INTRODUCTION

Somitogenesis is a dynamic morphogenetic process required for the generation of a metameric architecture in vertebrates. The paraxial mesoderm derived from the primitive streak or tailbud is aligned on both sides of the neural tube as the presomitic mesoderm (PSM). For formation of the metameric structure, mesenchymal PSM cells have to undergo two types of segmentation. One is initial segmentation, which is the segment border formation between the epithelial somites and PSM. The other is called resegmentation where individual vertebral units are formed. The initial segmentation process is accompanied by the mesenchymal-epithelial transition of PSM cells. Mesenchymal cells change their shape by epithelialization and are separated from caudal cells, which maintain mesenchymal morphology. The segment border formation occurs at fixed intervals and continues until the supply of paraxial mesoderm ends. Once the somites are formed, somitic cells start to differentiate, depending on their position within the somites. Cells facing the surface ectoderm differentiate into the dermomyotome, which then gives rise to the dermatome and myotome. The medial cells, under the influence of the notochord, differentiate into the sclerotome. The second segmentation, called resegmentation, occurs only in the sclerotome, during which the rostral and caudal halves within somites are segregated and re-fused with the next neighboring halves to form vertebrae. This process proceeds because of a difference in the property of cells between the rostral and caudal compartments within the somite. Embryonic manipulation and gene expression analysis have revealed that the rostrocaudal (RC) polarity is established in the anterior PSM prior to initial segmentation (Tam et al., 2000). Mesp2 is a member of the bHLH family of transcription factors. Expression is observed mainly during somitogenesis in the presomitic mesoderm, although a transient expression is also observed in nascent mesodermal cells at the onset of gastrulation. The Mesp2-null mouse shows two major defects in somitogenesis: the lack of the initial segment border, and the loss of rostral properties of the somite, resulting in the formation of a caudalized vertebrae (Saga et al., 1997). Genetic analysis revealed that Mesp2 plays a critical role in the establishment of RC polarity within somite primordia by regulating Dll1 expression through the Notch signaling pathway (Takahashi et al., 2000). To date, in any segmentation
mutant mouse, when RC polarity in PSM is disrupted, the segment border formation becomes disorganized. Therefore, establishment of RC polarity and initial segment border formation have not been genetically segregated.

Zebrafish mespb (formally known as mesp-b) was isolated on the basis of the homology of its bHLH region to that of Mesp2 (Sawada et al., 2000). mespb is segmentally expressed in one to three stripes in the anterior part of somite primordia, corresponding to the expression domain of mouse Mesp2. Ectopic expression of mespb in zebrafish embryos causes a loss of the posterior identity within the somite primordia, leading to a segmentation defect (Sawada et al., 2000). The Mesp2-null mouse shows the opposite result. Therefore, it is most likely that Mespb is a functional homologue of Mesp2. However, the homology is observed only in the bHLH region (74% identity) and sequences outside this motif are variable. Furthermore, fish and amphibian somites consist mainly of the myotomal component, and sclerotomal cells differentiate at later stages of somitogenesis. Thus, no resegmentation process has been reported in these animals (van Eeden et al., 1996). In order to determine whether zebrafish Mespb has a function similar to Mesp2 or whether these animals develop the Mesp-type transcription factor with a similar but distinct function, we examined the function of Mespb in mouse embryos using the gene knockin strategy.

During the course of the study, we generated the hypomorphic mesp allele in which endogenous Mesp2 is replaced with the mespb or neo-mespb gene. In a series of mespb-knockin mice, we observed dosage-dependent defects in RC polarity of the somite, resulting in varying degrees of vertebral fusions. Interestingly, however, initial segmentation occurs in RC-defective mice although the segment border was not maintained in the matured somites. These results, together with gene expression analysis, indicate that the Mesp family gene is involved in the different genetic cascades, one leading to the somite border formation and the other to the establishment of RC polarity required for resegmentation.

MATERIALS AND METHODS

Gene targeting

A zebrafish mespb knockin vector was constructed to insert mespb cDNA containing the complete coding region, at the start site of the Mesp2 coding region, using a common NcoI site at the ATG codon. The other parts, composed of the short and long arm regions of this vector, were almost the same as those in a targeting vector used for generating the Mesp1 knockin mouse (Saga, 1998), except that a floxed neo cassette was used and the poly(A) signal was separated from mespb cDNA by the neo cassette. The vectors were linearized and electroporated into TT2 ES cells (Yagi et al., 1993). Correctly targeted clones were then aggregated with ICR embryos to generate homologous recombination and subsequent excision of the floxed neo cassette for generating the Cre-recombinase. The CAG-Cre mouse was crossed with the floxed neo cassette for generating the Cre-recombinase. The F1 mice produced by the intercross of homozygous mespb/– mice showed kinked tails. In situ hybridization using the mespb-specific probe revealed that mespb knocked into the Mesp2 locus was expressed in a pattern similar to that of Mesp2 (Fig. 2B,C). Furthermore, based on the external appearance, clearly segmented somites were observed in the mespb/– embryos, suggesting that an almost complete rescue of the segmentation defect was achieved by introduction of mespb.

Dosage of Mespb is critical for the rescue of resegmentation defects of Mesp2-null mice

In addition to the lack of formation of initial segmentation, the Mesp2-null mouse lacks the rostral property of somites preventing the resegmentation process. As a result of this resegmentation defect, the mice exhibit extensive fusion of the pedicle and the lamina of the neural arch of the vertebrae (Saga et al., 1997). The homozygous mice died shortly after birth, while heterozygous Mesp2+/- (p2 single dose) mice developed normally. By breeding a mespb/+ mouse with a Mesp2+/- (+/-) mouse, mespb/– mice were generated. These mice died shortly after birth, indicating that, unlike Mesp2, one copy of the mespb gene is not sufficient to rescue Mesp2 deficiencies. Analyses of the skeletal phenotype of these mice revealed a fusion of both the rib and the vertebral column although the
severity was much milder than that observed in a Mesp2-null fetus (Fig. 2F-I). The result suggests a functional difference between Mesp2 and Mespb.

Comparison among mespb/mespb, mespb/– and −/− fetuses clearly revealed the dosage effect of the mespb gene (Fig. 3A-F). Two copies of mespb resulted in a clearer separation of the pedicles and the lamina, although partial fusion remained, particularly in the pedicles (Fig. 3B). In contrast, a single copy of mespb only partially rescued the skeletal anomaly caused by Mesp2 deficiency. Some separation of skeletal elements was generated within the fused vertebrae (Fig. 3C). For the neo-mespb/ne-mespb and neo-mespb/− fetuses, defects were more severe. The fusion of pedicle of the neural arch in the neo-mespb/ne-mespb or neo-mespb/− fetuses was more severe than that in mespb/− fetuses (Fig. 3D-E). In the neo-mespb/ne-mespb and ne-mespb/− embryos, mespb is expected to be transcribed in conjunction with a neo mRNA, which may affect the stable translation of the Mespb protein. The conjunct mespb-neo transcripts were detected by RT-PCR (data not shown) and by in situ hybridization using the mespb probe (Fig. 2D).

To understand the phenotype at the molecular level, we first analyzed the effect of Mespb on the establishment of RC polarity, because the skeletal malformations are a result of the loss of this RC polarity within the somite. Uncx4.1 serves as a good molecular marker for caudal half somites, and knockout mice of this gene lack the vertebral elements (especially pedicles) derived from the caudal sclerotome (Leitges et al., 2000; Mansouri et al., 2000). In wild-type embryos, Uncx4.1 is exclusively expressed in the caudal half of the segmented somites (Fig. 3G). Consistent with the degree of fusion of the pedicles, the expression pattern of Uncx4.1 in embryos with various mespb genotypes was affected in a dosage-dependent manner (Fig. 3H-K). In neo-mespb/− embryos that show the

Fig. 1. Comparison among members of the Mesp-related gene family (A) and strategy of gene replacement of mouse Mesp2 with zebrafish mespb (B,C). (A) Comparison of amino acid sequences in the bHLH motif. The percentage homology to Mesp2 within and outside the bHLH motif are shown. Differences are indicated in green (Mespb) and purple (Mesp1). (B) Knock-in strategy. The top line shows the genomic organization of the Mesp2 gene; the second shows the structure of the targeting vector; the third is the predicted structure of the Mesp2 locus following homologous recombination. Mesp2 exons (pink boxes) were completely deleted and replaced with the zebrafish mespb coding region flanked with floxed neo cassette and poly(A) signal (the arrowheads on the line represent loxP sites). Chimeric mice generated from recombinant ES cells containing targeted allele, Mesp2neo-mespb, were mated with CAG-Cre mice to excise the floxed neo cassette, resulting in the generation of the Mesp2neo-mespb allele. The probe used for Southern blot analysis is indicated. Restriction enzymes: B, BamHI; E, EcoRI; H, HindIII; H, HindIII; K, KpnI; P, PstI; S, SacI; Sm, Smal; X, XhoI. Arrows indicate PCR primers. (C) Genomic Southern blot analysis of Sall-digested DNA from embryos with various Mesp2 alleles. Arrowsheads show the 6.0 kb fragment of the wild-type allele, the 2.3 kb targeted Mesp2neo-mespb, and the 4.7 kb Cre-excised Mesp2mespb allele. Genotypes of progeny are indicated at the top of each lane. All represent genotypes of the Mesp2 allele.
very severe fusion of the pedicles, the expression pattern of Uncx4.1 was severely affected, expanding to the rostral half somites (Fig. 3K). However, as the dosage of the mespb gene increased, the expression of Uncx4.1 shifted caudally, and an almost normal expression pattern was observed in mespb/mespb embryos (Fig. 3H).

We have previously shown that the RC polarity of the somite is prefigured by the expression pattern of Dll1 in the anterior PSM (Takahashi et al., 2000). In wild-type embryos, Dll1 expression is uniform and intense in the caudal presomitic mesoderm (CPM) but it is markedly downregulated and localized in the caudal half somite primordia (Fig. 3M) (Bettenhausen et al., 1995). Because Mesp2 suppresses Dll1 expression in the presumptive rostral half of somite in PSM (Takahashi et al., 2000), Dll1 expression in the PSM was expanded rostrally in Mesp2-null embryos (Fig. 3R). As expected, the degree of rostral expansion of Dll1 expression was dependent on the dosage of mespb (Fig. 3N-Q). Thus mespb/mespb embryos showed an almost normal expression pattern (Fig. 3N), whereas the neo-mespb/– embryos exhibited an expanded Dll1 expression similar to that in the Mesp2-null embryos (Fig. 3Q). The difference in the expression patterns of Uncx4.1 and Dll1 in mice with different mespb alleles indicates that the mespb dosage is essential to both establish RC polarity in anterior PSM and maintain this RC polarity in the somites.

**Defective RC polarity reflects misregulation of Mesp2**

It has been shown that RC polarity within the somites is established by the autoregulated rostral restriction of Mesp2 within the somite primordia (Takahashi et al., 2000). Thus, we examined whether the defective RC polarity in mespb mice is correlated with misregulation of mespb in the PSM. We used a Mesp2-lacZ mouse (lacZ gene knocked into the Mesp2 locus). In this mouse line, the Mesp2 expression pattern is properly reproduced by the expression of lacZ transcripts (Fig. 4A,D). The β-gal activity was sustained in the somitic region because of the stability of the enzyme (Fig. 4H). Since Mesp2 expression, once detected in one-somite width, is rostrally restricted (Takahashi et al., 2000), β-gal activity shows a rostrocaudal gradient within each somite (Fig. 4H). In the absence of Mesp2, lacZ expression is not restricted to be localized to the rostral compartment of the somite (Fig. 4G), resulting in uniform β-gal staining in the somitic region (Fig. 4K). In the case of the mespb/L embryos that contain one mespb allele in the genome, the mespb (Fig. 4B) and lacZ (Fig. 4E) expression patterns were similar to that of endogenous Mesp2; a single discrete band ranging from one-somite to half somite width due to the transcriptional suppression in the caudal half (Haraguchi et al., 2001). The graded β-gal staining in the somitic region was observed, although it was not as clear as that in the Mesp2/L embryos (Fig. 4F). In contrast, in the neo-mespb/L embryos containing one neo-mespb allele, the expression pattern of both neo-mespb (Fig. 4C) and lacZ (Fig. 4F) transcripts were different from mespb (Fig. 4B) and lacZ (Fig. 4E) of mespb/L: two bands were observed and the anterior one was not localized in the rostral compartment. This result indicates that neo-mespb expression is extended without localization in the rostral half, resulting in the uniform pattern of β-gal staining (Fig. 4J). The result indicates that the amount
of Mespb protein provided by one neo-mespb locus is not sufficient to restrict mespb to the rostral compartment, a prerequisite for the establishment of RC polarity. In addition, the reduced amount of Mespb inneo-mespb or neo-mespb/L is unable to suppress Dll1 expression in the caudal halves of somites, resulting in the formation of the caudalized vertebrae as shown in Fig. 3.

**Initial segmental border is formed in the hypomorphic mice**

In spite of the loss of RC polarity and vertebral defects, the segmented somites appear to form in hypomorphic embryos, indicating segmentation without clear RC polarity. To confirm the segment border formation, the horizontal serial sections of embryonic tails at 11.5 dpc were compared among the various genotypes (Fig. 5). In sections of wild-type embryos, separated segmental borders were clearly observed between the somites (Fig. 5A). In contrast, neither segmental borders nor epithelial somites were observed in the Mesp2-null embryos (Fig. 5F). As expected from the external morphology, initial border formation was observed in mespb/mespb (Fig. 5B), mespb/– (Fig. 5C) and neo-mespb/neo-mespb (Fig. 5D) embryos. Moreover, in neo-mespb/– embryos, in which the segmented borders were not clear morphologically, the histological sections revealed that the initial segmental border is formed (Fig. 5E). However, in all hypomorphic embryos except for mespb/mespb, segregation is incomplete and somitic cells remain between newly formed somites. Furthermore, the segment borders tended not to be maintained. This was most obvious in neo-mespb/– embryos in which the somites finally fuse with each other. The somite fusions could be caused by...
the loss of RC polarity (Durbin et al., 2000). These results suggest that the formation and maintenance of somite borders are regulated by distinct mechanisms.

**Lfng, EphA4 and PAPC expression appears normal in mespb-embryos**

The above results led us to examine the expression pattern of Lunatic fringe (Lfng), EphA4 (Epha4) and protocadherin (PAPC; Pcdh8), which have been implicated in segment border formation in the rostral PSM (Johnston et al., 1997; Durbin et al., 1998; Schmidt et al., 2001; Kim et al., 2000). In the PSM, various expression patterns of Lfng are observed even at a similar developmental stage, due to cyclical expression linked to a segmentation clock (McGrew et al., 1998; Forsberg et al., 1998). The expression domain of Lfng in the caudal PSM appears to travel to the rostral region (indicated by bracket in Fig. 6A), while the rostral stripe gradually becomes thinner and finally stays at the future segmentation point (Fig. 6A, indicated by black arrowhead). The Fringe protein, which modifies the Notch receptor in the fly wing disc, has been implicated in border formation (Moloney et al., 2000). Similarly, it has been shown to function in the vertebrate somitogenesis; a Lfng-deficient embryo cannot form a clear segmental boundary (Evrard et al., 1998; Zhang and Gridley, 1998). In Mesp2-null embryos, Lfng expression in the rostral PSM expanded anteriorly, while caudal expression remained intact (Fig. 6F). To compare the expression patterns, we selected embryos that exhibited a similar expression profile with a thin band in the rostral PSM and a caudal broad band traveling rostrally (Fig. 6A). In all mespb embryos, namely, mespb/mespb (Fig. 6B), mespb/– (Fig. 6C), neo-mespb/neo-mespb (Fig. 6D) and neo-mespb/– (Fig. 6E), the definite bands of Lfng were detected in the rostral PSM close to the next segmental border. We then examined EphA4, which is normally expressed in a pattern similar to that of the rostral band of Lfng (Fig. 6G). EphA4 has been implicated in segment border formation because its misexpression induces ectopic segment border formation in segmentless zebrafish fss mutant embryos (Durbin et al., 2000). Importantly, EphA4 expression was severely downregulated in the Mesp2-null embryo (Fig. 6L). In contrast, relatively normal levels and patterns of EphA4 expression were observed in all mespb-mice irrespective of the dosage and nature of mespb (Fig. 6H-K). Similar results were obtained in the expression pattern of PAPC, which is also known as a key molecule for segment border formation (Kim et al., 2000). In wild-type embryos (Fig. 6M), PAPC is typically expressed as one or two defined bands in the rostral PSM in addition to a diffuse caudal expression in the middle PSM. In Mesp2-null embryos (Fig. 6R), the rostral band is missing and only broad expression is observed caudally. In the mespb-knockin embryos, the rostral band tends to be rescued, accompanied by the recovery of segmental border formation (Fig. 6N-Q). In addition to the induction, it is noted that the expression pattern of PAPC appears to be affected by the mespb dosage, such that the rostral band showed a diffused pattern at low mespb dosage, reflecting the RC polarity as well. The above results strongly suggest that a small amount of Mesp2 is sufficient to induce rostral expression of Lfng, EphA4 and PAPC and may drive segment border formation. The results also suggest that the rostral restriction of mespb expression is not necessarily required to elicit these gene expressions nor initiate segment border formation. This phenomenon is in contrast to that of the establishment of RC polarity, requiring a higher amount of Mesp2, suggesting the presence of distinct pathways controlling the two events.

**DISCUSSION**

**Establishment of hypomorphic allele**

The aim of this work was to determine whether zebrafish
**Fig. 6.** Gene expression implicated in the segmental border formation. The expressions of *Lfng* (A-F) *EphA4* (G-L) and *PAPC* (M-R) are compared in various *mespb* embryos at 11.5 dpc. *Lfng* is expressed in a highly dynamic manner. Therefore, 8-10 embryos of each genotype were analyzed by whole-mount in situ hybridization and we chose those with similar expression patterns for the comparison. The anterior thinner band of *Lfng* (black arrowhead) is rostrally extended in the *Mesp2*-null embryo. In contrast, the sharpness of the band was recovered in all *mespb* embryos. The rostral band of *EphA4* (green arrowhead) and *PAPC* (purple arrowhead) expression, which disappeared in the *Mesp2*-null embryo, is also present in *mespb* embryos.

**Fig. 7.** Possible models of events leading to the somite border formation and the establishment of RC polarity. (A) *Mesp2* or *Mespb* might regulate these two events using different genetic pathways. *Mesp2* is known to suppress *Dll1* via the Notch signaling pathway. (B) However, the pathway might be important for the normal expression of *EphA4*, *Lfng* and *PAPC*, which is required for the border formation. (C) Finally the suppression of *Dll1* required for the establishment of RC polarity might play a role in the border formation. In all cases, Notch signaling is required for the autoregulation of *Mesp2*, and RC polarity is required for the maintenance of the segmental border. Only the anterior most bands of *Lfng* and *PAPC* are Mesp-dependent.

**Table**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Expression Pattern</th>
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<tr>
<td><em>Lfng</em></td>
<td>Anterior thinner band rostrally extended</td>
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<tr>
<td><em>EphA4</em></td>
<td>Rostral band</td>
</tr>
<tr>
<td><em>PAPC</em></td>
<td>Rostral band</td>
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**Text**

**mespb** and mouse *Mesp2*, which share 74% identity of the bHLH region and have similar expression patterns and functions in somite formation, are indeed functional homologues. The *mespb/mespb* mice developed normally and overcame most of the deficiencies caused by the loss of *Mesp2*. However, we still observed a partial fusion of the vertebral and the truncation of the trunk. In addition, *mespb/−* embryos showed a severe defect in RC polarity within the somite, which was not observed in *Mesp2/−* embryos. This defect difference suggests a functional difference in either establishment or maintenance of RC polarity in the somite related with resegmentation between mouse *Mesp2* and zebrafish *Mespb*. The difference in the molecular nature could be attributable to the region outside the bHLH region since we previously observed a similar defect when *Mesp2* was replaced with *Mesp1*, the bHLH region of which has a 94% identity to that of *Mesp2* while the sequences outside this motif are diverse (Saga, 1998). However, we cannot rule out the possibility that a difference in the expression level and/or the stability of *mesp* mRNA and its protein may be the cause of this phenotype. Although transcriptional regulation is very important for the correct patterning of RC polarity, for technical reasons we have used the SV40 polyadenylation signal instead of the endogenous one in both *mespb* and *Mesp1*-knockin mouse, which may have affected the stability of the transcript.

Taking advantage of the nature of the *mespb* allele, we were able to generate a hypomorphic *Mesp* allele and analyze the dosage effect of the *Mesp* gene on somite formation. In various *mespb*-knockin mice, we observed the clear dosage effect on vertebral fusions. We think that this represents changes in the amount of the Mespb protein. In the *neo-mespb* embryo, *mespb* was transcribed with the *pgk-neo* cassette and the amount of neo-containing transcripts was comparable to that of *mespb* transcript in the *mespb* embryo. To date, we do not have a tool that can assess the translation
efficiency of the transcripts. However, when we compare the phenotypes and gene expressions between mespb/mespb and mespb/– mice or between neo-mespb/neo-mespb and neo-mespb/– mice, it is reasonable to conclude that the severity of the phenotype depends on the amount of the Mespb protein expressed.

**RC polarity and segment border formation**

One of the important findings in this study is that a hypomorphic embryo can form somite boundaries without clear RC polarity in the PSM, suggesting that segment border formation and establishment of RC polarity are genetically separate events. The two successive events are both affected in segmentation mutant mice including the Mesp2-null mouse (Harabe de Angelis et al., 1997; Koizumi et al., 2001; Evrard et al., 1998; Saga et al., 1997).

Experimentally, it is possible to separate segment border formation from RC polarity. In embryos from which the ectoderm is removed, normal RC polarity is established but no epithelial somites are generated indicating that the establishment of RC polarity is not directly linked to the border formation (Palmeirim et al., 1998; Correia and Conlon, 2000). Interestingly, no EphA4 expression was induced in embryos from which ectoderm had been removed (Schmidt et al., 2001), suggesting a direct relationship between EphA4 induction and segment formation. In our experiments, however, irrespective of defects in RC polarity, a relatively normal EphA4 segment formation. In our experiments, however, irrespective of defects in RC polarity, a relatively normal EphA4 segment formation is observed in all hypomorphic embryos, supporting the idea that these two events are independent of each other. Considering the absence of EphA4 expression in Mesp2-null embryos, EphA4 expression requires both an ectodermal signal and Mesp2.

Previously, we have reported that Mesp2 functions in generating RC polarity by suppressing Dll1 expression in the rostral half of a presumptive somite. This suppression is mediated by the Notch signaling pathway (Takahashi et al., 2000). In the present study, Dll1 expression was affected in mespb–/knockin embryos in a dosage-dependent manner. Particularly in neo-mespb/– embryos, extensive expansion of Dll1 expression was observed, indicating the lack of RC polarity. However, the initial segmental border was formed in the neo-mespb/– embryo, but not maintained. We believe that the failure to maintain the segmental border is due to the lack of RC polarity, since it was reported that the segmental border is maintained only when the rostral and caudal halves are confronted (Stern and Keynes, 1987). Therefore, the formation and maintenance of the segmental border must be regulated by different mechanisms: one is mediated by Lfng, EphA4 and PAPC to generate the segment border, and the other by Dll1 through the Notch signaling pathway to establish RC polarity (Fig. 7A). At the present, however, it is unclear at which level the two events bifurcate. It is also possible that the rostral localization of Lfng, EphA4 and PAPC expression could be mediated by the Notch signaling pathway (Fig. 7B). A preliminary study using the Mesp2-Notch1 mouse (Notch1 knocked in the Mesp2 locus) (Takahashi et al., 2000) suggests that PAPC expression is partly dependent on Notch signaling, indicating that other complicated pathways are involved in the regulation of these genes. Finally, it is formerly possible that the induction of LFng, EphA4 and PAPC requires RC polarity to some degree (Fig. 7C) since a subtle and undetectable RC polarity may exist that is sufficient for initial segmentation but not for establishment of RC polarity.

**Regulation of Mesp gene**

The autoregulation of Mesp2 transcription must be very important to establish RC polarity. Upon activation, Mesp2 is initially expressed in an area approximately one somite wide, defining the anterior limit at its initial expression. Next the transcription is suppressed only in the caudal half, generating RC polarity within a somite primordium (Takahashi et al., 2000). Indeed in this study, we observed that misregulation of mespb in the caudal half resulted in misestabishment of RC polarity. This is most likely to be caused by the reduced amount of Mesp2. Although the precise mechanism of the autoregulation is not yet known, Notch signaling has been implicated in this process, since no rostral restriction of Mesp2 expression is observed in Psen1-null embryos (Koizumi et al., 2001). We have already shown genetically that Psen1-dependent Notch signaling is required for the induction of Dll1 in the caudal half area of somite primordia (Takahashi et al., 2000). Thus, it is possible to speculate that this signaling also functions in suppressing Mesp2 in the caudal half leading to establish RC polarity.

At present, little is known about the direct targets of Mesp2 or Mesp, nor when and how long Mesp protein functions. Future studies should be focused on visualizing the protein molecules involved in the regulatory network to clarify the functional molecular cascade.

We thank Izumi Uehara, Mariko Ikumi, and Seiko Shinzawa for technical assistance. This work was supported in part by Grants-in-Aid for Scientific Research, and Special Coordination Funds for the Promotion of Science and Technology, from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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