anti-rabbit MAP2 mAb was from Sigma. Anti-mouse and anti-rabbit HRP-conjugated secondary antibodies were from Dako Australia Pty. Ltd. (Campbellfield, Australia). Secondary antibodies were goat anti-mouse IgG conjugated to Alexa Fluor-488 and 568 (Invitrogen). 4,6-Diamidino-2-phenylindole (DAPI) was from Sigma. Primary antibodies were goat anti-Neurogenin 2 (Cambridge, MA). The plasmids contained an IRES or IRES-GFP control vector and pCAG-IRES-APP695 were from Applied Biosystems. The expression vectors were from Sigma, biotin was from Sigma. Anti-mouse and anti-rabbit HRP-conjugated secondary antibodies were from Dako Australia Pty. Ltd. (Campbellfield, Australia). Secondary antibodies were goat anti-mouse IgG conjugated to Alexa Fluor-488 and 568 (Invitrogen). 4,6-Diamidino-2-phenylindole (DAPI) was from Sigma. The expression vectors were from Sigma, biotin was from Sigma.

**Neurosphere and Isolated NSPC Culture**—Primary neurosphere cultures derived from cerebral cortices of postnatal day 0 mice were prepared according to previously described procedures (19). Neurospheres were prepared by growing cells in suspension in 75-cm² cell culture flasks at a density of 400,000 cells in proliferation medium (DMEM supplemented with 2% (v/v) B27, 100 units/ml penicillin, 100 units/ml streptomycin, 20 ng/ml human bovine FGF, and 20 ng/ml human EGF). After 7 days in culture, neurospheres were dissociated mechanically by trituration, and cells were counted in a hemocytometer and then either reseeded as suspension cultures or replated as adherent cultures. All cultures were incubated in a humidified incubator at 37°C in an atmosphere containing 5% CO₂.

**Differentiation of NSPCs**—Neurospheres were mechanically dissociated, and then isolated cells were plated at a density of 10⁵ cells/well in 24-well plate. The cells were grown in a differentiation medium (DMEM supplemented with 2% (v/v) B27, 100 units/ml penicillin, 100 units/ml streptomycin, and 1% (v/v) fetal calf serum) for 5 days at 37°C in an atmosphere containing 5% CO₂. The cells were then fixed with 4% (v/v) paraformaldehyde in phosphate-buffered saline (PBS) (8 g/liter NaCl, 0.2 g/liter KCl, 1.44 g/liter NaH₂PO₄, and 0.24 g/liter KH₂PO₄, pH 7.2) for 15 min, permeabilized with 0.03% (v/v) Triton X-100 in PBS for 5 min, and incubated in 10% (v/v) sheep serum in PBS for 1 h to block nonspecific binding sites. Fixed cells were stained with a mouse anti-βIII tubulin antibody (1:1,000 diluted in 10% (v/v) sheep serum in PBS) and then incubated with a goat anti-mouse IgG conjugated to Alexa Fluor 488 or -568 (1:1,000 diluted in 10% (v/v) sheep serum in PBS) and DAPI at 1:10,000 dilution. βIII-tubulin + and DAPI + cells were counted under the 20× objective using a Zeiss Palm microscope.

**Alamar Blue Assay**—The number of viable cells was estimated using an Alamar Blue assay. Dissociated cells cultured adherently on poly-L-lysine-precoated 96-well plates were incubated for up to 5 days, and then 20 μl of Alamar Blue reagent (Invitrogen) was added into each well, and the cells were incubated for a further 4 h. The fluorescence intensity was determined using a FLUOSTAR Optima microplate fluorescence plate reader at an excitation wavelength of 540 nm and an emission wavelength of 590 nm. Cell number was expressed as the relative fluorescence intensity.

**PCR Array**—A mouse neurogenesis and neural stem cell PCR array in 384-well plate format (PAMM-4042ZG-Q; Qiagen, Chadstone, Australia) was used to assess gene expression changes. RNA was extracted from neurospheres cultures derived from n=3 independent mouse cohorts using a RNeasy mini kit (Qiagen). Each preparation of neurospheres contained ~10⁶ cells in proliferation medium. cDNA was reverse-synthesized from 400 ng of RNA with a RT² first strand kit (Qiagen). cDNA samples were added to the reaction plates, and the real-time amplification data (Ct values) were determined using a Roche Diagnostics LightCycler 480. Analysis of gene expression from real-time results was carried out using the RT² profiler PCR array data analysis v3.5 provided by Qiagen. Expression of the β-actin gene was used as a reference housekeeping gene.

**Immunoblotting**—The level of Ngn2 in NSPC cultures and in the brain cortex of the WT and APPKO mice was determined by western blotting. Cells were washed with PBS and then lysed as described previously (19). Cortices, 4 per group, derived from WT and APPKO mice were homogenized in RIPA buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Nonidet P-40, 0.1% SDS, and protease inhibitor mixture from Roche Diagnostics). Proteins were then separated on 12% sodium dodecyl sulfate-polyacrylamide gels before being transferred electrophoretically onto polyvinylidene difluoride membranes (Merck). The membranes were blocked for 2 h with 2% (w/v) skim milk powder in 50 mM Tris-buffered saline, pH 8, containing 0.05% (v/v) Tween 20 (TBS-Tween) and incubated overnight at 4°C with either anti-Ngn2 (1:1000 dilution) or anti-β-actin (1:10,000 dilution). Protein expression was detected using HRP-conjugated secondary antibodies (1:10,000 dilution). Chemiluminescence reactions were monitored using a CHEMI-SMART 5000, and images were collected using Chemi-Capt 5000. For quantification of immunoreactivity, images of blots were analyzed using ImageJ Version 1.46r (National Institutes of Health, Bethesda, MD).

**Cell Transfection**—NSPCs were electroporated with the appropriate plasmid using the Amaxa mouse neural stem cell nucleofector kit (VPG-1004, Lonza Ltd., Germany). Briefly, 2.5 × 10⁶ dissociated cells and 2 μg of plasmid were resuspended in 100 μl of nucleofector solution (Amaxa), then the cell/DNA suspension was transferred into a certified cuvette and electroporated with nucleofector device program A-033. Proliferation medium (500 μl) was added to the cuvette, and the suspension was gently transferred onto poly-L-lysine precoated coverslips in a 12-well plate containing 300 μl of proliferation medium prewarmed to 37°C. After 24 h the medium was changed to differentiation medium, and the cells were
incubated for 5 days at 37 °C in an atmosphere containing 5% CO₂. For siRNA transfections, 300,000 cells per well were plated onto poly-1-lysine-precoated coverslips maintained in proliferation medium for 48 h. After this time, the medium was changed to differentiation medium and the siRNA:Effectene (Qiagen) complex was added in a ratio of 20 nmol to 4 μl per well, and the cells were incubated for 5 days at 37 °C in an atmosphere containing 5% CO₂. Next, the cells were fixed in 4% (v/v) paraformaldehyde in PBS. Fixed cells were stained with a DAPI Sciences). confocal microscope with Volocity Software (PerkinElmer Life Australia) fluorescence microscope. Images of fluorescently objective using a Zeiss Palm microbeam IV (Carl Zeiss, Sydney, Australia) were collected using an UltraView downstream of the null hypothesis was 0.05. Data are presented as the means ± S.E. All results were obtained in at least three independent experiments.

RESULTS

Cystatin C Is Not Involved in Neuronal Differentiation—Previous studies from our group (19) showed that NSPCs derived from APPKO mice proliferated less rapidly and differentiated into neurons less readily than NSPCs from WT mice. The effect on proliferation was found to be mediated by the secretion of cystatin C. Therefore, to examine the possibility that cystatin C might also influence NSPC differentiation, we first compared the neural differentiation levels of NSPCs derived from P0 WT and APPKO mice. After 5 days in differentiation medium, the number of β-III-tubulin⁺ neurons was determined and expressed as a percentage of the total number of cells measured with DAPI. Similar to our previous study (19), we found that a smaller proportion of APPKO NSPCs were positive for β-III-tubulin⁺ than in WT cultures.

To examine the possibility that cystatin C is a mediator of APP-induced neuronal differentiation, cultures of WT and APPKO NSPCs were treated with cystatin C (100 ng/ml) and the cells were incubated for 5 days in differentiation medium. After this time, the percentage of β-III-tubulin⁺ cells present in the cultures was determined. We did not find a significant difference between the number of β-III-tubulin⁺ cells in
untreated WT and APPKO cultures when compared with those treated with cystatin C (Fig. 1, D and F). To ensure that the cystatin C was active, we conducted a parallel study in which we exposed proliferating NSPCs to the same concentration of cystatin C that we used in our differentiation assay. Using a fluorescence (Alamar Blue) assay to measure the number of viable cells (Fig. 1F), we observed an increase in the number of cells when the cells were incubated with 100 ng/ml cystatin C. This confirmed that although cystatin C could stimulate NSPC proliferation, the effect of APP on NSPC differentiation was not due to cystatin C.

Expression of Ngn2 Is Decreased in APPKO NSPCs—To identify a factor(s) that mediates APP-induced neuronal differentiation, the expression of neurogenesis-related genes in NSPCs derived from APPKO mice and from Tg2576 mice (which overexpress human APP) was analyzed using a mouse neurogenesis and neural stem cell-specific PCR array (RT2 Profiler PCR). RNA was extracted from NSPCs of APPKO and Tg2576 mice and from the corresponding WT control mice.

The expression levels of 84 genes that regulate neurogenesis including genes related to apoptosis, cell cycle, growth, and transcription were compared. We found that 18 of the 84 genes had expression levels that were either up- or down-regulated more than 2-fold in APPKO cells when compared with the corresponding WT cells (Table 1, Fig. 2). As expected, the measured App gene expression was at least 1800-fold lower in NSPCs of APPKO mice. Expression of the genes Bmp8, Mdk, Nf1, Nrp1, Odz1, Shh, and Th was significantly higher in the APPKO cells, whereas expression of Fgf2, Gdnf, Hey1, Hey2, Neurog2, Pax3, Pax5, and Pou3f3 was significantly lower. Ngn2 expression was 42-fold down-regulated in the APPKO relative to WT cells, which was a considerably lower level than for the other genes with the exception of App. Furthermore, Ngn2 expression was 2.5-fold up-regulated in the APP overexpressing Tg2576 cells relative to WT cells (Table 2, Fig. 2).

Taking into account the fact that Ngn2 expression correlated positively with APP expression in both the APPKO and Tg2576 mice, we decided to examine the role of Ngn2 in mediating APP-induced NSPC neuronal commitment and differentiation. We focused our study on APPKO mice in order to exclude the possibility of nonspecific transgene effects as might be expected from studying the effects of human mutant APP in Tg2576.

To confirm that the change in Ngn2 mRNA expression correlated with the level of Ngn2 (protein), we quantified the amount of Ngn2 by western blotting of NSPC lysates cultured for 5 days in proliferation medium. There was an ~50% lower level of Ngn2 in NSPCs derived from APPKO compared with the level in WT NSPCs (Fig. 3, A and C). When NSPCs were cultured in differentiation medium (Fig. 3, B and D), again we observed a lower level (40%) of Ngn2 in the APPKO cells.

To examine whether the expression of Ngn2 was also affected in vivo, we quantified the amount of Ngn2 by western

![FIGURE 2. Two-dimensional plot of gene expression levels in APPKO NSPCs (A) or Tg2576 NSPCs (B) versus WT NSPCs. Gene expression was calculated relative to the expression in the corresponding background strain (Tables 1 and 2) and is shown on a log_{10} scale. Eighty-four genes were analyzed. Gene expression relative to β-actin (reference gene) is shown. Analysis of gene expression from n = 3 independent experiments was carried out using the RT2 profiler PCR array data analysis v3.5 provided by Qiagen.](image-url)
blotting of brain cortex derived from APPKO and WT mice. We observed a 60% lower level of Ngn2 immunoreactivity in the brain cortex of APPKO mice compared with that of WT mice (Fig. 3, E and F). These results were consistent with the results obtained with NSPCs. We concluded that in the absence of APP there was a lower level of Ngn2 (protein) due to a decrease in the level of Ngn2 mRNA expression.

**Effect of Ngn2 on Neuronal Differentiation**—As NSPCs derived from APPKO mice had lower levels of Ngn2 and a decreased propensity to differentiate into neurons than NSPCs derived from WT mice, this suggested that APP might enhance neuronal differentiation through Ngn2. To confirm this hypothesis, we examined whether the expression of APP in NSPCs derived from APPKO mice could induce neuronal differentiation. The cells were transiently transfected with a pCAG-IRES-APP or a pCAG-IRES-GFP plasmid in order to express APP or GFP into the NSPCs. Then cells were cultured for 5 days in differentiation medium. After this, the percentage of transfected cells (APP⁺ or GFP⁺) that were also positive for MAP2 were analyzed, and the significance of the difference was determined with a χ² test (n = 34 cells counted). The results showed that 79 ± 5% of cells were APP⁺ MAP2⁺ compared with 15 ± 6% that were GFP⁺ MAP2⁺. The difference was statistically significant (p = 0.027), confirming that APP expression resulted in a recovery of neuronal differentiation of APPKO NSPCs.

The next step was to study whether Ngn2 was involved in the APPKO NSPC neuronal differentiation produced by APP. NSPCs derived from APPKO mice were transiently transfected with a pCAG-Ngn2-IRES-GFP or a pCAG-IRES-GFP control vector and cultured for 5 days in differentiation medium. Analysis of the pCAG-Ngn2-IRES-GFP-transfected cells showed that 95.6 ± 4.3% of the GFP-positive cells also stained positively for β-III-tubulin (300 cells counted across ×3 independent cultures). However, only 7.0% ± 0.6% of the cells that were transfected with pCAG-IRES-GFP (control plasmid) were β-III-tu-

**TABLE 2**

Analysis of gene expression in NSPCs from Tg2576 using a mouse neurogenesis and neural stem cell PCR array

Mouse PCR neurogenesis array results showing the expression of genes in Tg2576 NSPCs that are significantly up-regulated or down-regulated relative to NSPCs from the corresponding background strain WT mice. RNA was extracted from NSPC cultures derived from three independent mouse cohorts. Analysis and presentation of the data is the same as Table 1.

| Gene name                                      | Expression level (relative to β-actin) | Fold up-or down-regulation in Tg |
|------------------------------------------------|----------------------------------------|--------------------------------|
| Bone morphogenetic protein 4                  | 3.1 × 10⁻²                           | 0.05                           |
| Bone morphogenetic protein 8                  | 4.4 × 10⁻³                           | 0.27                           |
| CDK5 regulatory subunit associated protein 2   | 6.5 × 10⁻⁷                           | 0.26                           |
| Chemokine (C-X-C motif) ligand 1                | 6.1 × 10⁻⁹                           | 0.06                           |
| Dopamine receptor D2                          | 5.1 × 10⁻⁶                           | 0.07                           |
| Filamin, α                                    | 5.6 × 10⁻⁹                           | 0.01                           |
| Glial cell line-derived neurotrophic factor    | 3.9 × 10⁻⁹                           | 0.01                           |
| Hairy and enhancer of split 1                 | 9.3 × 10⁻⁹                           | 0.02                           |
| Hairy/enhancer-of-split related with YRPW motif 1 | 3.4 × 10⁻⁹                           | 0.02                           |
| Myocyte enhancer factor 2C                    | 1.0 × 10⁻⁶                           | 0.01                           |
| Neurogenic differentiation 1                   | 8.8 × 10⁻⁶                           | 0.01                           |
| Neurogenin 1                                  | 5.5 × 10⁻⁶                           | 0.02                           |
| Neurogenin 2                                   | 4.8 × 10⁻⁶                           | 0.01                           |
| Noggin                                        | 7.8 × 10⁻⁶                           | 0.02                           |
| Nuclear receptor subfamily 2, group E, member 3| 7.8 × 10⁻⁶                           | 0.02                           |
| POU domain, class 4, transcription factor 1    | 8.6 × 10⁻⁶                           | 0.02                           |
| Slit homolog 2                                 | 6.7 × 10⁻⁸                           | 0.001                          |

**FIGURE 3.** Western blotting analysis of Ngn2 in NSPC culture and brain cortex derived from WT and APPKO mice. The figure shows western blots (A, B, and E) and the corresponding quantification of immunoreactivity (C, D, and F). NSPCs from WT and APPKO mice were incubated in proliferation medium (A and C) or differentiation medium (B and D). Cells were lysed after 5 days, and the lysates were analyzed for Ngn2 by western blotting and for β-actin (loading control). E and F, analysis of in vivo expression. Brain cortices from WT and APPKO mice were homogenized, and the lysates were analyzed for Ngn2 by western blotting and for β-actin (loading control). Bars show the means ± S.E. (*p < 0.05; **p < 0.01 as determined by Student’s t test).
null
the number of β-III-tubulin+ cells in the cultures treated with APP fragments (Fig. 5). Thus the increase in neuronal differentiation of NSPCs observed in association with APP expression was unlikely to have been due to an increase in production of sAPPα or Aβ peptides.

**DISCUSSION**

The present study shows that neuronal differentiation is lower in NSPCs derived from APPKO mice and that this effect is due to decreased expression of Ngn2. Levels of Ngn2 correlated with APP expression. Furthermore, knockdown of Ngn2 in wild-type cells lowered neuronal differentiation, whereas transfection of APPKO NSPCs with either APP or Ngn2 resulted in recovery of neuronal differentiation. Taken together, the results demonstrate that APP contributes actively to neurogenesis during development.

Despite a large number of published studies on APP (31), the normal function of APP is largely unknown. Emerging evidence suggests that APP plays a role in neuronal growth and neural repair, although the precise mechanisms have been unclear (31). The expression of APP is regulated developmentally (28, 32) and is increased in association with neurite outgrowth and synaptogenesis (8, 26). Similarly, overexpression or knock-out of APP has been reported to disrupt a number of developmental functions including neuronal migration (9) and cell growth (33, 34). Secreted forms of APP are reported to have neurotrophic and neuroprotective effects (7, 8, 35, 36) and to stimulate proliferation of adult neural progenitors (37, 38). Additionally, there are reports that injection of soluble APPα after traumatic brain injury can improve neuronal survival and recovery (24, 39, 40). Collectively, these studies provide evidence that APP has a trophic function.

Some studies suggest that APP may play a role in the regulation of stem cell proliferation or differentiation. For example, APP is expressed in developing neuroblasts at the time of cell proliferation and differentiation (26, 31, 41). In the present study we found that in the absence of APP, there was a decrease in NSPC differentiation into neurons. These findings strongly support the idea that APP is involved in the regulation of NSPC neurogenesis. Indeed, APP is processed in a manner that is very similar to the protein Notch, which regulates NSPC proliferation (42). Therefore, APP may have a similar or related developmental function to that of Notch (43).

Previously we found that NSPC proliferation was regulated by APP through the secretion of cystatin C (19). However, in the present study we found that the APP-induced neuronal differentiation of NSPC was not due to cystatin C but rather to Ngn2. Ngn2 is a bHLH transcription factor that was first identified for its ability to promote neuronal differentiation in brain and spinal cord (44–46). Ngn2 also specifies phenotypic features of neurons (47–50), regulates axonal guidance (51) as well as dendritic morphologies of cortical neurons (52) and cortical neuron migration (53), and plays a crucial role along with other bHLH transcription factors during development (48, 54–56). Additionally, Ngn2 has an important function in stem-cell differentiation during neurogenesis driving neuronal differentiation (57–60). In this context, our present findings support the idea that the APP-induced differentiation of NSPCs is mediated by Ngn2.

In primary cultures of NSPCs derived from APPKO and WT mice, we found that Ngn2 mRNA expression and protein levels were decreased in APPKO cells consistent with the view that Ngn2 is involved in NSPC neuronal differentiation. We also analyzed the Ngn2 protein levels in brain cortices derived from APPKO mice and found a decrease in Ngn2 levels compared with cortices derived from WT mice, consistent with the results obtained in NSPC cultures. In support of the view that APP regulates neuronal differentiation of NSPCs via Ngn2, we found that transfection of a Ngn2 cDNA into NSPCs recovered the neuronal differentiation phenotype of the APPKO NSPCs. Furthermore, we found that knocking down the expression of Ngn2 gene in NSPCs derived from WT mice using a siRNA approach led to a decrease in neuronal differentiation. Taken together, the studies support the conclusion that APP increases neuronal differentiation by regulating Ngn2 expression.

Interestingly, Ngn2 expression was higher in APP-expressing cells than in APPKO cells when they were cultured in proliferation medium (i.e. before differentiation) (Fig. 3A). As the cells did not differentiate in proliferation medium, this implies that
APP-induced Ngn2 expression is not in itself a sufficient condition for neuronal differentiation.

Previously, Porayette et al. (30) reported that in human embryonic stem cells, proliferation and differentiation were mediated via APP cleavage (i.e., sAPPα). Our previous study (19) found no effect of sAPPα or Aβ peptides on NSPC proliferation. In the present study we similarly found no effect of sAPPα or Aβ peptides on neuronal differentiation. The reason for the differences between the previous study (30) and our own are unclear, but it seems possible that mechanisms of regulation of embryonic stem cells differ from those of the NSPCs used in our study. However, despite the finding that APP fragments did not have an effect on neuronal differentiation, this does not exclude the possibility that they may have effects on other events such as migration (9) or neurite outgrowth (4–8). Further studies are needed to examine this possibility.

It is not yet clear how APP regulates Ngn2 to increase NSPC neurogenesis. Some studies suggest that Ngn2 can be regulated through multisite phosphorylation of Ngn2 by kinases. Li et al. (46) reported that the capacity of Ngn2 to induce differentiation is regulated by glycoen synthase kinase 3β. For this reason, glycoen synthase kinase 3β may be a good candidate for further study because it may be regulated by APP as reported by Zhou et al. (61). On the other hand, Ngn2 expression can be regulated by other transcription factors such as Pax6, Nurr, and Mash1 (46, 55, 58, 62). As a consequence of this mechanism, the increased activity and expression of Ngn2 can induce stem cell differentiation. Studies have shown that Ngn2 expression can be regulated by retinoic acid, sonic hedgehog, and fibroblast growth factor (63). Whether these factors are involved in mediating the effect of APP on Ngn2 expression remains to be determined, as further studies will be required to define a specific mechanism through which APP regulates Ngn2.

Acknowledgments—We thank François Guillelmo (MRC–National Institute for Medical Research, London, UK) for the kind donation of the Ngn2 plasmid, Julian Heng (Monash University, Melbourne, Australia) for discussions regarding Ngn2, and Meredith Roberts-Thompson for work on the APPKO colony.

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