The Anaphase Promoting Complex Induces Substrate Degradation during Neuronal Differentiation

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The anaphase promoting complex (APC) is an E3 ubiquitin ligase required for the metaphase-to-anaphase transition and mitotic exit. However, APC also plays roles in G1, where it is regulated by Cdh1, and APC activity has also been detected in differentiated and non-proliferating cells, suggesting that it may play roles outside the cell cycle. Here, we report that disrupting APC^Cdh1 activity inhibits neurite outgrowth of both PC12 pheochromocytoma cells and primary cerebellar granule cells. APC^Cdh1 activity dramatically increases as PC12 cells differentiate in response to nerve growth factor. Furthermore, a key target degraded by APC^Cdh1 following nerve growth factor treatment is the F-box protein Skp2, and APC^Cdh1-mediated destruction of Skp2 is essential for proper terminal differentiation of neuronal precursors.

Ubiquitin-mediated proteolysis controls essentially every transition of the cell cycle (1, 2). For example, the metaphase-to-anaphase transition and mitotic exit are initiated after degradation of specific substrates (3), which are mediated by the anaphase promoting complex (APC)^E3 ubiquitin ligase (1). APC is a multisubunit ubiquitin ligase that associates with one of two known activators (Cdh1 (Cdc20 homolog 1) or Cdc20), which recruit substrates and bring them into close proximity to the ubiquitin-conjugating enzyme E2 (1). Following conjugation, substrates are ubiquitylated by APC, and ubiquitinated substrates are then degraded by the 26 S proteasome (1).

The essential role of APC^Cdc20 in initiating mitotic transitions is firmly established (1). However, the roles of the non-mitotic form of APC, APC^Cdh1, are just emerging (4), and they play roles outside the cell cycle. Here, we report that disrupting APC^Cdh1 activity inhibits neurite outgrowth of both PC12 pheochromocytoma cells and primary cerebellar granule cells. APC^Cdh1 activity dramatically increases as PC12 cells differentiate in response to nerve growth factor. Furthermore, a key target degraded by APC^Cdh1 following nerve growth factor treatment is the F-box protein Skp2, and APC^Cdh1-mediated destruction of Skp2 is essential for proper terminal differentiation of neuronal precursors.

UBIQUITIN-MEDIATED PROTEOLYSIS CONTROLS ESSENTIALLY EVERY TRANSITION OF THE CELL CYCLE (1, 2). FOR EXAMPLE, THE METAPHASE-TO-ANAPHASE TRANSITION AND MITOTIC EXIT ARE INITIATED AFTER DEGRADATION OF SPECIFIC SUBSTRATES (3), WHICH ARE MEDiators BY THE ANAPHASE PROMOTING COMPLEX (APC)^E3 UBIQUITIN ligase (1). APC IS A MULTISUBUNIT UBIQUITIN ligase that ASSOCIATES WITH ONE OF TWO KNOWN ACTIVATORS (CDH1 (CDC20 HOMOLOG 1) OR CDC20), WHICH RECRUIT SUBSTRATES AND Bring THEM INTO CLOSE PROXIMITY TO THE UBIQUITIN-CONJUGATING ENZYME E2 (1). FOLLOWING CONJUGATION, SUBSTRATES ARE UBIQUITYLATED BY APC, AND UBIQUITINATED SUBSTRATES ARE THEN DEGRADED BY THE 26 S PROTEASOME (1).

The essential role of APC^Cdc20 in initiating mitotic transitions is firmly established (1). However, the roles of the non-mitotic form of APC, APC^Cdh1, are just emerging (4), and they appear complex, as APC^Cdh1 is active throughout G1, in differentiating cells, and in terminally differentiated cells (5, 6). In G1, APC^Cdh1 is known to target essential DNA replication factors for destruction (7, 8). Thus, APC^Cdh1 activity may be responsible for G1 maintenance by inhibiting the initiation of DNA replication. Consistent with this notion, depletion of the APC activator Cdh1 induces premature entry into S phase (9, 10).

Although signaling pathways that regulate the mitotic form of APC are known (1, 4), those that control APC^Cdh1 activity during G1 have not been extensively characterized. Regulatory control is operational because APC^Cdh1 activity is high during early G1 but progressively decreases as cells approach S phase; therefore, in addition to intrinsic cell cycle controls, extrinsic pathways also likely control APC^Cdh1 activity during late G1 (11).

One extracellular signal that positively regulates APC^Cdh1 activity is transforming growth factor-β (TGF-β) (12). For example, TGF-β stimulation of epithelial cells induces hyper-activation of APC (12, 13), and these changes occur within minutes, suggesting that APC activation is an immediate response to TGF-β or is a cue to withdraw from the cell cycle. Furthermore, APC activity is necessary for mediating the TGF-β signal because inhibiting APC^Cdh1-mediated degradation of the substrate Sno-1 or Skp2 (S phase-associated kinase protein 2) diminishes TGF-β responsiveness of cells (13).

Drawing upon the studies linking APC^Cdh1 and the TGF-β pathway, we hypothesized that signals provoked by nerve growth factor (NGF) regulate APC^Cdh1 during neuronal differentiation. Indeed, we report here that the NGF signaling pathway rapidly induces APC^Cdh1 activity and that NGF induces the rapid degradation of APC substrates, including cyclin B1 and the F-box protein Skp2, which is necessary for degradation of the cyclin-dependent kinase inhibitor p27^kip1 (14). Furthermore, APC^Cdh1 activity and degradation of Skp2 is required for neurite outgrowth in PC12 pheochromocytoma cells and in primary cerebellar granule cells. Therefore, APC^Cdh1-mediated destruction of Skp2 appears to coordinate cell cycle exit and the terminal differentiation of neurons.

**EXPERIMENTAL PROCEDURES**

**Degradation and Ubiquitylation Assays**—In vitro degradation assays were performed essentially as described (15). Briefly, PC12 cells were treated for various times with NGF, and cell extracts were prepared after hypotonic swelling and repeated freeze–thaw cycles. Extracts were supplemented with ubiquitin (0.1 μg/ml), cycloheximide (1 μg/ml), and an energy-regenerating system. In vitro translated [35S-labeled] cyclin B, Δbox-cyclin B, Skp2, or Δbox-Skp2 proteins were incubated in 20 μl of cell extract for the indicated times at room temperature, and the reactions were stopped by adding SDS-containing sample buffer.

In vitro ubiquitylation assays were performed as described (10). PC12 cells were incubated in the presence or absence of...
NGF for 2 h. Lysates were then prepared from these cells as described above and incubated with 4 µg of anti-Cdc27 antibody complexed to protein A-Affi-Prep beads (Bio-Rad). Immunopurified APC was washed four times with buffer A (20 mM HEPES (pH 7.8), 100 mM KCl, and 5 mM MgCl2) supplemented with 1% Triton X-100, once with buffer A with 300 mM NaCl, and once with buffer A. Washed beads (5 µl) were incubated with 1 µl of 35S-labeled N-terminal cyclin B, Skp2, or Dbox-Skp2; UbcH10 (2.8 µM; Boston Biochem); E1 (1 µM; Boston Biochem); and an ATP-regenerating system in a final volume of 7.5 µl at room temperature with shaking. Ubiquitin (10 mg/ml; Boston Biochem) was also included. 1-µl aliquots of the ubiquitination reactions were removed at the indicated times and mixed with SDS-containing sample buffer. The relative extent of polyubiquitination was measured by quantifying entire lanes after subtracting the signal generated from input cyclin B (1 arbitrary unit = signal generated from input).

N-cyclin B1-Luciferase Assays—The first 100 amino acids of Xenopus cyclin B1 were fused to firefly luciferase (N-cyclin B1-luciferase) using the HindIII and Ncol sites of the pGL3 control vector (Promega, Madison, WI). Subconfluent HeLa cells (40,000/well) on a Corning 3917 96-well plate were reverse-transfected with 125 ng of N-cyclin B1-luciferase along with an equal amount of pcS2-Cyc-Myc (Myb-tagged vector–only control), pcS2-Myc-Xenopus CDH1, or pcS2-Myc-emi-1 using Trans-IT (Mirus Bio, Madison, WI) according to the manufacturer’s instructions. Cells were incubated at 37 °C and 10% CO2 in a humidified incubator for 20–24 h. Steady-state levels of luciferase were determined by adding an equal volume of britelite (PerkinElmer Life Sciences). Relative luciferase units of transfected cells were analyzed with Multi-Analyzer GT.

PC12 Cell Neurite Outgrowth Assays—PC12 cells were grown on 50 µg/ml laminin in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 5% horse serum, and streptomycin/penicillin. PC12 cells were transfected using FuGENE 6 (Roche Applied Science) in 6-well plates using 1 µg of total DNA (Cdh1, N-Cdh1, Skp2, or Dbox-Skp2 cotransfected with 125 ng of respective viral supernatants) were added. An additional 400 µl of medium was added to the slices 4 h later. The slices were then cultured for 48 h, fixed in 4% paraformaldehyde, and processed for GFP (Abcam) immunoreactivity. The Purkinje cell layer was identified by calbindin (Abcam) staining.

Cdh1 Knockdown Constructs—HeLa cells were transfected with Lipofectamine in 10-cm dishes with 4 µg of pcS2-FLAG-mouse CDH1 and 4 µg of either short hairpin RNA (shRNA) vector or three CDH1 shRNA knockdown constructs or Sport6 as a control. After 24 h, whole cell lysates were prepared, resolved on 4–20% Tris glycine gradient gels, and transferred to 0.2-µm nitrocellulose membranes. The membrane was probed with affinity-purified anti-FLAG monoclonal antibody M2 produced in mouse (F1804, Sigma) and horseradish peroxidase-conjugated donkey anti-mouse IgG used as the secondary antibody (Jackson ImmunoResearch Laboratories). The membrane was reprobed with rabbit anti-lamin A IgG (Santa Cruz Biotechnology, Santa Cruz, CA) as a loading control using horseradish peroxidase-conjugated donkey anti-rabbit IgG (GE Healthcare) as the secondary antibody. Blots were developed with ECL Plus (GE Healthcare). To characterize endogenous CDH1 expression after transfection with knockdown constructs, we cultured P19 cells and transfected them using FuGENE 6 with control shRNA (shRNA1) or shRNA4 and isolated cells 48 h after transfection. The sequences of CDH1 knockdown construct sequences attained from GenScript Corp. are as follows: control or shRNA1 vector only, pRNAT-H1.4/Retro(SD1253); shRNA2, ACGCGTCGTT-ATGAGTGCCAGTGGTGTTGTATCCGAAGCTCTC- AACTGCCCACCTCATATTATTCTTCACTCGAG; shRNA3, ACGCGTCGTTAAGAGTCTGTCTGTTGTATCCGAGACAGCAGTTTTCTTCACTCGAG; and shRNA4, ACGCGTCGTTAGTTGCTGTCTGTTGTATCCGAGACAGCAGTTTTCTTCACTCGAG; and shRNA4, ACGCGTCGTTAGTTGCTGTCTGTTGTATCCGAGACAGCAGTTTTCTTCACTCGAG.

Production of Retroviruses—Retroviruses were isolated from tissue culture supernatants of 293T transfected cells. 293T cells were grown in 60-mm dishes and transfected with 2 µg of pCL-Eco and 2 µg of retroviral construct (pRNAT-H1.4/Retro for shRNA constructs and pMX-GFP or pMX-GFP-N-Cdh1) using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s directions. Virus-containing supernatant was collected 48 and 72 h post-transfection and used in slice culture assays. The medium of transfected cells was changed 4 h after transfection to granule cell medium (Eagle’s basal medium, 10% horse serum, 5% fetal calf serum, 1% glucose, 2 mM L-glutamine, and 100 units/ml penicillin, and 100 µg/ml streptomycin).

RESULTS

NGF Induces APC<sup>Cdhl</sup> Activity in PC12 Cells—Among many possible systems to evaluate the role of APC in cell differentiation, neuronal differentiation seemed particularly attractive given the high levels of APC expression in the brain (6). The pheochromocytoma cell line PC12 has been widely used as a model system of neuronal differentiation because, in response to NGF, it recapitulates many of the morphological features of differentiating neurons, including the outgrowth of neurites (6). To initially evaluate the activity of APC during NGF-induced differentiation, concentrated somatic cell extracts from NGF-treated or untreated PC12 cells were prepared and tested for their in vitro degradation activity using the...
model mitotic APC substrate cyclin B1. Cyclin B1 degradation increased dramatically 15 min after NGF addition (Fig. 1, A and B) and remained high for at least 3 h (supplemental Fig. S1; data not shown). Furthermore, cyclin B1 degradation was contingent upon the presence of the N-terminal destruction box sequence in cyclin B1, as a mutant form of cyclin B1 lacking the destruction box (Δdbox-cyclin B) was not efficiently degraded (Fig. 1, A and B). Moreover, increased cyclin B degradation was accompanied by enhanced APC-dependent ubiquitination (Fig. 1C) and was not due to overt changes in the cell cycle, as these events were manifest long before changes in the cell cycle were observed (Fig. 1D). Furthermore, the increase in cyclin B1 degradation was not due to changes in proteasome activity as determined by a succinyl-LLVY-7-amino-4-methylcoumarin fluorogenic substrate assay (data not shown).

**APC<sub>Cdh1</sub> Targets Skp2 for Destruction after NGF Treatment of PC12 Cells—**NGF stimulation provokes G<sub>1</sub> arrest and terminal differentiation of PC12 cells (17). Among the known G<sub>1</sub> substrates of APC is the F-box protein Skp2 (10, 18). Skp2 harbors a highly conserved N-terminal destruction box that is present in most APC substrates. Furthermore, APC targets F-box proteins for degradation during G<sub>1</sub> (10, 18). Skp2 regulates the turnover of the cyclin-dependent kinase inhibitor p27<sup>Kip1</sup>, a potent inducer of cell cycle exit and differentiation in various systems (19, 20).

To initially test whether APC targets Skp2 for degradation during NGF-induced differentiation, we prepared concentrated cell extracts from either NGF-treated or untreated PC12 cells. As observed for cyclin B1, Skp2 degradation was accelerated after 1 h of NGF stimulation (Fig. 2, A and B, and supplemental Fig. S1). Furthermore, a Skp2 mutant lacking the destruction box (Δdbox-Skp2) was not degraded as efficiently as wild-type Skp2, suggesting that APC is required for degradation of Skp2 in these extracts, although other pathways may also be involved (Fig. 2A). Finally, the role of APC in Skp2 degradation was indicated by the ability of excess N-terminal cyclin B (N-cyclin B) to inhibit Skp2 degradation (Fig. 2B) and by the inability of Δdbox-Skp2 to be efficiently ubiquitinated by extracts isolated from NGF-treated PC12 cells (supplemental Fig. S2).

**APC<sub>Cdh1</sub> Activity Is Required for Neurite Outgrowth of PC12 Cells—**The unexpected finding that NGF rapidly induced significant increases in APC-mediated cyclin B1 and Skp2 degradation suggested that APC might play critical roles in NGF-mediated differentiation. To test this hypothesis, we disrupted APC function by evaluating the effects of a dominant-negative variant of the APC activator Cdh1, termed N-Cdh1, and the inhibitor of the APC Emi-1 (early mitosis inhibitor 1) (21, 22). We initially tested their activity by cotransfection of HeLa cells with an expression vector encoding a fusion of the N terminus of cyclin B and firefly luciferase (Fig. 3), which acts as an accurate surrogate target of APC and a direct measure of APC activity (23). As expected, overexpression of Cdh1 repressed N-cyclin B1-luciferase levels, whereas overexpression of N-Cdh1 or Emi-1 led to marked increases in N-cyclin B1-luciferase (Fig. 3).
Therefore, the N-Cdh1 mutant and Emi-1 indeed act as effective inhibitors of APC by disrupting its association with endogenous substrates, as has been shown previously by multiple groups (21, 22).

To test the effects of disrupting APC activity on NGF-induced differentiation, PC12 cells were transfected with Cdh1, N-Cdh1, or Emi-1 (Fig. 4) along with the gene encoding GFP. As a control, PC12 cells were transfected with wild-type Cdh1 or GFP alone. The transfected PC12 cells were then induced to differentiate with NGF for 48 h, and the effects on differentiation were determined by scoring neurite outgrowth. Notably, enforced expression of either N-Cdh1 or Emi-1 significantly inhibited (by at least 50%) the differentiation of PC12 cells compared with cells expressing comparable levels of wild-type Cdh1 or those expressing GFP alone (Fig. 4, A and B; data not shown). Again, these effects were not a consequence of simply inducing cell cycle arrest, as fluorescence-activated cell sorter analysis of transfected cells indicated no overt differences in their cell cycle profiles (supplemental Fig. S3). Furthermore, overexpression of N-Cdh1 or Emi-1 did not induce apoptosis (data not shown). Therefore, APC activity contributes to neurite formation in differentiating neuronal cells.

**APC Cdh1 Activity Is Required for Migration of Cerebellar Granule Cells**—To test the function of APC Cdh1 in a more physiologically relevant context, we tested the role of APC Cdh1 in cerebellar granule cell differentiation using an in vitro cerebellar slice culture system, which has been utilized to study cerebellar granule cell differentiation and migration (24). Cerebella from P5 or P6 mice were isolated, sliced, and then cultured for several days submerged in slice culture medium, and their differentiation state was assessed as they migrated within these slices. In this system, undifferentiated progenitors reside in the periphery of the cerebellum and then migrate inward as they differentiate, from the external granule layer (EGL) to the molecular layer and eventually the internal granule layer (24). Thus, simply by marking a cell’s position within the cerebellum, one can determine its differentiation state (Fig. 5A).

To utilize the cerebellar slice system, we first generated a retrovirus encoding a GFP fusion protein fused to the dominant-negative N-Cdh1 form of the APC activator Cdh1 (GFP-N-Cdh1). P5 or P6 cerebellar slices were infected with GFP-N-Cdh1- or GFP only-expressing retroviruses, cultured for 48 h, and then stained for GFP using an anti-GFP antibody to detect infected cells. Notably, N-Cdh1 overexpression significantly compromised cerebellar granule cell migration and differentiation in slice cultures, and most of these failed to migrate from the slice edge (Fig. 5B). By contrast, control cerebellar cells expressing GFP alone migrated away from the EGL inward toward the Purkinje cell layer (data not shown). Finally, cerebellar cells overexpressing GFP-N-Cdh1 had significantly shorter neurites relative to control cells expressing GFP alone and slightly lower...
expression of the neuron-specific marker doublecortin (Fig. 5B and supplemental Fig. S4) (25). Therefore, APC^Cdh1 activity is also required for the migration and differentiation of primary cerebellar neurons.

Cdh1 Is Essential for Cerebellar Neuron Migration—A caveat of our N-Cdh1 studies is that overexpression of this dominant-negative form of Cdh1 could have off-target effects. Therefore, we also tested the requirement for the APC^Cdh1 complex in neuronal differentiation by selectively knocking down Cdh1 expression. Three shRNA-expressing retroviruses that specifically target mouse Cdh1 and also express coral GFP in cis from an internal ribosome entry site were generated and tested for their ability to knock down cotransfected mouse Cdh1 in HeLa cells or endogenous Cdh1 in P19 mouse teratocarcinoma cells. One of these Cdh1-directed shRNAs (shRNA4) depleted Cdh1 (Fig. 6, A and B).

To test the effects of Cdh1 knockdown in cerebellar cell migration, P5 cerebellar slice cultures were infected with retroviruses expressing CDH1 shRNA (shRNA4) or control shRNA, and the effects on cell migration from the outer EGL to the Purkinje cell layer were assessed by following coral GFP expression, which was detectable in cell bodies. Notably, knockdown of Cdh1 impaired cerebellar cell migration relative to control shRNA (Fig. 6C). The strength of signal for coral GFP was reduced compared with enhanced GFP, so we therefore could not follow its distribution in neurites throughout slice cultures. outgrowth (data not shown), suggesting that supraphysiologically levels of Skp2 override the degradative machinery to impair morphological differentiation. Regardless, the data support the conclusion that Skp2 degradation, which is directed by the APC^Cdh1 complex, is required for proper migration and differentiation of both normal and transformed neuronal progenitor cells.

DISCUSSION

APC^Cdh1 Activity Is Required for Cell Cycle Exit and Differentiation—The studies presented herein support the emerging theme that APC^Cdh1 is required for the initiation and/or execution of differentiation programs. Indeed, similar to what we have observed in neuronal cells, other recent studies have demonstrated a required role for APC and the degradation of Skp2 during myogenesis and lens epithelial differentiation (13, 26, 27). Collectively, these findings indicate that APC^Cdh1 function is required to coordinate differentiation programs with cell cycle exit and that Skp2 is a key substrate in this response.

In addition to intrinsic cues, our studies suggest that APC^Cdh1-mediated Skp2 degradation is regulated by extrinsic cues because APC^Cdh1 activity and Skp2 degradation are augmented by NGF-directed signaling. Precisely how NGF stimulates APC^Cdh1 activity is not clear, but this may be due to overt changes in the expression of APC components such as Skp2 Degradation Contributes to Neurite Outgrowth—Our N-Cdh1 overexpression and Cdh1 knockdown studies suggest that APC^Cdh1 activity is generally required for neuronal morphological differentiation. Because APC^Cdh1-mediated destruction of Skp2 increased following NGF treatment of PC12 cells (Fig. 2 and supplemental Fig. S1), we tested whether Skp2 degradation is required for neuronal morphological differentiation. PC12 cells were transfected with either wild-type Skp2 or the Skp2 mutant that is resistant to degradation (Δdbox-Skp2), and cells were expanded in culture. These cells were then treated with NGF for 48 h. Overexpression of either wild-type Skp2 or GFP did not affect neurite formation (Fig. 7A). However, overexpression of Δdbox-Skp2 severely impaired neurite outgrowth (Fig. 7A). Therefore, Skp2 destruction appears to be required for neurite outgrowth in PC12 cells. In analyses of the role of Skp2 degradation in neurite outgrowth in primary P5 granule cells, we observed that overexpression of either wild-type Skp2 or Δdbox-Skp2 impaired neurite
APC4, Cdc23, and Cdh1. (The mRNA levels of APC4, CDC23, and CDH1 increased 2–3-fold as judged by Affymetrix analysis of mRNA isolated from PC12 cells treated with NGF for 24 h.) Thus, we posit that NGF signaling may lead to modification of components of the APC<sup>Cdh1</sup> complex that increase its intrinsic E3 ubiquitin ligase activity toward Skp2. In turn, Skp2 degradation then leads to increases in p27<sup>Kip1</sup> levels, which are well known to set the threshold for cell cycle exit and differentiation (Fig. 7 <sup>B</sup> and supplemental Fig. S1), particularly during neuronal differentiation programs (17, 20). Consistent with this notion, we found that Emi-1-dependent inhibition of neurite outgrowth can be partially overcome by enforced p27 expression (supplemental Fig. S5).

The observation that p27 expression only partially rescues the neurite outgrowth defect observed following inactivation of the APC<sup>Cdh1</sup> complex suggests that there are other APC<sup>Cdh1</sup> substrates that must be degraded for cell cycle exit and differentiation to proceed. Indeed, APC<sup>Cdh1</sup> targets the transcriptional regulators Id2 and Sno-N for destruction in neuronal systems (28, 29), and T-cadherin is degraded via APC in PC12 cells, an event that is key for NGF-dependent neurite outgrowth (30). Furthermore, there appears to be hierarchy in the degradation of APC substrates, where disabling the destruction of one substrate can influence the timing or efficiency of degradation of others (31). Such control could also be directed by signaling pathways operational during differentiation that affect the specificity or affinity of APC<sup>Cdh1</sup> for its substrates.

**APC<sup>Cdh1</sup> Has Distinct Functions in Proliferation Versus Differentiation**—We found that disrupting APC activity inhibits neurite outgrowth and cerebellar cell migration in slice cultures. These findings conflict with those of Bonni and co-workers (32), who reported that the lengths of neuronal processes were augmented by knockdown of rat Cdh1. One possible explanation is that we targeted APC<sup>Cdh1</sup> in proliferating cells using retroviruses, whereas the transfection procedure of Bonni and co-workers targeted all cells, including those that were terminally differentiated. Thus, the timing of APC inhibition may direct outcome, where APC<sup>Cdh1</sup> is required for cell cycle exit, migration, and neurite outgrowth during the early stages of cell cycle withdrawal yet controls the extension of processes once cells have differentiated. Consistent with the
notion of distinct temporal roles for APC-Cdh1 in neuronal differentiation are the findings that APC inhibition can also lead to apoptosis of terminally differentiated cells and cell cycle exit defects in SH-SHY cells or in neural stem cells of Cdh1 heterozygous mice (33–35). Furthermore, the fact that mice lacking both alleles of Cdh1 in neurons were not reported may be a strong indication that Cdh1 plays an essential role in proliferation and cell cycle exit of neuronal precursors in vivo.

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