Presenilin 1 in migration and morphogenesis in the central nervous system

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Summary

Morphogenesis of the central nervous system relies in large part upon the correct migration of neuronal cells from birthplace to final position. Two general modes of migration govern CNS morphogenesis: radial, which is mostly glia-guided and topologically relatively simple; and tangential, which often involves complex movement of neurons in more than one direction. We describe the consequences of loss of function of presenilin 1 on these fundamental processes. Previous studies of the central nervous system in presenilin 1 homozygote mutant embryos identified a premature neuronal differentiation that is transient and localized, with cortical dysplasia at later stages. We document widespread effects on CNS morphogenesis that appear strongly linked to defective neuronal migration. Loss of presenilin 1 function perturbs both radial and tangential migration in cerebral cortex, and several tangential migratory pathways in the brainstem. The inability of cells to execute their migratory trajectories affects cortical lamination, formation of the facial branchiomotor nucleus, the spread of cerebellar granule cell precursors to form the external granule layer and development of the pontine nuclei. Finally, overall morphogenesis of the mid-hindbrain region is abnormal, resulting in incomplete midline fusion of the cerebellum and overgrowth of the caudal midbrain. These observations indicate that in the absence of presenilin 1 function, the ability of a cell to move can be severely impaired regardless of its mode of migration, and, at a grosser level, brain morphogenesis is perturbed. Our results demonstrate that presenilin 1 plays a much more important role in brain development than has been assumed, consistent with a pleiotropic involvement of this molecule in cellular signaling.

Key words: Presenilin 1, Neuronal migration, Morphogenesis, Cortical development, Midbrain, Dopaminergic neurons, Cerebellum, Precerebellar nuclei, Facial branchiomotor neurons, γ-Secretase, Mouse

Introduction

Presenilin 1 and 2 (Psen1 and Psen2) are polytopic membrane proteins that are mutated in the majority of pedigrees with early-onset familial Alzheimer’s disease (Price and Sisodia, 1998). Compelling evidence has accumulated supporting a role for Psen1 in intramembranous, ‘γ-secretase’ processing not only of the β-amyloid precursor protein (APP) (De Strooper et al., 1998; Naruse et al., 1998), but also an increasing list of various type I membrane proteins, including Notch1 (De Strooper et al., 1999; Struhl and Greenwald, 1999), Erb-B4 (Ni et al., 2001), N- and E-cadherins (Marambaud et al., 2002), low density lipoprotein receptor-related protein (May et al., 2002), CD44 (Lammich et al., 2002), nectin 1α (Kim et al., 2002), and DCC (Taniguchi et al., 2003) (for a review, see Sisodia and St George-Hyslop, 2002). Although it has become clear that the biochemical function of presenilin is reflected in the phenotype of Psen1 mutants (Wong et al., 1997), focusing on CNS morphogenesis and neuronal migration.

In the present study, we asked if the emerging complexity of the biochemical function of presenilin is reflected in the phenotype of Psen1 mutants (Wong et al., 1997), focusing on CNS morphogenesis and neuronal migration.

One of the best-studied examples of radial migration is the formation of the cerebral cortex (Marín and Rubenstein, 2003; Rakic, 2003). Molecular and cellular studies coupled with analyses of natural and targeted mutations in the mouse have indicated that radial migration is under the control of at least two signaling pathways, the one involving reelin and its receptors (Tissir and Goffinet, 2003), the other dependent upon Cdk5 and its regulatory subunits p35 and p39 (Dhavan and Tsai, 2001; Ohshima and Mikoshiba, 2002). Tangential migration, however, is a broad term used to group together various forms of neuronal movement along the anteroposterior...
or dorsoventral axis of the neural tube. Paradigms of tangential migration include interneurons migrating from the ganglionic eminences into the cortex and the olfactory bulb; neuronal precursors of the cerebellum and the precerebellar system migrating from the rhombic lip; and facial motoneurons migrating within the brainstem. Tangential migration has been associated with a host of molecular signals, including motogenic factors, extracellular matrix and cell-adhesion molecules, and many of the same chemoeffector and chemorepulsive signals implicated in axon guidance (Marín and Rubenstein, 2001; Marín and Rubenstein, 2003).

We examined the development of the cerebral cortex as an example of both radial and tangential migration, and hippocampal dentate gyrus precursors, the external granular layer of the cerebellum, the precerebellar system and facial branchiomotor neurons as varied examples of tangential migration. In addition to widespread neuronal migration defects in the cortex, hippocampus, midbrain, cerebellar system and hindbrain, we documented defects in morphogenesis of the mid-hindbrain region. We found that both general modes of migration are disturbed in the Psen1 mutants, suggesting that the many disparate molecular mechanisms directly or indirectly governing neuronal migration are simultaneously affected.

Materials and methods

Mice

The Psen1 mutation (Wong et al., 1997) was maintained on C57BL/6 background. A total of 34 litters between E10.5 and E17.5 (155 wild-type or heterozygous and 65 homozygous embryos) were analyzed.

Retrograde labeling and photoconversion

Embryos were fixed overnight in phosphate-buffered 4% paraformaldehyde. A solution of Dil (Molecular Probes) (0.5% in ethanol, further diluted 1:10 in 0.3 M sucrose) was injected into the VIIth cranial nerve root exposed from the ventral aspect after dissection; DiO (Molecular Probes) was injected into the medial mass in the mutants. To label pia-attached radial glia, Dil crystals were placed along the pia. Photoconversion of Dil into a stable product was performed as described (Louvi and Wassef, 2000).

BrdU labeling

For labeling of dividing cells at E17.5, pregnant females were injected intraperitoneally with a solution of BrdU (15 mg/ml in saline) at 20 μg/g of body weight and sacrificed 4 hours later. BrdU incorporation was detected with anti-BrdU-FITC antibody (Beckton-Dickinson) as described (Tole et al., 1997).

In situ hybridization and immunohistochemistry

Both were performed as described (Louvi and Wassef, 2000). RNA probes used were for the following genes: β-tubulin (type III), Cdh6, Cdh8, Dab1, Dlx2, ephrin A5, F-spondin, Gad67, Gata3, Gbx2, Hes5, Hoxb1, Isl1, Lmx1a, Lmx1b, Math1, NeuroD, Pax6, Phox2b, p75, Prox1, reelin, Rora, Scip, Tag1, Thb1 and Th. RC2 mAb (1:2) was from Developmental Studies Hybridoma Bank.

Results

Defects in facial branchiomotor neuron migration

Facial branchiomotor (FBM) neurons are born in ventral rhombomere (r) 4 and undergo complex migration before they reach their final destination close to the pial surface of r6, where they form the facial motor nucleus (Altman and Bayer, 1982). Observation of Psen1 mutants revealed a cellular mass, accumulating medially on the ventricular surface of the ventral hindbrain from embryonic day (E) 11.5 onwards. This mass consisted of postmitotic neurons, as indicated by class III β-tubulin in situ hybridization and anti-neurofilament immunofluorescence; TUNEL assay revealed no differences in cell death in this area in comparison with wild-type embryos (Fig. 1A,B; data not shown). Floor-plate markers, including Shh and netrin, were unaffected and inter-rhombomere boundaries appeared undefined in the Psen1 mutants at E10.5 (data not shown). Rhombomere identity markers indicated that the mass accumulated in r4, but was nevertheless negative for Hoxb1, the r4 marker (Murphy et al., 1989). Its spatial and temporal coordinates suggested that it might correspond to FBM neurons amassing ectopically. In situ hybridization with probes for Phox2b, Tag1 (Ctn2 – Mouse Genome Informatics) and Ret, established markers of migratory FBM neurons (Garel et al., 2000), indicated that FBM neurons were born in ventral r4 of Psen1 mutants, executed their differentiation program by modulating gene expression, but failed nevertheless to engage in caudal migration through r5 and r6 (Fig. 1C,D; data not shown). A subset of inner ear efferent (contralateral vestibulocoustic or CVA) neurons, also born in ventral r4, project axons contralaterally and eventually migrate across the midline (Simon and Lumsden, 1993; Bruce et al., 1997). CVA neurons were generated normally in the Psen1 mutants, as revealed by Gata3 expression at E11.5 (Nardelli et al., 1999; Pata et al., 1999) (Fig. 1E,F), but failed to send axons contralaterally, and presumably to migrate across the midline (see below).

To corroborate these findings, we injected DiI into the common facial/vestibulocoustic nerve root at E11.5, in order to trace migrating neurons. In wild-type embryos, a cluster of FBM neurons migrates tangentially along the floor plate in r5 and r6, and visceral motoneurons (VMN) migrate dorsally within r5 (Fig. 1G). Observation at a different focal plane revealed CVA neurons projecting contralaterally across the floor plate (Fig. 1G, inset). In Psen1 mutants, FBM neurons failed to initiate tangential migration and, as a result, accumulated medially at the most distal extent of the leading processes that appeared punctuated. Moreover, CVA neurons did not project contralaterally. Small focal injections of DiO into the ectopic mass labeled axons extending only ipsilaterally towards the exit point of the VIIth nerve (Fig. 1H).

Despite the defect documented at the onset of FBM neuron migration, a small fragmented facial (VII) nucleus was eventually formed, as demonstrated by in situ hybridization with Isl1 (Ericson et al., 1992) at E16.5 (Fig. 1J), indicating that at least a subset of FBM neurons complete their migratory routine. Therefore, in the absence of Psen1, FBM neurons differentiate, but fail to migrate properly. This defect thus reflects an involvement of Psen1 in tangential neuronal migration.

Further potential migration defects were noted, but studied in less detail. Two other hindbrain somatic motor nuclei were abnormal: the nucleus abducens (VI), differentiating within r5/6, was fragmented; while the hypoglossal (XII) nucleus appeared fused in an aberrant dorsomedial position in r8. Extreme disorganization of hindbrain motoneurons was already obvious at E11.5, evidenced by Isl1 expression (data

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Finally, Pax6 expression in neuronal progenitors was downregulated in the hindbrain of Psen1 mutants at E11.5 (Fig. 1K,L). Unexpectedly, Pax6-positive cells formed discrete streams in ventral r3 and r5/6 (arrows in Fig. 1L). Taken together, these data suggested that the null mutation in Psen1 affected the migration of somatic motoneurons in the hindbrain. These observations correlate well with the strong expression of Psen1 in most hindbrain nuclei and the facial nucleus in particular (Tanimukai et al., 1999) (A.L., unpublished).

Defects in glia-guided radial migration of cortical neurons

In light of our observations and reports that have indicated cortical dysplasia with focal heterotopias in three independently generated Psen1 strains (Hartmann et al., 1999; Handler et al., 2000; Yuasa et al., 2002), we analyzed cortical development, as an example of radial migration. Psen1 mutants are smaller than wild type, with abnormal overall brain morphology, in part because of severe CNS hemorrhage (Wong et al., 1996; Shen et al., 1996). Because they die perinatally, our analyses were limited to late embryonic stages. Cortical stratification was perturbed in the Psen1 mutants at E16.5, as evidenced in Nissl preparations (Fig. 2A-D). Psen1 itself is expressed in wild-type cortex in the ventricular zone (VZ), the intermediate zone (IZ) where cells migrate, the subplate (SP) and the cortical plate (CP) at E17.5 (Fig. 2E,F). Neuronal differentiation, assessed by class III β-tubulin gene expression, appeared to have proceeded at comparable levels in wild-type and Psen1 mutant littermates at late stages (data not shown). Indeed, neuronal differentiation occurs prematurely (and in a region-specific manner) at early stages in Psen1 mutants, but reverts to wild-type rates after E12.5 (Handler et al., 2000).

In cerebral cortex, radial glial cells are thought both to generate neurons and guide their radial migration (Noctor et al., 2001). A single-dose BrdU pulse at E17.5 labeled the nuclei of dividing cells – presumed to be radial glia – in the VZ. Labeled cells formed an orderly, thin band along the ventricular surface of wild-type cortex (Fig. 2G) but in the
mutant, patches of dense labeled nuclei alternated with patches of sparse labeling (Fig. 2H), suggesting that radial glia progenitor cells were abnormally positioned or dysfunctional. Previous studies have documented only mild and localized differences in BrdU labeling patterns in the \textit{Psen1} mutants at early stages (Handler et al., 2000).

To assess migration to and within the cortical plate at E17.5, we used a panel of layer-specific markers for genes [reelin, \textit{Scip} (Pou3f1 – Mouse Genome Informatics), \textit{Tbr1} and \textit{p75} (Ngfr – Mouse Genome Informatics)]. The earliest generated marginal zone (MZ), which is identified by reelin expression, formed normally in the \textit{Psen1} mutants, indicating that migration of Cajal-Retzius cells was unaffected. The layer of Cajal-Retzius cells appeared nevertheless disrupted, perhaps owing to overall reduction of cell density in the MZ (Hartmann et al., 1999). Expression of \textit{Scip} is confined to a subpopulation of prospective layer V neurons, but is also high in the subventricular (SVZ) and intermediate (IZ) zones at late embryonic stages (Frantz et al., 1994). In \textit{Psen1} mutants, \textit{Scip} expression was indeed detected within the CP; strikingly, however, many neurons expressed \textit{Scip} ectopically along a radial path, as though unable to execute their migratory program properly (Fig. 2I-L). At late embryonic stages, \textit{Tbr1} is expressed in the subplate and future layer VI, as well as in superficial layers I-III (Bulfone et al., 1995). Expression of \textit{Tbr1} in the \textit{Psen1} mutants again revealed apparent abnormal migratory behavior of differentiating cortical neurons (Fig. 3A,B). Expression of \textit{p75}, which was confined to the subplate and layer VI at this stage (Mackarehtschian et al., 1999), was not only downregulated in the \textit{Psen1} mutants, but also appeared patchy, lacking normal gradients, and severely disorganized (Fig. 3C,D). Furthermore, expression of cadherin 6 and cadherin 8, which were detected, respectively, in future layers II-IV and V/VI (Inoue et al., 1998), was downregulated overall in the CP and nearly absent in the IZ of \textit{Psen1} mutants (Fig. 3E,F; data not shown).

A common denominator in gene expression pattern changes described above was lower than normal intensity in the VZ of the \textit{Psen1} mutants (Fig. 2I-L; Fig. 3A-F). We analyzed expression of radial glial markers, on one hand, and of \textit{Dab1}, on the other, as components of the reelin pathway are expressed in the VZ (Tissir and Goffinet, 2003). \textit{Dab1} was downregulated in the CP of \textit{Psen1} mutants at E16.5, and nearly undetectable in the VZ (Fig. 3G,H). Interestingly, \textit{Dab1} has recently been shown to colocalize with radial glial markers in the VZ (Luque et al., 2003), and to influence neuronal migration by controlling the timing of detachment of migrating neurons from radial glia (Sanada et al., 2004). \textit{Pax6}, which is localized in radial glia in the E16 cortex (Götz et al., 1998), was severely downregulated in the \textit{Psen1} mutants (Fig. 3J,L). Finally, the dramatic downregulation of \textit{Hes5}, a target of the Notch pathway in neural progenitors and a faithful read-out of its activity (Ahmad...
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et al., 1995; Ohtsuka et al., 1999), indicated that Notch signaling was suboptimal in the Psen1 mutants (Fig. 3K,L). This result is consistent with findings described above, in that activation of Notch signaling promotes context-dependent radial glial fate (Gaiano et al., 2000).

The molecular data described above, in conjunction with the abnormal distribution of BrdU at E17.5, pointed, albeit indirectly, to a defect in radial glia. To directly examine its morphology, we labeled pia-attached radial glia by placing small DiI crystals along the pial surface of the brain at E16.5. In wild type, radial glia processes were rather straight and regular, resembling well-combed hair, and extended to the ventricular surface. By contrast, in the Psen1 mutant, radial glial processes appeared tangled (Fig. 4A-D). Morphological changes in radial glia were confirmed by RC2 immunohistochemistry (Fig. 4E,F). To assess the association of neurons with radial glia, DiI was photoconverted and sections were processed to show Scip expression. In wild-type cortex, Scip-expressing neurons were smoothly juxtaposed to radial glia processes (Fig. 4G). In the mutant, thick masses of Scip-expressing cells and their processes overlaid clumps of radial glia, suggesting stalled or defective migration (Fig. 4H). Both molecular and morphological observations therefore pointed to defects in cortical proliferation and radial migration, at least in part due to abnormalities in radial glial cells.

Corticogenesis also depends on the tangential migration of some cell types, notably of interneurons from the ganglionic eminences. Expression of Dlx2 (Porteus et al., 1994) identified in wild-type embryos a well-developed region adjacent to the ganglionic eminences (Fig. 5A), from which a faint stream of cells appeared to be migrating into the cortex through the SVZ (arrow in Fig. 5A). In Psen1 mutants, however, the Dlx2-positive region was abnormal (Fig. 5B), with more migrating cells evident (arrow in Fig. 5B). Expression of glutamic acid decarboxylase (Gad67; Gad1 – Mouse Genome Informatics) revealed that Gad67-positive cells, normally engaging in tangential migration through deep layers, had instead aggregated superficially (Fig. 5C,D; arrow in Fig. 5D).

Finally, the dentate gyrus, another site of tangential migration, appeared poorly formed as assessed by NeuroD (Neurod1 – Mouse Genome Informatics) and Prox1 expression (data not shown). Taken together, our observations suggest that cortical plate formation, which relies on radial migration, and tangential migration of interneurons from the basal telencephalon into the cortex were both affected in the absence of Psen1.

Migration defects in the midbrain

We next analyzed the effects of Psen1 loss of function in the midbrain, where neuronal migration has not been studied extensively. We noticed that expression of F-spondin (Spon1 – Mouse Genome Informatics), which is implicated in spinal cord commissural axon pathfinding (Burstyn-Cohen et al., 1999) was severely diminished in the Psen1 mutants at E10.5 and E11.5 (Fig. 6A,B; data not shown). However, ventral midbrain expression of other markers (Shh, Lmx1a, Lmx1b, Pax6)
between E10.5 and E12.5 appeared normal (Fig. 6C,D; data not shown). Moreover, the anlagen of the oculomotor complex (III) and the trochlear (IV) motor nucleus, identified, respectively, by Isl1/Gata3/Phox2b expression, or Phox2b expression alone (Pattyn et al., 1997; Nardelli et al., 1999; Agarwala and Ragsdale, 2002) formed in the Psen1 mutants (Fig. 6E,F; data not shown); the former, however, was smaller and had fewer Isl1/Gata3-positive cells migrating anteriorly (Fig. 6F).

Midbrain dopaminergic (DA) neurons are born ventrally and thought to migrate extensively to their final positions (Altman and Bayer, 1981). Migrating DA neurons were identified by tyrosine hydroxylase (Th) expression (Specht et al., 1981). DA neurons appeared fused across the midline in the Psen1 mutants at E11.5 (Fig. 6G,H), where Th-expressing cells also persisted later (Fig. 6I,J). Thus, DA neurons appear to represent yet another example of a cell type that differentiates but does not migrate properly, becoming stalled at abnormal positions.

Finally, the two bilateral streams of cells emigrating caudally from the mesencephalic tract of the trigeminal nerve (tmesV) into dorsal r1, identified by Isl1 expression at E11.5 (Fedtsova and Turner, 2001), were considerably underdeveloped (data not shown).

Defects in tangential migration in the brainstem

Next, we focused on the development of the cerebellum and precerebellar system for several reasons: the rhombic lip is a site of tangential (posterior to anterior and circumferential) migrations par excellence; Psen1 is expressed in the cerebellum, the pontine nucleus and the inferior olive (Lee et al., 1996; Tanimukai et al., 1999); and, finally, the caudal midbrain appears to have overgrown at the expense of an abnormal cerebellum.

Prenatal cerebellar development relies on tangential migration of the proliferative granule cell precursors (GCPs) from the anterior rhombic lip over the developing cerebellum, and ascension of Purkinje cells (PCs) from the neuroepithelium along radial glia fibers (Altman and Bayer, 1997). In Psen1 mutants, the cerebellum was smaller with its two halves remaining separate posteriorly, indicating incomplete fusion at the midline (Fig. 7A-H). To examine GCPs at E17.5 we analyzed Math1 (Atoh1 – Mouse Genome Informatics) (Bena-Rie et al., 1997) and Pax6 (Engelkamp et al., 1999). A
well-developed external granule layer (EGL) covered the cerebellum in wild type; in the Psen1 mutants, however, GCPs failed to reach the anterior-most part of the cerebellum (Fig. 7A-B,E-F,G-H), where a GCP-free medial region developed (arrow in Fig. 7B,D). In addition, PCs, which are identified by Rora expression (Hamilton et al., 1996), had clustered beneath the displaced EGL (Fig. 7C,D,I,J; compare 7A with 7C, 7B with 7D, 7G with 7H, and 7H with 7I).

As noted further below, these defects in cerebellar morphogenesis are likely to represent the combinatorial outcome of abnormal proliferation as well as differentiation and migration.

Development of the precerebellar system, however, relies on tangential migrations from the posterior rhombic lip (Altman and Bayer, 1997; Rodriguez and Dymecki, 2000). For example, pontine nuclei are formed by long-range migration of postmitotic precursors along a superficial circumferential trajectory. We used Pax6 as a marker of the pontine migratory stream and nuclei proper (Engelkamp et al., 1999; Yee et al., 1999). Pontine nuclei were underdeveloped in the Psen1 mutants, as evidenced by morphology and Pax6 expression and the migratory stream was underpopulated with migratory precursors, only a few of which appeared to reach their final destination in the pons (Fig. 7K,L). Instead, a cluster of Pax6-positive cells accumulated ectopically (Fig. 7L, arrow). Finally, the inferior olive appeared poorly assembled (data not shown). Thus, the pontine nuclei appear to represent another example of a tangential migration defect in the absence of Psen1.

**Defects in morphogenesis of the mid/hindbrain**

Gross morphological and gene expression analyses indicated defects in the derivatives of the mid/hindbrain (see Fig. 7A-D). A single-dose BrdU pulse at E17.5 identified the compromised EGL in the Psen1 mutants (Fig. 8A,B), and numerous proliferating cells in the GCP-free ectopic region rostral to the ventricular view; anterior towards the top. The oculomotor complex, which is detected by Gata3 expression, is smaller in the mutant (F) in comparison with wild type (E). (G-I) Midbrain dopaminergic neurons (DA), detected by TH expression at E11.5, develop close to the ventral midline in the wild-type (G) but are continuous across the midline in the mutant (H). (IJ) At late embryonic stages (E17.5), DA neurons remain clustered at the midline of the mutant (J). Notice ectopic cluster of TH-positive neurons (arrow in J). Coronal sections through E11.5 (G,H) and E17.5 (LJ) embryos. aq. aqueduct; red arrowheads in HJ indicate the midline.

To assess early stages of cerebellar morphogenesis, we analyzed Math1 and Pax6 expression. Expression of Math1 was normal at E10.5 but became significantly enhanced in the mutants at E11.5 and persisted in the medial rhombic lip, where it is normally downregulated (Louvi et al., 2003). At E14.5, the rhombic lip appeared thinned and, on the other, the isthmic organizer was deregulated, indicating that the inferior colliculus was indeed expanded in the Psen1 mutants (Fig. 8E,F).

**Discussion**

Loss of Psen1 function results in multiple abnormalities in brain structure at the end of embryogenesis. We have
documented widespread, albeit not universal, defects in neuronal migration and CNS morphogenesis in the \textit{Psen1} mutants affecting both radial as well as tangential migration and leading to abnormal morphogenesis of the cerebral cortex and the brainstem. The sites where these \textit{Psen1}-associated defects are seen correlate well with regions of high levels of \textit{Psen1} mRNA expression, consistent with the notion that \textit{Psen1} may indeed be implicated in these processes, but also suggesting a far broader role of \textit{Psen1} in brain development than previously assumed. Importantly, our analysis indicates that the phenotype we document is likely to be the consequence of abnormalities in a diverse set of cellular pathways that are linked directly or indirectly to \textit{Psen1} function.

\textbf{\textit{Psen1} and \textit{Cdk5} mutants: similarities and implications}

The array of neuronal migration defects in the \textit{Psen1} mutants is strikingly reminiscent of those observed in embryos with targeted mutations in the \textit{Cdk5} mutants affecting both radial as well as tangential migration and leading to abnormal morphogenesis of the cerebral cortex and the brainstem. The sites where these \textit{Psen1}-associated defects are seen correlate well with regions of high levels of \textit{Psen1} mRNA expression, consistent with the notion that \textit{Psen1} may indeed be implicated in these processes, but also suggesting a far broader role of \textit{Psen1} in brain development than previously assumed. Importantly, our analysis indicates that the phenotype we document is likely to be the consequence of abnormalities in a diverse set of cellular pathways that are linked directly or indirectly to \textit{Psen1} function.

The array of neuronal migration defects in the \textit{Psen1} mutants is strikingly reminiscent of those observed in embryos with targeted mutations in the \textit{Cdk5/p35/p39} pathway (reviewed by Dhavan and Tsai, 2001; Ohshima and Mikoshiba, 2002). In the cerebral cortex, \textit{Cdk5} mutants exhibit defects confined to the late-migrating, glia-guided cortical neurons. \textit{Cdk5} and \textit{Psen1} mutants share a surprisingly similar defect in the positioning of FBM, complete with the appearance of an ectopic mass of postmitotic neurons in the ventral hindbrain. Dhavan and Tsai (Dhavan and Tsai, 2001) have previously suggested that \textit{Cdk5} and its regulatory subunits p35 and p39 are crucial regulators of neuronal migration. Indeed, \textit{p35} mutants display an atypical mode of migration in the cortex, associated with disrupted neuronal-glial interactions (Gupta et al., 2003). The similarities in the mutant phenotypes we document indicate the possible convergence of different pathways towards similar intracellular factors and could be the manifestation of molecular interactions between the \textit{Psen1} and \textit{Cdk5} ‘pathways’. \textit{Psen1} has been reported to interact with \textit{Cdk5} in many ways. On the one hand, \textit{Cdk5/p35} has been reported to bind and phosphorylate \(\beta\)-catenin and to regulate \(\beta\)-catenin/\textit{Psen1} interactions (Kesavapany et al., 2001). \textit{Cdk5/p35} is, on the other hand, involved in the regulation of N-cadherin-mediated adhesion in cortical neurons, and N-cadherin itself is a \(\gamma\)-secretase substrate (Kwon et al., 2000; Marambaud et al., 2002). \textit{Dab1}, which is downregulated in \textit{Psen1} mutants, is also a substrate for \textit{Cdk5/p35}, and interacts with \textit{APP} in yeast two-hybrid screens (Howell et al., 1999; Keshvara et al., 2002). Finally, deregulation of \textit{Cdk5} activity through accumulation of \textit{p25} (a cleavage product of \textit{p35}) has been implicated in Alzheimer’s disease itself (Tseng et al., 2002).
In comparing Psen1-related neuronal migration phenotypes to the phenotypic consequences of other mutations, it is noteworthy that recent observations suggest an intersection of pathways controlling molecular mechanisms of neuronal migration and axonal transport. In legs at odd angles (Loa/Loa) embryos, which carry a missense mutation in dynein cytoplasmic heavy chain, migrating FBM neurons bifurcate and a double nucleus eventually forms (Hafezparast et al., 2003), not unlike the fragmented facial nucleus of the Psen1 mutants. Cytoplasmic dynein is a major motor complex involved in retrograde transport with reported roles in neuronal migration, neurite outgrowth and axonal transport of microtubules, neurofilaments and organelles (reviewed by Morris, 2000; Terada and Hirokawa, 2000). Interestingly, the dynein pathway component Nudel is a substrate for Cdk5 (Niethammer et al., 2000; Sasaki et al., 2000), suggesting a further link. Moreover, embryos lacking another motor protein, kinesin KIF1Bβ, also display defective development of the facial nucleus (Zhao et al., 2001). Notably, Psen1 has recently been implicated in anterograde (kinesin-based) axonal transport (Pigino et al., 2003).

**Psen1 and Small eye mutants: the role of Pax6**

It is quite clear that the abnormalities we observe in the Psen1 mutants cannot be explained only by the Psen1/Cdk5 relationship. Our analysis revealed a second process that appears to be affected by Psen1 malfunction, namely Pax6 regulation. We showed that, in the Psen1 mutants in general, Math1 expression is enhanced in the Psen1 mutants (B, upper panel) in comparison with wild type (A, upper panel). At E13.5, strong Math1 expression persists in the medial rhombic lip of the mutant (arrow in B, middle panel) but becomes downregulated in the wild type (A, middle panel). At E14.5, Math1-positive cells are spreading over the cerebellum in the wild type (A, lower panel), but accumulate medially in the mutant (B, lower panel). Notice the thinned rhombic lip and overall abnormal morphology of cerebellum in the mutant.

**Fig. 8.** Morphogenetic defects in the mid/hindbrain region. (A,B) BrdU incorporation at E17.5 detects proliferating cells in the EGL of wild type (A) and Psen1 mutants (B). In the mutant, proliferation is highest in medial ectopic tissue (white arrow in B) and also in the caudal midbrain (black arrow in B). (C,D) Expression of reelin is detected in the EGL of wild type (C) and mutant (D), and reveals, in addition, a dramatic overgrowth of the caudal midbrain in the mutant (asterisk in D) and a medial mass forming between the developing cerebellum and the caudal midbrain (delineated by the two white lines in D). (E,F) Ephrin A5 expression identifies the inferior colliculus in wild type (E) and its expansion in the mutant (F). Sagittal (A-D) or coronal (E,F) sections through caudal midbrain and cerebellum at E17.5.

**Fig. 9.** Early development of the cerebellum. (A,B) Incorrect regulation of Math1 expression in the anterior rhombic lip. At E11.5, Math1 expression is enhanced in the Psen1 mutants (B, upper panel) in comparison with wild type (A, upper panel). At E13.5, strong Math1 expression persists in the medial rhombic lip of the mutant (arrow in B, middle panel) but becomes downregulated in the wild type (A, middle panel). At E14.5, Math1-positive cells are spreading over the cerebellum in the wild type (A, lower panel), but accumulate medially in the mutant (B, lower panel). Notice the thinned rhombic lip and overall abnormal morphology of cerebellum in the mutant.

(C-F) Downregulation of Gbx2 expression in anterior hindbrain. (C,D) Gbx2 expression is downregulated in the mutant. Dorsal view of wild type (C) and mutant (D) embryos after whole-mount in situ hybridization. Gbx2 expression is lower in dorsal r1 of the mutant (arrow in D). (E,F) Flat-mount preparations of hindbrain following in situ hybridization with Gbx2. Notice downregulation and incorrect pattern of expression (asterisks in F) in the mutant anterior hindbrain.
radial glia are lost in the Sey/Sey cortex (Götz et al., 1998). Moreover, in the Sey/Sey mouse, loss of Pax6 leads to excessive migration of interneurons from basal brain into the cortex (Chapouton et al., 1999; Yun et al., 2001), hindbrain motor neurons are incorrectly specified (Erickson et al., 1997; Osumi et al., 1997) and rhombic lip-derived structures severely affected (Engelkamp et al., 1999). Widespread downregulation of Pax6 expression as such documented in the Psen1 mutants is therefore expected to affect much the same processes. Thus, the defects in radial-glia dependent cortical migration, radial glia differentiation in the VZ, incorrect processes. Thus, the defects in radial-glia dependent cortical migration, radial glia differentiation in the VZ, incorrect migration of interneurons into the cortex and of pontine precursors in the precerebellar system could be consequences of insufficient levels of Pax6 expression. Although no direct relationship between Pax6 and Psen1 has been described, studies in Drosophila have linked Notch signaling with the regulation of the Pax6 ortholog eyless (ey) during eye development, suggesting that ey may act, in some fashion, downstream of Notch signal input (Kurata et al., 2000; Kumar and Moses, 2001; Kenyon et al., 2003). One possibility, therefore, is that Psen1 influences Pax6 function via the Notch pathway.

Morphogenesis of the cerebellar system, tangential migrations and guidance molecules

We found that loss of Psen1 function leads to abnormalities in glia-guided radial migration, as well as tangential migration, and to defects in cerebellar morphogenesis. We determined that the latter is accompanied by the downregulation of Gbx2 in the anterior hindbrain. Otx2 and Gbx2 act antagonistically to position the isthmic organizer (reviewed by Wurst and Bally-Cuif, 2001), and although Otx2 appears unaffected in the Psen1 mutants, Gbx2 downregulation implies that the isthmic organizer might be deregulated. Interestingly, in the absence of functional Gbx2, the inferior colliculus is dramatically thickened (Wassarman et al., 1997), a phenotype also observed in the Psen1 mutants. In addition to Gbx2 downregulation, incorrect regulation of Math1 may partly account for the cerebellar morphogenetic phenotype: downregulation of Math1 in the medial rhombic lip is essential for cerebellar midline fusion (Louvi et al., 2003). What could account for the failure of CGPs to reach the anterior-most part of the cerebellum? Although we have not examined this issue in detail, we note that the guidance molecule ErbB4, subject to γ-secretase processing (Ni et al., 2001), is expressed in CGPs and in the caudal rhombic lip (Dixon and Lumsden, 1999), explaining perhaps part of the migration defects we observe. In addition, it is known that precerebellar nuclei precursors interpret netrin as a chemotactant (reviewed by Wingate, 2001) and this property could account for the defects in the pontine migratory stream and nuclei. Interestingly, all netrin receptors, the migratory pontine cells express on their leading processes solely DCC (Yee et al., 1999) and not surprisingly, pontine nuclei are absent in netrin 1 and Dcc mutant mice (Serafini et al., 1996; Fazeli et al., 1997). Moreover, cells migrating to the inferior olive express Dcc (in addition to other netrin receptors) and netrin signaling plays a role in the finer subdivision of this nucleus. Although netrin expression appears unaffected in the ventral hindbrain of Psen1 mutants, Dcc was recently shown to undergo Psen1-dependent γ-secretase processing (Taniguchi et al., 2003), suggesting that the interpretation and/or reception of netrin signaling is defective in the absence of functional Psen1.

Presenilins and the cytoskeleton: a mechanistic explanation?

As radial and tangential modes of migration are affected in the Psen1 mutants, it seems plausible that fundamental cellular mechanisms required for cell movement might be perturbed in the absence of functional Psen1 protein. In preparation to move, cells extend a leading process sensing the immediate environment, followed by translocation of the nucleus into the leading process and subsequent retraction of the trailing process. The first step heavily depends on polymerization and reorganization of actin microfilaments and is controlled by Rho family GTPases (Ridley, 2001), while the second step relies on microtubules (Morris et al., 1998; Lambert de Rouvroit and Goffinet, 2001; Nadarajah and Parmavelas, 2002). Evidence suggests that Psen1 may indeed interact with cytoskeletal elements. First, in hippocampal cultures, Psen1 associates with microtubules and microfilaments in a developmentally regulated manner and is localized in lamellipodia and filopodia of neuronal growth cones (Pigino et al., 2001). Second, the microtubule-associated protein Tau can associate with Psen1 in cultured cells and to a lesser extent in brain extracts (Takashima et al., 1998). Third, presenilins can interact in vivo and in vitro with at least two actin-binding family members, filamin A and filamin homolog 1 (Zhang et al., 1998). In Drosophila, filamin interacts genetically and physically with presenilin (Guo et al., 2000). More importantly, in humans, mutations in filamin A prevent migration of cerebral cortical neurons causing periventricular heterotopia (Fox et al., 1998). Finally, in Drosophila, Psn (presenilin) mutations disrupt the spectrin cytoskeleton (López-Schier and St Johnston, 2002), whereas Psen1-dependent γ-secretase cleavage of E-cadherin leads to its dissociation from the cytoskeleton (Marambaud et al., 2002). Interestingly enough, α-spectrin accumulates in cytoplasmic inclusions in the brains of individuals with Alzheimer’s disease (Sangerman et al., 2001). Thus, evidence, albeit circumstantial, exists to suggest a link between presenilins and the cytoskeleton that could provide a mechanistic explanation for some of the migration defects seen in the Psen1 mutants.

In conclusion, our analysis established a novel role for Psen1 in neuronal migration and morphogenesis while revealing the complex relationship between Psen1 and specific cellular events and biochemical pathways that affect migration. The extent to which this complex phenotype directly or indirectly reflects the diversity of substrates that can be affected by γ-secretase remains to be determined. Nevertheless, our data implicate Psen1 with a roster of specific cellular pathways and demonstrate that the Psen1 loss-of-function phenotypes reflect the many developmental processes simultaneously affected by this mutation.

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