Alzheimer’s disease-related presenilins are thought to be involved in Notch signaling during embryonic development and/or cellular differentiation. Proteins mediating the cellular functions of the presenilins are still unknown. We utilized the yeast two-hybrid system to identify an interacting armadillo protein, termed p0071, that binds specifically to the hydrophilic loop of presenilin 1. In vivo, the presenilins constitutively undergo proteolytic processing, forming two stable fragments. Here, we show that the C-terminal fragment of presenilin 1 directly binds to p0071. Nine out of 10 armadillo repeats in p0071 are essential for mediating this interaction. Since armadillo proteins, like β-catenin and APC, are known to participate in cellular signaling, p0071 may function as a mediator of presenilin 1 in signaling events.

Mutations in the two presenilin genes (PS1 and PS2) are the most common cause of early-onset familial Alzheimer’s disease (for review, see Ref. 1). Encoded by genes on chromosomes 14 and 1, respectively, PS1 and PS2 are highly homologous proteins predicted to contain eight transmembrane helices (2, 3). Immunocytochemical analysis has shown that PS1 is localized mainly in the endoplasmic reticulum and, to a lesser extent, in the Golgi compartment (4, 5). More than 40 missense mutations have been identified in PS1 (6, 7), and these mutations account for up to 60% of early-onset cases of familial Alzheimer’s disease (8). Many of these mutations occur within transmembrane domains or immediately adjacent to the predicted hydrophilic loop between transmembrane domains 6 and 7 (9). A large family from Colombia has an E280A mutation in the hydrophilic loop of PS1; affected individuals show massive deposits of Aβ42 peptide in many brain regions (10). The mutant gene products of PS1 and PS2 cause dysfunction and/or death of vulnerable populations of nerve cells, with the resulting clinical syndrome of progressive dementia.

To examine the in vivo role of PS1 in mammalian development, mice with a targeted disruption of the PS1 gene were generated (11, 12). Homozygous mutant mice failed to survive beyond the first 10 min after birth. The most striking phenotype observed in PS1 null embryos was a severe perturbation in the development of the axial skeleton and ribs. The failed development of the axial skeleton in PS1 null mutants was traced to defects in somitogenesis; at embryonic days 8.5 and 9.5, somites were irregularly shaped and misaligned along the entire length of the neural tube and largely absent in the most caudal regions. The abnormal somite patterns in PS1 null embryos are highly reminiscent of somite segmentation defects in mice with functionally inactivated notch-1 and dll-1 genes (13, 14). Indeed, the expression of mRNA that encodes Notch-1 andDll-1 is strongly reduced in the presomitic mesoderm of PS1 null mice (12).

This raises the possibility that the presenilins play an important role in Notch signaling during embryonic development and/or cellular differentiation. In support of this notion, Sel-12, the presenolin homolog in Caenorhabditis elegans, functions as a co-receptor for the nematode notch receptor, Lin-12 (15), and a misfunction of the sel-12 gene causes constitutive activation of lin-12, resulting in an egg-lyting defect (15). How these functions contribute to the pathological properties of mutant presenilins in Alzheimer’s disease remains to be determined.

To elucidate the normal biological role of the presenilins, efforts have been made to identify molecules that interact with the presenilins. In three recent reports (16–18), PS1 was shown to interact with different members of the catenin family of proteins, termed β- and δ-catenin. Pathogenic mutations in the PS1 gene reduced the ability of PS1 to stabilize β-catenin and caused an increased degradation of β-catenin in the brains of transgenic mice (17).

The catenins are mammalian homologs of the Drosophila armadillo protein predicted to participate in the Wingless signaling pathway. The Notch and Wingless signaling pathways have been described to be mutually inhibitory and are functionally associated with the protein called Dishevelled (19). Interestingly, the human homolog of Dishevelled has been mapped to a region of chromosome 3 that has recently been linked to late-onset Alzheimer’s disease (6, 20).

We have now used yeast two-hybrid screens to identify molecules that bind to the hydrophilic loop of PS1. A single interacting protein was discovered, termed p0071, identical to a recently reported novel member of the armadillo family (21). The p0071 sequence covers three distinct domains: the N- and C-terminal domains lack significant homology to other known proteins, whereas the central domain represents a stretch of 10 armadillo repeat domains with strong homology to δ-catenin, p120, and B6P/plakophilin-1 (18, 21). The interaction of PS1 with p0071 was confirmed by three independent methods, i.e. yeast two-hybrid retransformation assays, binding to GST1 fusion proteins, and immunoprecipitation from brain and cotransfected COS cells. The interaction is mediated by the C-terminal fragment of PS1 constitutively formed by proteolytic

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1 The abbreviations used are: GST, glutathione S-transferase; PCR, polymerase chain reaction; aa, amino acid(s); PAGE, polyacrylamide gel electrophoresis.
processing under in vivo conditions (22). Neither the hydrophobic N or C terminus of PS1 nor the loop of PS2 shows an interaction with p0071. Nine out of 10 armadillo repeat domains in p0071 are essential for mediating this interaction, consistent with the paradigm that individual motifs or couples of these motifs function as protein modules in signaling cascades establishing protein-protein interactions (23).

Our studies provide compelling evidence that a novel member of the armadillo family, termed p0071, interacts in a specific manner with PS1. In contrast to the recently reported armadillo protein δ-catenin, which interacts with PS1 and shows a brain-specific expression pattern (18), p0071 and δ-catenin are broadly expressed in a variety of tissues (1, 21), fitting well with the widespread expression of PS1 (24). Thus, it seems that differential interactions modulated in a tissue- or developmental stage-specific manner may be the molecular basis for the different functions of the normal and mutant developmental stage-specific manner may be the molecular basis for the different functions of the normal and mutant proteins—

#### MATERIALS AND METHODS

**cDNA Cloning, Construction of Yeast Bait and Prey Vectors, Construction and Quantitation of Eukaryotic COS Cell Expression, and Sequencing**—Full-length cDNAs for rat PS1 and PS2 were generated by polymerase chain reaction (PCR) using primers specific for the respective 5′- and 3′-ends of the presenilin coding regions and rat brain cDNA (CLONTECH) as template. For amplification by PCR, Pfu polymerase (Stratagene) was used. Subcloning into pBlue-script SK II and sequencing confirmed the identity of PS1 and PS2. These presenilin constructs served as templates to amplify DNA fragments with specific primers containing the restriction enzyme sites BamHI at the 5′- and EcoRI at the 3′-end, respectively, for subcloning into the yeast bait vector pLexN (25).

**Yeast prey vector constructions**—were obtained by subcloning of p0071 DNA fragments into pVP16 (25) using BamHI and NorI as restriction sites. p0071 fragments were generated by PCR as described above. A partial p0071 clone obtained by a yeast two-hybrid screen of a rat brain library was used as template.

A PS1 construct with its hydrophilic loop fused to GST was generated by subcloning an EcoRI-SalI fragment into the vector pGEX-5X-1 (Amersham Pharmacia Biotech). It was expressed in Escherichia coli (XL1-Blue) and purified as described (26).

His6-tagged p0071 was created by cloning a 2.5-kilobase pair BamHI-HindIII fragment into pQE-9 (Amersham Pharmacia Biotech). The final construct was obtained by PCR amplification introducing a Kozak sequence (CCACCATGA) and subsequent cloning into the pCMV-based vector pcDNA3 (Invitrogen). Full-length PS1 was amplified by PCR using a sense primer with a Kozak sequence and a PS1 construct as template (see above). Subsequently, a 1.4-kilobase PCR fragment was cloned into the HI-EcoRI site of pDNA3. These two pcDNA3 constructs were used for transfection of COS cells. Plasmid DNA was transfected into COS cells using DEAE-dextran, and transfected COS cells were harvested 48 h after transfection (27).

The following primers were used for amplification by PCR: for PS1, 5′-ACCGGATCCGGTCTGCTCAATGACGATACCTCGAC (sense, starting at aa 1), 5′-GGGAATTCATTGAAATTTGATGGAAT (antisense, ending at aa 468), 5′-GGGAATTCGCTAGCTAATTANTTC (antisense, starting at aa 468), 5′-CCTGAGATATCGACGTATCTCAACC (sense, starting at aa 107), 5′-ATGGATGATCTTTGGC (antisense, starting at aa 510), 5′-ATGGATGATCTTTGGC (sense, starting at aa 552), 5′-ATGGATGATCTTTGGC (antisense, starting at aa 584), 5′-ATGGATGATCTTTGGC (sense, starting at aa 629), 5′-ATGGATGATCTTTGGC (antisense, starting at aa 659), 5′-ATGGATGATCTTTGGC (sense, starting at aa 690), 5′-ATGGATGATCTTTGGC (antisense, starting at aa 904), and 5′-ATGGATGATCTTTGGC (sense, starting at aa 963). The identity of most DNA constructs was confirmed by sequencing. DNA sequencing was performed by the dyeoxyxide nucleotide chain termination method using fluorescently labeled primers and an ABI370A DNA sequencer.

**Binding of Reconstituted p0071 to Immobilized GST Fusion Proteins**—COS cells transfected with His6-p0071 in pcDNA3 were solubilized in phosphate-buffered saline containing 1% Triton X-100 and 1 mM phenylmethylsulfonyl fluoride and centrifuged at high speed (10,000 × g) to remove insoluble material. The hydrophilic loop of PS1 fused to GST was expressed in E. coli and purified according to standard procedures (Amersham Pharmacia Biotech). Glutathione beads with immobilized GST fusion protein were incubated with His6-p0071-transfected COS cells at 4 °C for 1 h. After four washing steps with phosphate-buffered saline containing 1% Triton X-100 and 1 mM phenylmethylsulfonyl fluoride, SDS sample buffer was added to the beads. Proteins eluted from these beads were analyzed by SDS-PAGE and immunoblotting using the enhanced chemiluminescence method as described previously (30).

**RESULTS**

**Yeast Two-hybrid Screen for PS1-interacting Proteins**—Many of the mutations causing an early onset of familial Alzheimer’s disease are located in the proximal part of the hydrophilic loop in PS1 (1), implying an important functional role for this domain. Therefore, the loop domain was chosen as bait for a yeast two-hybrid screen. Yeast transformants containing the loop domain were screened for proteins interacting with the hydrophilic loop of PS1 encompassing residues 263–407. Only a single positive clone was identified from this screen, termed pPrey D1. Retransformations of yeast carrying a combination of pPrey D1 and a set of different bait plasmids demonstrated that β-galactosidase activation occurred only
Presenilin 1 Interacts with Armadillo Protein p0071

FIG. 1. PS1 interacts directly with armadillo protein p0071. A yeast-two-hybrid screen with a bait plasmid encoding the hydrophilic loop of PS1 and a prey cDNA library from rat brain was performed. As prey, a single positive clone encoding p0071 was identified. The column diagram shows β-galactosidase activities of yeast clones harboring the respective bait and prey plasmids. The interaction with p0071 was specific for the hydrophilic loop of PS1 (second column), β-galactosidase activity depended on both of them (second column). There was no detectable β-galactosidase activity when one of these proteins was missing (first and third columns). Neither the N terminus of PS1 (fourth column) nor its C terminus (fifth column) showed any interaction with p0071.

When pPrey D1 was cotransfected with a bait construct containing the hydrophilic loop of PS1 and a prey cDNA library from rat brain was performed. As prey, a single positive clone encoding p0071 was identified. The column diagram shows β-galactosidase activities of yeast clones harboring the respective bait and prey plasmids. The interaction with p0071 was specific for the hydrophilic loop of PS1 (second column). β-Galactosidase activity depended on both of them (second column). There was no detectable β-galactosidase activity when one of these proteins was missing (first and third columns). Neither the N terminus of PS1 (fourth column) nor its C terminus (fifth column) showed any interaction with p0071.

Confirmation of the Interaction between PS1 and p0071—To confirm the validity of the interaction, three additional independent sets of experiments were performed. The binding of p0071 to an immobilized GST fusion protein containing the hydrophilic loop of PS1 was analyzed by SDS-PAGE and subsequent immunoblotting (Fig. 2). For this purpose, a His6 tag at the N terminus of p0071 was used as a recognition site for a commercially available monoclonal antibody. His6-tagged p0071 interacted specifically with the hydrophilic loop of PS1 fused to GST (Fig. 2A, second lane). No binding to GST was observed (Fig. 2A, first lane), demonstrating the specificity of the interaction.

We conclude from this experiment that p0071 interacts in vitro with the hydrophilic loop of PS1. To demonstrate an interaction in vivo, we used COS cells and brain tissue for co-immunoprecipitation experiments. COS cells were transfected with His6-tagged p0071, full-length PS1, or a combination of both. A polyclonal antibody raised against the N terminus of PS1 specifically recognized a 43-kDa protein in transfected COS cells and a 28-kDa protein in both transfected and untransfected cells (Fig. 3A). The relative molecular masses of these proteins perfectly matched the expected sizes of full-length PS1 and the N-terminal fragment of PS1, respectively (22).

This antibody was used to immunoprecipitate solubilized PS1 from transfected COS cells. The immunoprecipitates were analyzed by SDS-PAGE and immunoblotting for His6-p0071. His6-tagged p0071 and PS1 co-immunoprecipitated from cotransfected COS cells (Fig. 3B, third lane), demonstrating an interaction of both proteins in vivo. No interaction was observed in single His6-p0071-transfected cells (Fig. 3B, second lane), although COS cells express endogenous PS1 (22). Most likely, endogenous PS1 was cleaved by a constitutively active protease, forming an N- and a C-terminal fragment (22). Since the antibody used for immunoprecipitation is directed against the N-terminal part of PS1, binding of p0071 to the C-terminal part would not be detectable. However, in transfected cells overexpressing PS1, full-length PS1 is the predominant form (Fig. 3A) (22).

The question now arises whether PS1 and p0071 interact in a human tissue, e.g. in human brain. To address this question, PS1 was immunoprecipitated from solubilized human brain tissue (cortex) using the monoclonal antibody APS 18 (31). APS 18 was reported to be specific for the C-terminal fragment of human PS1 (31). The immunoprecipitates obtained with the PS1 and p0071 antibodies were analyzed by gel electrophoresis and immunoblotting (Fig. 3, C and D). Indeed, p0071 and PS1 co-immunoprecipitated, strongly supporting the notion that both proteins form a stable complex in the brain. Taken together, we conclude from these experiments that PS1 and the armadillo protein p0071 specifically interact in vitro and in vivo.

Determination of the Binding Domains in p0071 and PS1—To determine the domains mediating the interaction between p0071 and PS1, we made a series of bait and prey vector constructs with inserts shortened at their respective 5’- and 3’-ends. We then measured the β-galactosidase activities for various combinations of these constructs by the yeast two-hybrid method.

p0071 is composed of three distinct domains: unique N- and C-terminal parts encompassing residues 1–509 and 988–1211, respectively, and a highly conserved middle part comprising a set of 10 armadillo repeat domains (21). As depicted in Fig. 4, armadillo repeat domains 2–10 are necessary and sufficient to mediate the interaction with the hydrophilic loop of PS1. The unique N- and C-terminal parts of p0071 are not necessary for interaction, although it seems that these parts might strengthen the interaction (for comparison, see constructs A and E). Since the highly conserved armadillo repeats mediate the interaction, it is not surprising that other members of the armadillo family, like β- and δ-catenin, can also bind to PS1 (16–18).

Under in vivo conditions, PS1 is specifically cleaved by a protease, leading to the formation of stable N- and C-terminal fragments (22). These two fragments stay together and form a tight complex with an unknown biological function (31). The question now arises as to which part of the complex interacts with p0071. Therefore, we generated multiple bait vector constructs. Two of these constructs start with the residues reported recently (34) as the major physiological cleavage sites in PS1. As shown in Fig. 5 (constructs D and E), the C-terminal fragment of PS1 mediates the interaction with p0071. Interestingly, the strength of the interaction was enhanced by shortening the loop fragments (for comparison, see constructs A, B, D, and E). The N-terminal presenilin fragment did not bind to p0071 (construct C). Thus, only one part of the complex consisting of the presenilin fragments is responsible for the interaction.
**Fig. 2.** The immobilized hydrophilic loop of PS1 binds to p0071. A, a GST fusion protein containing the hydrophilic loop of PS1 was immobilized on glutathione beads. These beads were incubated with a Triton X-100 extract from COS cells transfected with His₆-p0071. Proteins bound to these beads were analyzed by SDS-PAGE and immunoblotting for the His epitope. The hydrophilic loop of PS1 interacted specifically with His₆-p0071 (second lane). It did not interact with endogenous COS cell proteins (third lane). GST alone did not bind to His₆-p0071 (first lane). B, electrophoretically separated GST and GST-PS1 encompassing residues 263–407 were stained with Coomassie Blue. Equal amounts of both proteins were used for binding assays.

**Fig. 3.** PS1 and p0071 co-immunoprecipitate from transfected COS cells and human brain tissue. A, extracts from COS cells transfected with full-length PS1 were immunostained with a polyclonal antibody raised against the N terminus of PS1. The N-terminal (N-term.) fragment of PS1 was detected in both untransfected and transfected cells (marked by an asterisk), whereas full-length PS1 was found only in transfected cells (second lane, marked by an arrow). B, COS cells were transfected with PS1 (first lane), His₆-p0071 (second lane), or a combination of both proteins (third lane). PS1 was immunoprecipitated from solubilized COS cells with a specific PS1 antibody (specificity of this antibody is shown in A). From these cells, immunoprecipitates were analyzed by SDS-PAGE and immunoblotting for the His₆ epitope. Immunoprecipitates from cotransfected COS cells contained His₆-p0071 (third lane). His₆-p0071 was not found in immunoprecipitates from COS cells transfected with either His₆-p0071 (second lane) or PS1 (first lane), demonstrating that PS1 and p0071 interact specifically in cotransfected cells. The arrow and the asterisk indicate the positions of His₆-p0071 and the heavy chain of the antibody used for immunoprecipitation, respectively. C, PS1 was immunoprecipitated from solubilized human brain tissue (cortex) with the monoclonal antibody APS 18, specific for the C-terminal fragment of human PS1. Analysis of these immunoprecipitates with a polyclonal antibody raised against p0071 revealed a strong band with a molecular mass of 130–140 kDa (second lane, marked by an arrow). This band was absent when a monoclonal antibody directed against the synaptic vesicle protein synaptophysin was used for immunoprecipitation (first lane, control). Please note that endogenous p0071 (C) is bigger than recombinant p0071 (B) because a partial cDNA clone (aa 104–963) was used for expression in COS cells. D, p0071 was precipitated from solubilized brain tissue using the polyclonal antibody mentioned above. These immunoprecipitates contained the C-terminal fragment (C-term.) of PS1 (second lane, marked by an arrow). Full-length PS1 did not co-immunoprecipitate. The C-terminal fragment of PS1 was not detectable using the preimmune serum instead of the p0071 antiserum (first lane, control).
Comparison of PS1 and PS2 with Regard to p0071 Binding—As mentioned above, the presenilins exist in two distinct isoforms, PS1 and PS2. The amino acid sequences of both isoforms are very similar, implying that they are derived from a common ancient precursor. The presenilin isoforms seem to function in a common and distinct manner (24). Therefore, we asked whether the interaction with p0071 is a common function of both presenilin isoforms.

The presenilin loop sequences from different species are depicted in Fig. 6 using the CLUSTAL method for alignment. Residues shared by at least three of the four presenilins are shown on a black background. The sequences at both ends of the loop are highly conserved (Fig. 6A). In contrast, in the

![Fig. 4. The armadillo repeats of p0071 mediate interaction with PS1. N- and C-terminal deletion mutants of p0071 were coexpressed in yeast together with the hydrophilic loop of PS1. A, the numbers on both sides of the p0071 constructs represent the N- and C-terminal amino acids of the various constructs, respectively. The armadillo repeats of p0071 are depicted as black boxes. B, the interaction of various p0071 constructs with the hydrophilic loop of PS1 was determined by measuring the specific activity of the reporter gene β-galactosidase. β-Galactosidase activity was measured in triplicates and is shown as the mean ± S.D. The shortest fragment of p0071 that could interact with PS1 encompassed residues 552–963 (construct E), showing that 8.5 out of 10 armadillo repeats are necessary and sufficient to mediate the interaction.](image1)

![Fig. 5. The C-terminal part of PS1 binds specifically to p0071. N- and C-terminal deletion mutants for the hydrophilic loop of PS1 were generated and coexpressed in yeast together with p0071. A, the numbers on both sides of the PS1 constructs specify the N- and C-terminal amino acids of individual constructs, respectively. The arrows point to two major physiological cleavage sides of PS1. B, the interaction of these PS1 constructs with p0071 was determined by measuring the specific activity of the reporter gene β-galactosidase. β-Galactosidase activity was measured in triplicates and is shown as the mean ± S.D. The N-terminal part of the PS1 loop failed to bind to p0071 (construct C), whereas the C-terminal part bound specifically to p0071 (construct E). A prey construct devoid of p0071 (construct F) did not show any interaction with the C-terminal part of PS1.](image2)
A

rat presenilin 1
rat presenilin 2
mouse presenilin 1
human presenilin 1

rat presenilin 1
rat presenilin 2
mouse presenilin 1
human presenilin 1

rat presenilin 1
rat presenilin 2
mouse presenilin 1
human presenilin 1

rat presenilin 1
rat presenilin 2
mouse presenilin 1
human presenilin 1

rat presenilin 1
rat presenilin 2
mouse presenilin 1
human presenilin 1

rat presenilin 1
rat presenilin 2
mouse presenilin 1
human presenilin 1

B

specific activity (nmol/min/mg protein)

loop of presenilin 2 and prey vector

loop of presenilin 2 and p0071

loop of presenilin 1 and p0071

Fig. 6.
mediate the interaction with PS1 (Fig. 4). As recently reported, armadillo repeats 2–10 are necessary and sufficient to function in signaling cascades involving imperfect 42-amino acid repeats (36). Based on the The members of the armadillo family are characterized by a series of imperfect 42-amino acid repeats involved in inductive signaling events during development (35).

B to p0071 (see Fig. 5). In this region, rat PS1 and PS2 are very divergent, explaining the inability of PS2 to bind to p0071 (see Fig. 5 and the second column).

We conclude from this experiment that the presenilin-p0071 interaction is confined to PS1, suggesting that the presenilin isoforms differ in their functions. Indeed, quantitative reverse transcription-PCR studies revealed that PS1 and PS2 mRNAs are expressed at significantly different levels among tissues and in brain development (24). Furthermore, the functional inactivation of the mouse PS1 gene leading to a lethal phenotype could not be compensated by the PS2 gene (11, 12), implying that the presenilin isoforms have common and distinct functions.

**DISCUSSION**

The presenilins PS1 and PS2 are the major genes responsible for early-onset familial Alzheimer’s disease (1). The aim of this study was to find interacting proteins for PS1 because most cellular functions of proteins are mediated by protein-protein interactions. Thus, the identification of interacting molecules helps us to understand the biological function of a protein. Therefore, we performed a yeast two-hybrid screen for the hydrophilic loop of PS1, located between transmembrane domains 6 and 7. In this screen, we identified a recently discovered novel member of the armadillo family, termed p0071 (21), that interacts in a specific manner with PS1. We confirmed the interaction by three independent methods, i.e. yeast two-hybrid retransformation assays, binding to GST fusion proteins, and immunoprecipitations from human brain and transfected COS cells.

As mentioned, p0071 belongs to a protein family named after the *Drosophila* segment polarity gene armadillo, which is involved in inductive signaling events during development (35). The members of the armadillo family are characterized by a series of imperfect 42-amino acid repeats (36). Based on the modular composition of the armadillo family members, it was suggested that individual motifs or couples of these motifs function in signaling cascades establishing protein-protein interactions (23). Therefore, we were interested in determining the binding motif in p0071.

Using the yeast two-hybrid method, we could demonstrate that armadillo repeats 2–10 are necessary and sufficient to mediate the interaction with PS1 (Fig. 4). As recently reported, β-catenin interacts with conductin via its armadillo repeats 3–7 (37). Thus, the interactions mediated by p0071 or β-catenin support the above-mentioned paradigm of how armadillo proteins mediate protein-protein interactions.

The armadillo family members β/δ-catenin and p0071 show a high degree of amino acid identity in the armadillo repeat domain located in the middle of the respective molecules. For instance, p0071 and δ-catenin are 69.2% identical in their armadillo repeat domains. This high degree of identity in the domains mediating protein-protein interactions predicts that the respective binding partners are similar or even identical. Thus, it is not surprising that β/δ-catenin can also interact with PS1 (16–18).

The question now arises as to what the biological significance is of multiple interacting molecules. Most likely, the interactions of PS1 with different members of the armadillo family may be a function of the cell type or the developmental stage of an organism. In support of this hypothesis, δ-catenin shows an almost brain-specific expression pattern (18), whereas p0071 and β-catenin are widely expressed in all tissues (1, 21), reminiscent of the PS1 expression pattern (24).

A direct interaction of PS1 with members of the armadillo family has a number of implications. β-Catenin and its *Drosophila* homolog armadillo are multifunctional proteins: both are key components of cell-cell adhesive junctions and also participate in transduction of Wingless-Wnt cell-cell signals. Wingless-Wnt signals direct many key developmental decisions, regulating anterior-posterior pattern in flies and vertebrates. β-Catenin/armadillo is a key effector of signal transduction. In the absence of signals, levels of free β-catenin/armadillo are low: the Wingless-Wnt signal stabilizes free β-catenin/armadillo. Stabilized β-catenin binds to a transcription factor of the Tcf-Lef family and enters the nucleus, inducing expression of genes involved in proliferation or apoptosis (38).

The high degree of similarity between the armadillo family members implies that p0071 acts in a similar manner. Indeed, p0071 contains a nuclear targeting signal (KKKKKKKR) involved in its armadillo repeat domain. Thus, it is tempting to speculate that p0071, in concert with a transcription factor, enters the nucleus and alters gene expression. The function of PS1 might be to form a complex with p0071 in the endoplasmic reticulum or Golgi compartment and to control its targeting and/or proteolytic degradation. The targeted disruption of the PS1 gene in mice strongly reduces the expression of Notch-1 in the presomitic mesoderm (12). In addition, the abnormal somite patterns in PS1 null embryos are highly reminiscent of somite segmentation defects described in mice with a functionally inactivated notch-1 gene (13). Thus, the members of the armadillo family might be mediators of PS1 function in signaling cascades. Further experiments will be needed to elucidate the precise function of the presenilins and armadillo proteins in the Wingless and Notch signaling pathways.

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