The role of presenilin 1 during somite segmentation

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SUMMARY

The Notch signalling pathway plays essential roles during the specification of the rostral and caudal somite halves and subsequent segmentation of the paraxial mesoderm. We have re-investigated the role of presenilin 1 (Ps1; encoded by Psen1) during segmentation using newly generated alleles of the Psen1 mutation. In Psen1-deficient mice, proteolytic activation of Notch1 was significantly affected and the expression of several genes involved in the Notch signalling pathway was altered, including Delta-like3, Hes5, lunatic fringe (Lfng) and Mesp2. Thus, Ps1-dependent activation of the Notch pathway is essential for caudal half somite development. We observed defects in Notch signalling in both the caudal and rostral region of the presomitic mesoderm. In the caudal presomitic mesoderm, Ps1 was involved in maintaining the amplitude of cyclic activation of the Notch pathway, as represented by significant reduction of Lfng expression in Psen1-deficient mice. In the rostral presomitic mesoderm, rapid downregulation of the Mesp2 expression in the presumptive caudal half somite depends on Ps1 and is a prerequisite for caudal somite half specification. Chimaera analysis between Psen1-deficient and wild-type cells revealed that condensation of the wild-type cells in the caudal half somite was concordant with the formation of segment boundaries, while mutant and wild-type cells intermingled in the presomitic mesoderm. This implies that periodic activation of the Notch pathway in the presomitic mesoderm is still latent to segregate the presumptive rostral and caudal somite. A transient episode of Mesp2 expression might be needed for Notch activation by Ps1 to confer rostral or caudal properties. In summary, we propose that Ps1 is involved in the functional manifestation of the segmentation clock in the presomitic mesoderm.

Key words: Somite, Presenilin 1, Mesp2, Notch1, Lunatic fringe, Dll1, Segmentation, Mouse

INTRODUCTION

In vertebrates, somite formation precedes the segmental appearance of the vertebral column, trunk musculature and peripheral nerves. The somites are generated by segmentation of the paraxial mesoderm on either side of the neural tube and notochord, where they form as pseudoepithelial spheres. Newly formed somites are a mosaic of presumptive rostral and caudal cells. A number of differences exist between the rostral and caudal sclerotome halves, reflected, that is, by the specific expression of several marker genes (Stern and Keynes, 1987; Yamaguchi et al., 1992; Candia et al., 1992; Bettenhausen et al., 1995; Rovescalli et al., 1996; Mansouri et al., 1997; Saga et al., 1997). The rostrocaudal polarity of each somite is already established within the presomitic mesoderm. The specification of the rostral and caudal compartments is plays an important role in the formation of segment boundaries (Aoyama and Asamoto, 1988; Swiatek et al., 1994; Conlon et al., 1995; Hrabe de Angelis et al., 1997; Saga et al., 1997).

Insight into the segmentation of the somites has recently been obtained from observations of the rhythmic expression of hairy-related genes (chick hairy1 and chick hairy2, avian homologues of mouse Hes1), of lunatic fringe (Lfng) in chick presomitic mesoderm, and of Hes1 and Lfng in mouse presomitic mesoderm (Palmeirim et al., 1997; McGrew et al., 1998; Aulehla and Johnson, 1999; Jouve et al., 2000). Since the Lfng gene product is essential for normal segmentation of somites, a molecular clock represented by the cyclic expression of Lfng seems to be involved in somite segmentation (Zhang et al., 1998; Evrard et al., 1998). The Lfng gene product apparently functions via the Notch signalling pathway during segmentation of the somitic mesoderm for the following reasons: First, fringe products are required for Delta-dependent proteolytic activation of Notch by presenilins in both invertebrates and vertebrates (Panin et al., 1997; Cohen et al., 1997; Irvine 1999; Moloney et al., 2000; Brückner et al., 2000; Hicks et al., 2000). Second, segmentation of the somitic mesoderm is affected in Notch1, Delta-like1 (Dll1), Dll3 and presenilin 1 (Ps1; encoded by Psen1 – Mouse Genome Informatics) -deficient mice (Swiatek et al., 1994; Conlon et al., 1995; Hrabe de Angelis et al., 1997; Wong et al., 1997; Shen et al., 1997; Kusumi et al., 1998). Therefore, the Notch
pathway is essential for the establishment of rostrocaudal polarity under the control of the segmentation clock.

Psen1 has been identified as a major causal gene of early-onset familial Alzheimer’s disease (Sherrington et al., 1995). Ps1 processes the amyloid precursor protein (APP) and Notch family proteins as a γ-secretase (De Strooper et al., 1998; De Strooper et al., 1999; Wolfe et al., 1999; Struhl and Greenwald, 1999; Ye et al., 1999). Ps1 regulates the nuclear translocation of the Notch intracellular domain (NICD) via the proteolytic cleavage of the C-terminal fragment (CTF) of Notch in a Delta- and fringe-dependent manner. In Psen1 deficient mice, the specification of the caudal half somite is affected and, subsequently, segmentation of the somitic mesoderm is impaired (Wong et al., 1997; Shen et al., 1997). In our previous studies, we showed that Mesp2, which encodes a bHLH transcriptional regulator expressed in the rostral region of the presomitic mesoderm is required for specification of the rostral half somite (Saga et al., 1997). Phenotypic analyses of Mesp2/Psen1 double mutants suggest that the sequential repression and activation of the Notch pathway by Mesp2 and Ps1, respectively, coordinates the establishment of the rostral and caudal halves of the somites (Takahashi et al., 2000). Although a functional antagonism between Mesp2 and Ps1 seems to be essential for somite segmentation, the molecular basis for this antagonism is not well understood.

In this study, we report the generation of a new allele of the Psen1 mutation with slightly hypomorphic skeletal phenotypes, and suggest that Ps1 is involved in the repression of Mesp2 expression in the presumptive caudal somite halves. Chimaera analysis revealed the segregation of Psen1-deficient and wild-type cells immediately after the transient expression of Mesp2 and before the formation of the segment boundaries, suggesting a requirement of Mesp2 expression for Notch activation.

MATERIALS AND METHODS

Generation of a new allele of the Psen1 mutation

A genomic clone covering exon 9 of Psen1 gene was isolated from the 129/SvJ mouse genomic library in AFIXII (Stratagene). The 6 kb genomic fragment containing exons 7 and 8 and a 2.1 kb fragment amplified from a genomic clone by PCR were used for long and short arms, respectively (Fig. 1A). A NotI/KpnI restricted insert fragment containing the long arm fragment with the neo-resistance gene (Neo+) cassette derived from the pMC1Neo and DT-A(B) vector were introduced to allow negative and positive selection (Oriental Kobo, Japan, Yagi et al., 1993). The targeting vector was linearised with NotI and used for the electroporation of embryonic stem (ES) cells. Genomic DNA from the 264 G418-resistant clones was digested with BglII and analyzed by Southern blot using a 442 base pair (bp) 3' probe (nucleotides 45509-45950; GenBank, AF007560) (Fig. 1A,B). Four homologous recombinants were obtained and germline probe (nucleotides 45509-45950; GenBank, AF007560) (Fig. 1A,B). Four homologous recombinants were obtained and germline probe (nucleotides 45509-45950; GenBank, AF007560) (Fig. 1A,B). Four homologous recombinants were obtained and germline probe (nucleotides 45509-45950; GenBank, AF007560) (Fig. 1A,B).

Detection of proliferating and apoptotic cells

Proliferating cells in 11.5 dpc embryos were labelled with Bromodeoxyuridine (Brdu; Sigma) and immunodetected with anti-Brdu antibody (clone B44; Becton Dickinson), as described previously (Wallin et al., 1994).

In vitro culture of explants

Embryos at 9.5 dpc were harvested and the tail region was sectioned at 6 μm. The in situ TdT-mediated dUTP-fluorescein-labelled nick end labelling (TUNEL) was performed on sections using cell death detection kits (Boehringer Mannheim).

Generation of the chimaeric embryos and β-galactosidase staining

Chimaeras were generated as depicted in Fig. 8A. Briefly, eight-cell embryos of the mutant mice were obtained from crosses of Psen1ΔE9 heterozygous (Psen1ΔE9/+;ROSA26Δ/+), and Psen1null mutant heterozygous (Psen1ΔE9/+;ROSA26Δ/+). Eight-cell embryos of wild type were obtained from crosses of males heterozygous for ROSA26 gene trap insertion with BDF1 females (Zambrowicz et al., 1997). Chimaeric embryos were generated by aggregating eight-cell embryos of wild type with and prospective mutant mice, harvested at 11.5 dpc, and fixed in 37°C in DMEM supplemented with 20% foetal calf serum in the hanging drops.
RESULTS

Generation of a new allele of the Psen1 mutation lacking exon 9

We have generated a new allele of the Psen1 mutation lacking exon 9, which encodes a proteolytic cleavage site in the Ps1 protein, and designate this mutant allele as Psen1<sup>AE9</sup> (Fig. 1A; Wolfe et al., 1999). In the offspring of heterozygous intercrosses, 4.2 kb wild-type and 6.0 kb targeted alleles were detected by a 3’ probe following BglII digestion (Fig. 1B). Mutant allele-specific bands were amplified by PCR from genomic DNA of Psen1<sup>+/-AE9</sup> and Psen1<sup>AE9/AE9</sup> foetuses (Fig. 1C).

The expression of Psen1 or Psen1<sup>AE9</sup> transcripts was examined by RT-PCR. As expected, 507 bp fragments representing Psen1<sup>AE9</sup> transcripts were amplified from Psen1<sup>AE9/AE9</sup> embryonic RNA, while 594 bp was detectable in expected molecular mass of about 50 kDa. We were totally abolished, and that no of the 30 kDa NTF and 20 kDa CTF CTF of Ps1 revealed that the expression analysis using antisera against NTF and Psen1 fragment was hardly visible in Psen1<sup>+/AE9</sup> embryos. Western blot analysis using antisera against NTF and CTF of Ps1 revealed that the expression of the 30 kDa NTF and 20 kDa CTF were totally abolished, and that no uncleaved Ps1 protein with the expected molecular mass of about 50 kDa was detectable in Psen1<sup>AE9/AE9</sup> foetal tissues (Fig. 2B). In Psen1<sup>+/AE9</sup> foetuses, the expression of NTF and CTF was half of that in the wild type. Therefore, the expression of the uncleaved Ps1 protein derived from the Psen1<sup>AE9</sup> locus could not be detected, and the Psen1<sup>AE9</sup> mutation was suggested to be almost equivalent to a null mutation. Indeed, the generation of NICD was significantly impaired in Psen1<sup>AE9/AE9</sup> foetal brain (Fig. 2C). The trace amounts of NICD observed in Psen1<sup>AE9/AE9</sup> foetuses might be due to residual Ps1<sup>AE9</sup> or presenilin 2 (Ps2) gene products (Levitan et al., 1996; Donoviel et al., 1999; Herreman et al., 1999).

Fig. 1. Generation of a new allele of Psen1 mutation lacking exon 9. (A) Maps of the wild-type Psen1 locus, the targeting vector, Psen1<sup>AE9</sup> allele and Psen1<sup>AE9/AE9</sup> allele. Black boxes represents the exons in Psen1 locus. A white box indicates Neo<sup>+</sup> cassette. The targeting vector shows the replacement of exon 9 and flanking genomic sequences by the Neo<sup>+</sup> cassette (neo-tk). Arrows indicate the sites for PCR primers used for genotyping of wild type, Psen1<sup>AE9</sup> and Psen1<sup>AE9/AE9</sup> alleles, and grey bars represent expected size of PCR products from each allele. The DNA fragment used for external probe is indicated by a horizontal black bar (probe). EcoRI, RI; HindIII, H; BamHI, B; BglII, Bgl. (B) DNA blot analysis by BglII digestion clearly distinguished homozygous, heterozygous and wild-type embryos. The external probe detects 4.2 kb BglII fragment in wild-type allele and 6.0 kb in Psen1<sup>AE9</sup> allele. (C) PCR analysis also distinguished homozygous, heterozygous and wild-type embryos. Genotyping was performed by PCR using three primers: Ps1/PCR#1 and Ps1/PCR#2 for wild type (giving 656 bp product) and Ps1/PCR#1 and Ps1/PCR#3 for Psen1<sup>AE9</sup> allele (giving 251 bp PCR product).
Therefore, the phenotype of 
\( Psen1^{DE9/DE9} \) mice is slightly milder than that of \( Psen1^{+/+} \) mice. We suggest that the \( Psen1^{DE9} \) mutation is a hypomorphic rather than null mutation. As uncleaved Ps1 is able to generate NICD trace amounts of uncleaved Ps1, gene products are probably expressed in \( Psen1^{DE9/DE9} \) mice, as suggested by RT-PCR, and can partially restore some of the defects observed in null mutants (Levitan et al., 1996). These axial skeletal abnormalities are a result of defects in the specification of the caudal half of the somitic mesoderm in \( Psen1^{-/-} \) mice (Wong et al., 1997; Shen et al., 1997). As expected, segmentation of the sclerotome was totally impaired in \( Psen1^{DE9/DE9} \) 9.5 dpc embryos as seen in \( Psen1^{+/+} \), and the expression of Uncx4.1, a marker for the caudal half somite, was completely missing in \( Psen1^{DE9/DE9} \) as well as \( Psen1^{+/+} \). However, NICD (105 kDa) was detected in \( Psen1^{+/+} \) exclusively, but not in \( Psen1^{DE9/DE9} \). Nonspecific signals that are due to secondary antibody are marked by asterisks.

Table 1. Rib abnormality in \( Psen1^{+/+} \) and \( Psen1^{DE9/DE9} \) mice

<table>
<thead>
<tr>
<th>Condition</th>
<th>( Psen1^{+/+} ) (n=10)</th>
<th>( Psen1^{DE9/DE9} ) (n=10)</th>
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<tbody>
<tr>
<td>Ribs</td>
<td>13 (8.7±0.6)</td>
<td>9.9 (±0.7)</td>
</tr>
<tr>
<td>Ribs to sternum</td>
<td>7 (5.5±0.6)</td>
<td>6.1 (±0.5)</td>
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Values are means (±s.d.)

Fig. 2. The expression of Ps1 and NICD in \( Psen1 \) mutant mice. (A) RT-PCR analysis for \( Psen1 \) expression. An upstream primer specific for exon 7 and downstream primer for exon 11 as shown were used. The RT reaction was conducted either in the presence (+) or absence (−) of reverse transcriptase. An expected, a 594 bp band was found in wild type and in heterozygous mutant. In homozygous mutant, a 507 bp product and two additional bands were found. The bands on the lower panel indicate PCR products derived from the G3PDH mRNA as a control. Sequence analysis indicated the 507 bp product amplified from \( Psen1^{DE9/DE9} \) embryos lack 87 nucleotides encoded by exon 9 and is able to encode expected mutant protein. (B) Western blot analysis for Ps1 expression in 14.5 dpc \( Psen1^{DE9/DE9} \), \( Psen1^{DE9/DE9} \) or \( Psen1^{+/+} \) foetus. The arrowheads indicate the Ps1 CTF and NTF. (C) Western analysis for NICD expression in 15 dpc \( Psen1^{+/+} \) or \( Psen1^{DE9/DE9} \) foetal brain. The processed C-terminal fragment of Notch1 (120 kDa) were detected in \( Psen1^{DE9/DE9} \) as well as \( Psen1^{+/+} \). However, NICD (105 kDa) was detected in \( Psen1^{+/+} \) exclusively, but not in \( Psen1^{DE9/DE9} \). Nonspecific signals that are due to secondary antibody are marked by asterisks.

The expression of genes required for the segmentation and induction of the somitic mesoderms in \( Psen1^{+/+} \) embryos

A molecular clock represented by the cyclic expression of Hes1 and Lfng in the tail bud mesoderm plays a decisive role for segmentation of the somitic mesoderm (Jouve et al., 2000; McGrew et al., 1998). As both Hes1 and Lfng are involved in the Notch signalling pathway, and as the generation of NICD is affected in \( Psen1 \) mutants, we examined the expression of...
Presenilin 1 in somite segmentation

genes involved in the Notch signalling pathway, including Notch1, Notch2, Dll1, Dll3, Hes1, Hes5, Lfng and Mesp2, in Psen1+/− embryos. The expression of Notch1, Notch2 and Dll1 was not significantly affected in Psen1+/− embryos, although the anterior boundaries of Notch1 and Notch2 expression were diffuse (Fig. 5A-D). Dll3 expression in the presomitic mesoderm was clearly increased in Psen1+/− embryos (Fig. 5G,H). A similar observation has been reported in Dll1-deficient mice (Barrantes et al., 1999). The expression of Hes5, one of the genes responsive to the Notch signalling pathway, was totally lacking. However, we could not detect expression of Hes1 in the presomitic mesoderm of wild-type embryos (Fig. 5I,J; K. K. and H. K., unpublished; Nishimura et al., 1998). This might be due to a problem in the sensitivity of our experiments. The expression of Lfng and Mesp2 were also significantly reduced in the presomitic mesoderm in Psen1+/− embryos (Figs 6A, 7A). Therefore, the expression of several genes involved in the Notch signalling pathway is affected in the presomitic mesoderm of Psen1+/− embryos.

We further investigated the expression of other classes of genes involved in segmentation and induction of the somitic mesoderm. Inductive signals from the surface ectoderm required for segmentation are represented by the expression of Paraxis (Sosic et al., 1997). Paraxis expression is not significantly affected in Psen1+/− embryos (Fig. 5K,L). Inductive signals mediated by the FGF family proteins are also known to play an essential role during segmentation of the somitic mesoderm (Yamaguchi et al., 1994; Deng et al., 1994). The expression of Fgfr1 and Fgf18 was not significantly affected in Psen1+/− embryos (Fig. 5M-P). We also investigated the expression of genes during the induction of presomitic mesoderm, because cyclic expression of Hes1 and Lfng has been described in mesenchymal cells in the tail bud region (McGrew et al., 1998; Aulehla and Johnson, 1999; Jouve et al., 2000). We have looked at the expression of several genes in the tail bud and early somitic mesoderm, including Wnt3a, Evx1, Hoxb4 and Mfhi1. The expression of these genes is not affected in Psen1+/− embryos (Fig. 5Q,R; K. K. and H. K., unpublished). Thus, the signals required for early induction of the somitic mesoderm or for segmentation emanating from the surface ectoderm or mediated by FGF family proteins are apparently not affected in Psen1+/− embryos. We suggest that
defects in the proteolytic activation of Notch family proteins by Ps1 could be causative factors for the defects in segmentation in Psen1-deficient mice.

**Lfng expression in the presomitic mesoderm is dependent on the Ps1 gene product**

The expression of Lfng in the paraxial mesoderm of wild-type embryos demarcates mutually distinct portions of the presomitic mesoderm because of its cyclic expression as reported previously with 30 minutes chromogenic reaction (Fig. 6A, parts a-d) (McGrew et al., 1998; Aulehla and Johnson, 1999). However, we could not see Lfng expression in Psen1-/- embryos following the same chromogenic reaction (Fig. 6A, parts e-h). Thus, Lfng expression in the presomitic mesoderm is significantly downregulated in Psen1-/- embryos. Interestingly, we did not observe the reduction of

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**Fig. 4.** Localization of proliferating and apoptotic cells in the presomitic and somitic mesoderm of Psen1+/+ mice. (A) Localization of BrdU-labelled cells in wild-type (a) and Psen1+/+ (c) mice. An arrowhead indicates a newly generated segment boundary. (b,d) Higher magnification views as indicated by boxes in (a) and (c), respectively. (B) Localization of TUNEL-positive cells in wild type (a) and Psen1+/+ (c) mice. (b,d) Higher magnification views as indicated by boxes in (a) and (c), respectively. Scale bars: 100 μm.

**Fig. 5.** The expression of mesodermal markers in wild type and Psen1+/+ embryos. (A,B) Notch1 expression in 9.5 dpc embryos. (C,D) Notch2 expression in 9.5 dpc embryos. (E,F) Dll1 expression in 9.5 dpc embryos. (G,H) Dll3 expression in 9.5 dpc embryos. Dll3 is significantly increased in the presomitic mesoderm of Psen1-/- embryos. (I,J) Hes5 expression in 11.5 dpc embryos. (K,L) Paraxis expression in 9.5 dpc embryos. (M,N) Fgfr1 expression in 9.5 dpc embryos. (O,P) Fgf18 expression in 10.5 dpc embryos. (Q,R) Wnt3a expression in 9.5 dpc embryos.
Presenilin 1 in somite segmentation

**Fig. 6.** Cyclic expression of Lfng in the presomitic mesoderm was retained but significantly reduced in Psen1−/− embryos. (A) The expression of Lfng in the presomitic mesoderm of the wild-type (a-d) and Psen1−/− (e-h) embryos. To allow the quantitative comparison, chromogenic reaction was limited to 30 minutes. Under this condition, Lfng expression could not be observed in any of Psen1−/− embryos (15 specimens), while dynamic expression of Lfng was clearly seen in the wild type. (B) Progression of wave front of cyclic expression of Lfng in the wild type (a-d) and Psen1−/− (e-h) embryos. Two hours chromogenic reaction allowed us to see Lfng expression in the presomitic mesoderm of Psen1−/− embryos (e-h). Specimens were aligned to show the rostrally directed progression of the wave front of Lfng expression as indicated by brackets. For wild-type embryos (left), specimens after 30 minutes reactions were shown. (C) Comparison of Lfng expression between uncultured (left) and cultured (right) halves with various times as shown. In Psen1−/− embryos (d-f), Lfng expression is indicated by brackets.

Lfng expression in the neural tube of Psen1−/− embryos (K. K. and H. K., unpublished). Chromogenic reaction for over 2 hours allowed us to see Lfng expression in Psen1−/− embryos (Fig. 6B, parts e-h). Specimens were aligned to show the rostrally directed progression of the wave front of Lfng expression. The anteriorly directed progression of Lfng expression was seen in wild-type and Psen1−/− embryos. This suggests that the cyclic expression of Lfng in the presomitic mesoderm is retained in Psen1−/− embryos. We further compared the duration of one cycle of Lfng expression between wild-type and Psen1−/− embryos. After bisection of the caudal regions of 9.5 dpc embryos at the midline with a microscalpel, one side was immediately fixed and the other was cultured for various periods from 60 to 120 minutes as reported previously (Aulehla and Johnson, 1999). For wild-type samples, more than ten specimens were examined for every time point. After 110 minutes of culture, four out of ten specimens exhibited almost identical expression patterns between uncultured and cultured specimens, while the position of the Lfng stripe in the rostral region of the somitic mesoderm shifted caudally (Fig. 6C, part b). After both 100 and 120 minutes in culture, one out of ten specimens exhibited identical patterns (Fig. 6C, parts a,c). We conclude that Lfng expression oscillates every 110 minutes on average under our culture conditions. Similarly, two out of six specimens obtained from Psen1−/− embryos exhibited almost identical expression patterns after 110 minutes in culture (Fig. 6C, part e) but identical pattern was not seen after 100 and 120 minutes (Fig. 6C, parts d,f). Thus, the amplitude of oscillation of Lfng expression is significantly affected while the duration is unaffected in Psen1−/− embryos.

**Rapid repression of Mesp2 expression in the presumptive caudal half somite is dependent on Ps1**

Mesp2 is also involved in the Notch pathway and is essential for the specification of the rostral half somite, and subsequently for segmentation (Saga et al., 1997). Mesp2 expression first appears one segment in width, and subsequently for segmentation (Saga et al., 1997). Mesp2 expression first appears one segment in width, and subsequently for segmentation (Saga et al., 1997). Rapid repression of Mesp2 expression has been suggested to be important for the establishment and maintenance of Ps1-dependent activation of the Notch signalling pathway (Takahashi et al., 2000). This led us to re-examine Mesp2 expression in Psen1−/− embryos. In 9.5 dpc wild-type embryos, Mesp2 expression in the presomitic mesoderm varies in width and strength due to the rapid downregulation in the presumptive caudal half somite (Fig. 7A, parts a-d). In Psen1−/− embryos, Mesp2 expression appears at almost the same positions as in the wild type, but the level is significantly reduced (Fig. 7A, parts e-h). Importantly, the width of the
Impairment of rapid downregulation of Mesp2 expression in the presumptive caudal half somite in Psen1−/− embryos.

(A) Mesp2 expression in the presumptive mesoderm of 9.5 dpc wild-type (a-d) and Psen1+/− (e-h) embryos. (B) Comparison of Mesp2 expression between uncultured (left) and cultured (right) halves with 60 or 120 minutes culture of 9.5 dpc wild-type (a-d) and cultured (right) halves with 60 or 120 minutes culture of 9.5 dpc wild-type (a-d) and Psen1−/− (e-h) embryos.

Mesp2 stripe does not vary while the strength of the stripe varies in Psen1−/− embryos. This suggests that the rapid repression of Mesp2 expression in the presumptive caudal half somite might be impaired in Psen1−/− embryos. To examine this possibility, we examined changes in the Mesp2 stripe after 60 and 120 minutes of culture in 24 wild-type or Psen1+/− embryos, and nine Psen1−/− embryos. In the wild type, the width and strength of the Mesp2 stripe changed clearly within 60 or 120 minutes (Fig. 7B, parts a-d), and in some cases the stripe shifted posteriorly (Fig. 7B, parts a,d). A rapid repression in the caudal half of the stripe is obvious as shown in Fig. 7B, part c. In contrast, we found no significant alterations in the width of Mesp2 stripe in Psen1−/− embryos while strength of the stripe changed (Fig. 7B, parts e-h). The position of the stripe shifted posteriorly in some cases (Fig. 7B, parts e,h). In conclusion, the rapid repression of Mesp2 expression in the presumptive caudal half somite is affected in Psen1−/− embryos, while the Mesp2 stripe appears at an almost normal position.

**Distribution of Psen1ΔE9−/− cells in the somitic and presomitic mesoderm of chimaeric embryos**

Although impairment in the Notch signalling is obvious in the tail bud mesoderm of Psen1-deficient mice, it has not yet been determined when it is functionally manifested. To address this, we have generated chimaera composed of Psen1ΔE9−/− and wild-type cells and, have analyzed chimaerism in the presomitic and somitic mesoderm. The wild-type cells were genetically labelled by β-galactosidase expression derived from the ROSA26 locus (Fig. 8A). The availability of Psen1ΔE9+ and Psen1null alleles allowed us to distinguish Psen1ΔE9−/− chimaera from Psen1+/ΔE9+ or Psen1+/− chimaera (Fig. 8B). We generated 74 chimaeras, seven of which were Psen1ΔE9−/− chimaeras with β-galactosidase expression. The tail regions of the chimaeras were dissected and subjected to a histological analysis in order to examine the formation of segment boundaries. The newly formed somites and presumptive somites undergoing segmentation are designated S0 and S-1, respectively (Fig. 8C). In this definition, the region of the Mesp2 stripe corresponds to S-2. Five out of seven of the Psen1ΔE9−/− chimaeras exhibited a moderate contribution of Psen1ΔE9−/− cells (Fig. 8D, parts a,e). Two showed a strong contribution of Psen1ΔE9−/− cells (Fig. 8D, part h). In all of the moderate Psen1ΔE9−/− chimaeras, we observed stripes of β-galactosidase-positive cells derived from the wild type in the somitic mesoderm, while the striped distribution was never seen in Psen1+/ΔE9+, Psen1+/− or wild-type chimaeras (Fig. 8D, parts a, e, E, parts a,e; K. K. and H. K., unpublished).

β-Galactosidase-positive cells were metamERICALLY distributed in the somitic mesoderm of moderate Psen1ΔE9−/− chimaeras as confirmed by low magnification histological analyses (Fig. 8D, parts b,f). In strong chimaeras, a metameric distribution of β-galactosidase-positive cells was vaguely visible, and histological investigation revealed the periodic appearance of colonies of β-galactosidase-positive cells (Fig. 8D, parts h,i). Stripes of β-galactosidase-positive cells were seen exclusively in the segmented region, but not in the presomitic mesoderm of either moderate or strong chimaeras. Interestingly, three out of five moderate chimaeras were segmented in the somitic mesoderm, but two were not (compare Fig. 8D, parts c,g). The segmented Psen1ΔE9−/− chimaeras allowed us to examine the localization of β-galactosidase-positive cells in each segment. β-Galactosidase-positive cells, indeed, clearly distributed to the caudal region of each segment while the segment borders were less obvious than in wild-type chimaeras (Fig. 7D, part c, E, part c). Within the newly segmented somite (S0), β-galactosidase-positive cells were clearly distributed in the caudal region, suggesting the exclusion of Psen1ΔE9−/− cells from the caudal region, while Psen1ΔE9−/− and wild-type cells still intermingled in the S-1 and S-2 regions (Fig. 8D, part d). In Psen1+/ΔE9+, Psen1+/− or wild-type chimaeras, β-galactosidase-positive cells were distributed almost evenly within each segment (Fig. 8E, parts c,d,g). Another piece of evidence obtained from the trunk region of strong chimaeras also suggests the predominant localization of wild-type cells...
in the caudal half somite. \(\beta\)-Galactosidase-positive cells in the strong chimaeras localise exclusively in the neural arch anlage, which are known to be derived from the caudal half somite (Fig. 8D, part k) (Goldstein and Kalcheim, 1991). This implies that the colonies of \(\beta\)-galactosidase-positive cells seen in the sclerotomal region of strong chimaeras might possess cellular properties specific for the caudal half somite, although the segment boundaries are not formed.
DISCUSSION

Periodic activation of the Notch pathway in the presomitic mesoderm

In the present study, we generated an allelic mutation of Psen1 that shows slightly hypomorphic phenotypes in the axial skeleton. Establishment of the caudal half somites and subsequent segmentation were totally impaired, as reported previously (Wong et al., 1997; Shen et al., 1997; Takahashi et al., 2000). Defects in the generation of NICD and mis-expression of Dll3, Hes5 and Lfng in the presomitic mesoderm in Psen1-deficient mice suggest that the defects in the somite segmentation are most probably due to impairment in the Notch signalling pathway.

The significant reduction of Lfng expression in Psen1 mutant embryos indicates that the Ps1 gene product is essential for maintaining the amplitude of Lfng expression in the presomitic mesoderm. Thus, the expression of Lfng is suggested to be under the control of NICD. As the fringe gene product is needed for the generation of NICD, the molecular circuitry, including the Notch family proteins. Ps1 and Lfng, and presumably Delta family proteins, seem to be important for the maintenance of the amplitude of the periodic activation of the Notch pathway in the presomitic mesoderm (Panin et al., 1997; Cohen et al., 1998; Brückner et al., 2000; Hicks et al., 2000). We have also demonstrated that the duration of cyclic activation of the Notch pathway, as represented by Lfng expression, is not altered significantly in Psen1 mutants. Thus, the molecular mechanisms that generate the periodic expression of Lfng, which presumably include the clock mechanism, are apparently independent of the Ps1 gene product. Importantly, the dynamic expression of Hes1 and Lfng is lost in the presomitic mesoderm of Dll1 mutants (Barrantes et al., 1999; Jouve et al., 2000). As both Hes1 and Lfng expression are regulated by the molecular clock linked to segmentation, the initial oscillation of the segmentation clock could be dependent on Notch signalling in the presomitic mesoderm. Because Ps2, a homologue of Ps1 is also able to function in the presomitic mesoderm. Because Ps1 and Lfng, and presumably Delta family proteins, seem to be important for the maintenance of the amplitude of the periodic activation of the Notch pathway in the presomitic mesoderm (Panin et al., 1997; Cohen et al., 1998; Brückner et al., 2000; Hicks et al., 2000). We have also demonstrated that the duration of cyclic activation of the Notch pathway, as represented by Lfng expression, is not altered significantly in Psen1 mutants. Thus, the molecular mechanisms that generate the periodic expression of Lfng, which presumably include the clock mechanism, are apparently independent of the Ps1 gene product. Importantly, the dynamic expression of Hes1 and Lfng is lost in the presomitic mesoderm of Dll1 mutants (Barrantes et al., 1999; Jouve et al., 2000). As both Hes1 and Lfng expression are regulated by the molecular clock linked to segmentation, the initial oscillation of the segmentation clock could be dependent on Notch signalling in the presomitic mesoderm. Because Ps2, a homologue of Ps1, is also able to function as a γ-secretase and is expected to play redundant roles, the remaining activity of Lfng expression might be due to the function of Ps2 (Donoviel et al., 1999; Herreman et al., 1999). It will be necessary to examine Lfng expression in Psen1/Pesen2 double homozygous embryos.

Specification of the caudal half somite involves a Ps1-dependent rapid downregulation of Mesp2

Mesp2 is a transcriptional regulator essential for specification of the rostral half somite as revealed by experiments involving Mesp2-deficient mice (Saga et al., 1997). The induction of Mesp2 expression in the S-2 region is, at least in part, dependent on Notch signals, because Mesp2 expression is significantly downregulated not only in Psen1- but also Dll1-, Notch1-, RBPJκ- and Dll3-deficient mice (this study, Barrantes et al., 1999; Y. Takahashi and Y. S., unpublished). Based on the present study, we suggest that the rapid downregulation of Mesp2 in the presumptive caudal half is also dependent on Notch activation mediated by Ps1. Derepression of Mesp2 expression in the presumptive caudal half somites seems to be involved in the development of caudal half somite defects in Psen1 mutants because the effects of the Psen1 mutation can be restored by a Mesp2 mutation, as previously observed in Psen1/Mesp2 double homozygotes (Takahashi et al., 2000). As Mesp2 has been demonstrated to repress Dll1 expression, the rapid repression of Mesp2 mediated by Ps1 might allow the establishment and stabilisation of the molecular circuitry essential for caudal half somite specification, including Dll1, Notch1 and Ps1.

A previous study using the Mesp2lacZ allele demonstrated that the downregulation of Mesp2 in the segmented region is dependent upon Mesp2 itself, not Ps1 (Takahashi et al., 2000). The downregulation of Mesp2 in the presumptive rostral and caudal halves might therefore be dependent on Mesp2 and Ps1, respectively.

Functional manifestation of the Notch activation induces homophilic condensation of like half somite cells

The chimera assay revealed that segment border formation is concordant with the condensation of wild-type cells into the caudal compartment of forming somites while Psen1-deficient and wild-type cells intermingle in the presomitic region. This implies that segregation of presomitic cells into prospective rostral and caudal half compartments takes place in the S-1 region of chimaeras because the transient expression of Mesp2 in the S-2 region is essential for the rostral half specification (Takahashi et al., 2000). This is in good agreement with the observation that striped expression of Dll1 in the caudal half somites is first established in the S-1 region (Takahashi et al., 2000). Since Ps1 is not essential for the proliferation or survival of presomitic and somitic mesoderm, exclusion of Psen1-deficient cells from the prospective caudal half somite might be due to cell-sorting machinery, rather than defects in proliferation or survival. This is supported by previous observations that indicate counteracting cell adhesive properties of rostral and caudal half cells (Stern and Keynes, 1987). Thus, Dll1- and Ps1-dependent activation of the Notch pathway might induce or stabilise the homophilic condensation of caudal half somite cells, and, at the same time, exclude Psen1-deficient Dll1-negative cells from the caudal half. This is reminiscent of recent observations on avian trunk neural crest cells that neurogenic precursors expressing Notch1 proteins in nascent crest populations are eliminated by Dll1-expressing cells (Wakamatsu et al., 2000; Maynard et al., 2000). In contrast, cyclic activation of the Notch pathway in the caudal region of presomitic mesoderm, as represented by cyclic expression of Lfng, is still latent in the segregation of Psen1-deficient and wild-type cells. A transient episode of Mesp2 expression might be needed for the functional manifestation of Notch activation to segregate prospective rostral or caudal half somite cells.

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