A novel signal induces a segmentation fissure by acting in a ventral-to-dorsal direction in the presomitic mesoderm

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Abstract

We describe here a novel inductive action that operates during somitic segmentation in chicken embryos. We previously reported that the posterior border cells located at a next-forming boundary in the anterior end of the presomitic mesoderm (PSM) exhibit an inductive activity that acts on the anterior cells to cause the formation of a somitic fissure (Sato, Y., Yasuda, K., Takahashi, Y., 2002. Morphological boundary forms by a novel inductive event mediated by Lunatic fringe and Notch during somitic segmentation. Development 129, 3633–3644). In this study, we have found a second inductive action along the dorso-ventral (D–V) axis during fissure formation. When relocated into a non-segmenting region of PSM, the ventral-most cells taken from the presumptive boundary are sufficient to induce an ectopic fissure in host cells. The ventrally derived signal acts in a ventral-to-dorsal direction but not ventrally, regardless of where the ventral cells are placed. This directional signaling is governed, at least in part, by the signal-receiving cells of the PSM, which we found to be polarized along the D–V axis, and also by intimate cell–cell interactions. Finally, we have observed that morphological segmentation is able to rearrange the anterior and posterior regionalization of individual somites. These findings suggest that discrete unidirectional signals along both the antero-posterior and the D–V axes act coordinately to achieve the formation of the intersomitic fissure, and also that fissure formation is important for the fine-tuning of A–P regionalization in individual somites.

Keywords: Somite; Segmentation; Induction; Polarity; Quail-chick chimera

Introduction

During early morphogenesis in vertebrate development, a variety of boundaries must be delineated to produce distinct organs. It is widely accepted that prior to the morphological boundary formation, selector genes, in most cases transcription factors, define the flanking regions, and morphological changes subsequently commence in accordance with the identity of each of the regions established by the selector genes (Blair, 2003; Dahmann and Basler, 1999; Irvine and Rauskolb, 2001; Lawrence and Struhl, 1996; Mann and Morata, 2000, references therein). In many cases, “border cells”, which are induced to emerge at the interface between the flanking regions, take an initiative in the actual morphological changes and separation between the neighboring tissues. Although the mechanisms by which selector genes define flanking fields have extensively been studied in organogenesis, the cellular mechanisms directly underlying the morphological changes remain poorly understood.

Somitogenesis, the basis for the formation of axial bones and all the skeletal muscles in the trunk, provides a unique and powerful advantage for investigating the mechanisms of boundary formation. When a somite forms from the presomitic mesoderm (PSM; unsegmented), the cellular mass of a somitic unit separates from the anterior end of the
PSM one pair at the time, producing a spherical structure enclosed by epithelial cells (Brand-Saberi and Christ, 2000; Nakaya et al., 2004; Stockdale et al., 2000; Takahashi, in press). Recurrence of this event as the amniote embryo grows leads to an array of segmented somites aligned along the antero-posterior (A–P) axis. We previously reported that the posterior border cells, located posteriorly adjacent to the presumptive boundary, are specified to produce the activity, designated as the “segmenter”, which acts on the anterior cells to make a fissure between the anterior and posterior border cells (Sato et al., 2002). This action is mediated by Notch signals. We also showed that the segmenter activity operates in a unidirectional manner, from posterior to anterior tissues (Sato et al., 2002). Thus, the posterior border cells play an important role in the separation of consecutive somites.

In the present study, we demonstrate a second novel inductive signal that acts along the dorso-ventral (D–V) axis to make each fissure. The ventrally located cells at the presumptive boundary are sufficient to induce an ectopic fissure when relocated into a non-segmenting region. Unexpectedly, these ventral cells act solely in a dorsal direction but not ventrally. Furthermore, the formation of a fissure is concomitant with a rearrangement of the A–P identity in a single formed somite. We will discuss the two inductive activities along the A–P and D–V axes, acting coordinately to make a morphological boundary during somitogenesis.

Materials and methods

Embryological manipulation and histological analyses

Chicken and Japanese quail embryos of 18–20 somites were used. Tissue transplantations, labeling with Dil and DiO (Molecular Probes), paraffin sectioning, and histological staining with QCPN antibody (DSHB) were performed as previously described (Sato et al., 2002). Silver foil of 5 μm thickness and isopore membrane filter (Millipore; pore size 0.8 μm) were used as a barrier. Histological sections were photographed under Nomarski optics.

In situ hybridization

The preparation of RNA probes of L-fringe and Delta1, and whole-mount in situ hybridizations were performed as previously described (Sato et al., 2002). For double staining with QCPN antibody and RNA probe, embryos were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) followed by frozen sectioning. Section in situ hybridization was performed basically as described in Tonegawa et al. (2003) except for excluding the step of Proteinase K treatment. After the color reaction, the sections were washed in PBS and processed to QCPN-antibody staining, detected by goat anti-mouse Alexa 568 (Molecular Probes).

Results

A non-boundary-forming region of PSM is induced to form a morphological boundary when placed at the prospective boundary

In the present study, we designate a next-forming boundary as level −1, and the level one somite unit posterior to level −1 as level −2 as shown previously (Sato et al., 2002). To understand the differences between the segmenting and non-segmenting sites in the anterior PSM, we dissected a tissue region containing a non-boundary-forming site (level −1.5) from a donor quail embryo, and transplanted it into level −1 of a chicken host embryo with its D-V orientation unchanged. During the first series of experiments, a dorsal portion of level −1 was removed from the host prior to the transplantation, with the ventral cells remaining. The transplantation resulted in the formation of an ectopic fissure observed in the middle within the graft. The position of the induced fissure was in register with a host somitic boundary (45/65; Fig. 1B, arrow). Transplantation of a −1.5 region into the −1.5 level as a control produced no ectopic fissure (n = 12, data not shown). These observations suggest that the non-boundary region at −1.5 is capable of producing a morphological boundary if it receives a signal emanated from a boundary-forming region.

We reasoned that the host cells remaining in the ventral region of level −1 were responsible for this inductive action. To test this, we examined whether an ectopic fissure would form in the graft in the absence of the host ventral cells. We removed both the dorsal and ventral cells from the −1 region of a host using a glass capillary prior to the transplantation (Fig. 1D). In these embryos, no fissure was observed in the graft, resulting in the formation of an elongated somite fused with the grafted and host somites along the A–P axis (n = 11; Fig. 1E). This suggests that the ventral cells at −1 are required for the fissure induction observed above. During the course of experiments, two specimens in which an ectopic fissure was formed in the graft retained host ventral cells only posteriorly to level −1 and not anteriorly (Fig. 1C, arrowhead). This implies that the ventral posterior border cells at level −1 take an initiative in inducing a fissure formation.

We next asked whether a younger/immature PSM was also susceptible of the inductive activity produced by the −1 ventral cells. We carried out a similar transplantation as shown earlier (Fig. 1A), but we this time used a tissue taken from around level −5.5 of PSM as a donor graft. This manipulation produced no ectopic boundary within the donor tissue (n = 4; Fig. 1F). Therefore, a young PSM is not capable of interpreting the ventrally derived signal of level −1. It also suggests that for the inductive event to occur at the next-forming boundary, both the signal production from the ventral cells and a susceptibility of the dorsally located cells in receiving these signals need to act coordinately.
The ventral cells of the prospective boundary are sufficient to induce an ectopic fissure

We next determined whether the −1 ventral cells are sufficient to induce a fissure in a non-segmenting site. We transplanted these cells into the ventral side at −1.5 of a host PSM. This manipulation resulted in the formation of an ectopic fissure, producing two miniature somites at the treated site (n = 5). In three out of these five specimens, the grafted cells were located solely in the posterior site adjacent to the ectopic fissure (Fig. 2A) with no quail cells found anteriorly in all serial sections examined. In the other two embryos, the donor cells straddled the ectopic boundary (Fig. 2B). The former phenomenon (the grafted cells being seen at the posterior side of the fissure) suggests that the ventral posterior border cells at level −1 are sufficient to induce a somitic fissure, and this notion is consistent with aforementioned observation (Fig. 1C). The latter phenomenon (where the grafted tissue straddled the ectopic boundary) can be accounted for in the way that, within the graft, the cells that derived from the posterior–ventral site of a donor also acted as a segmenter, an activity acting in a posterior-to-anterior direction to form a fissure as we previously reported (Sato et al., 2002). In the controls, a small tissue taken from donor −1.5 was transplanted into host −1.5, and displayed no appreciable effect (n = 5; Fig. 2G). From these observations, we conclude that, during normal somitogenesis, the ventral cells at the next-forming boundary are important and sufficient to send a signal to produce a fissure in the dorsal region of PSM. In addition, it is likely that the posterior border cells are involved in this activity, although it does not exclude a possible contribution of the anterior border cells.

The fissure-forming signal acts unidirectionally in a ventral-to-dorsal direction

During a series of transplantation with the −1 ventral cells into the −1.5 region, we unexpectedly found that the transplanted cells solely affected the dorsally located cells but not ventrally located neighbors. As shown in Figs. 2C–D, when the grafted cells were placed in the middle region of PSM along the D–V axis, a fissure was found only in the dorsal aspect of the donor cells (n = 6). The ectopic fissure formed in the dorsal aspect was explicitly detected by its epithelial morphology as shown in Figs. 2C′–D′. Likewise, when a donor was located in the dorsal-most region of PSM, a fissure formed only within the grafted cells and not in the ventral host cells (n = 10; Figs. 2E–F). Thus, the inductive event occurred only in the dorsal aspect of the graft regardless of the position where the graft was placed. In this series of transplantation (−1 into −1.5), in which we used a graft as an inducer, it was important to reduce the size of the graft, making it practically difficult to control the D–V orientation of the graft. It therefore appeared unlikely that the unidirectional inductive event observed here was attributed to the D–V orientation of the graft.

To understand what determines the unidirectional action of the fissure-forming signal, we inverted the D–V orientation of a donor tissue taken from the level −1.5, and placed it into the host −1 region in a way similar to the experiment as shown in Fig. 1A. Like Fig. 1A, a tissue piece dissected from donor −1.5 was relatively large, enabling us to control the D–V orientation. In contrast to the result shown in Fig. 1A, no ectopic fissure was found in the graft (n = 7; Fig. 2I). Thus, the D–V polarity of the PSM that receives the ventral signal is critical to make an intersomitic boundary.
We also asked whether the dorsoventrally surrounding tissues would exert any influence on the V-to-D signaling. When an ectodermal signal was blocked by inserting a piece of silver foil with the −1.5 region being transplanted into −1 (the same experiment as Fig. 1A), an ectopic fissure formed within the host PSM, producing two miniature somites. Out of 5 such specimens, 3 embryos exhibited the grafted cells solely in the posterior region to the ectopic cleft (A), whereas the remaining 2 embryos had the quail cells straddling the fissure (B). (C, D) When placed into an intermediate position along the D–V axis, −1 ventral cells of a donor induced a fissure in a dorsal region (arrow), but not ventrally. High magnification of a rectangle was shown in C’ and D’ with a trace of cell shapes, highlighting the morphology of the cells facing the cleft. (E, F) When the cells were placed into the dorsal-most position of a host, the grafted cells exhibited a fissure within themselves (arrow), and no fissure was formed in the host area at the ventral aspect of the graft. (G) In a control experiment where −1.5 ventral cells of a donor were transplanted into the ventral region of host −1.5, no ectopic boundary formed. (H) Control transplantation with −1.5 ventral cells of a donor into a dorsal area of host −1.5 gave no ectopic fissure. (I) A donor −1.5 was inverted along the D–V axis and placed into the host −1 with the host ventral cells remaining. No ectopic fissure was induced to form in the graft (bracket). (J) An interference with ectodermal signals by making a barrier with a piece of silver foil did not affect the inductive event that would occur when the donor −1.5 was placed to the host −1. An induced fissure within the donor cells is indicated by an arrow, and the position where the silver foil piece had been present was shown by a dotted line (the silver foil was lost during specimen preparation). (K) The endoderm did not affect the segmentation boundary (arrows). A portion of the ectoderm was replaced by a piece of endoderm (shown as a pink dotted line in the diagram, and by a bracket in the photo).

Fig. 2. The ventral cells at level −1 induce a fissure in a non-segmenting region in a V-to-D direction. (A, B) When a small population of cells taken from −1 of a donor was placed into the ventral region at −1.5 of a host, an ectopic fissure (arrow) was induced to form dorsally in the host PSM, producing two miniature somites. Out of 5 such specimens, 3 embryos exhibited the grafted cells solely in the posterior region to the ectopic cleft (A), whereas the remaining 2 embryos had the quail cells straddling the fissure (B). (C, D) When placed into an intermediate position along the D–V axis, −1 ventral cells of a donor induced a fissure in a dorsal region (arrow), but not ventrally. High magnification of a rectangle was shown in C’ and D’ with a trace of cell shapes, highlighting the morphology of the cells facing the cleft. (E, F) When the cells were placed into the dorsal-most position of a host, the grafted cells exhibited a fissure within themselves (arrow), and no fissure was formed in the host area at the ventral aspect of the graft. (G) In a control experiment where −1.5 ventral cells of a donor were transplanted into the ventral region of host −1.5, no ectopic boundary formed. (H) Control transplantation with −1.5 ventral cells of a donor into a dorsal area of host −1.5 gave no ectopic fissure. (I) A donor −1.5 was inverted along the D–V axis and placed into the host −1 with the host ventral cells remaining. No ectopic fissure was induced to form in the graft (bracket). (J) An interference with ectodermal signals by making a barrier with a piece of silver foil did not affect the inductive event that would occur when the donor −1.5 was placed to the host −1. An induced fissure within the donor cells is indicated by an arrow, and the position where the silver foil piece had been present was shown by a dotted line (the silver foil was lost during specimen preparation). (K) The endoderm did not affect the segmentation boundary (arrows). A portion of the ectoderm was replaced by a piece of endoderm (shown as a pink dotted line in the diagram, and by a bracket in the photo).

The ventral part of level −1 is not the only source of the inductive signal

Although the results shown above indicate that the −1 ventral cells are capable of inducing an ectopic boundary in the −1.5 region, they do not necessarily mean that these cells are required for the normal fissure formation at level −1. To know whether the ventral-most cells are the only source for the V-to-D acting signal, we attempted to block the ventral-derived signal with a piece of silver foil. Prior to manipulation with normal embryos, the insertion of this barrier was confirmed to block the ventral signal that would otherwise act on the −1.5-derived donor tissue (same experiment as Fig. 1A) (n = 7; Fig. 3A). This observation
not only corroborates our previous finding that the ventral cells at \(-1\) are required for the induction of an *ectopic* fissure as mentioned earlier (Fig. 1), but also suggests that these ventral cells act directly on dorsally adjacent cells. Interestingly, a piece of Millipore membrane also blocked the signal \((n = 5; \text{Fig. 3B})\), implying that the V-to-D signal is mediated by intimate cell–cell interactions rather than by diffusible morphogen molecules.

We next examined whether the normally developing somites would be affected by the insertion of a piece of foil, separating the dorsal PSM from the ventral one. The barrier was placed over a distance of several prospective somite units (from level \(-5\) to level \(0\), Fig. 3C) to assure that any possible ventral signals could be blocked. As shown in Figs. 3D and E, somitic fissures formed correctly in both the dorsally and ventrally separated areas of PSM, and the positions of these fissures were in register between these two portions \((n = 11; \text{Fig. 3E, arrows})\). These observations suggest that, during normal somitogenesis, the ventral-most cells are not the single source for the V-to-D signal, and also that multiple signal sources may be distributed along the D–V axis at \(-1\) (see also the model shown in Fig. 6). Supporting this, we also found that a dorsal portion taken from \(-1\) was capable of inducing an ectopic fissure when relocated into the ventral \(-1.5\) \((n = 4; \text{data not shown})\).

**Fissure formation does not require interactions along the medio-lateral axis**

Together with our previous findings of the inductive signal acting in a posterior-to-anterior direction at level \(-1\) (Sato et al., 2002), the present study has demonstrated that signals involved in the somitic fissure formation operate unidirectionally along the two dimensions, A–P and D–V axes, with precisely regulated mechanisms. We therefore asked whether the third axis, the medio-lateral \((M–L)\), was also important. We transplanted a narrow strip of tissue dissected either from medial \((n = 5)\) and lateral \((n = 3)\) areas straddling level \(-1\), and transplanted it into their corresponding region at \(-1.5\) (Figs. 4A, B). In no case did we observe a fissure ectopically formed in adjacent host tissues along the M–L axis. This finding indicates that signaling along the M–L axis is not involved in the somitic fissure formation, at least, in the anterior end of the PSM.

The morphological boundary formation was concomitant with a rearrangement of the A–P characters of a somite

A formed somite displays overt A–P compartments with characteristic identities, and these A–P characters are irreversibly determined after somites form (Aoyama and Asamoto, 1988). Whereas Keynes and Stern (1984) carefully proposed that the acquirement of the A–P identities occurs at or before overt segmentation, most recent reports are in favor of the A–P determination before segmentation, although they are not necessarily controversial with the possibility of “at the segmentation” (Palmeirim et al., 1998;
To clarify this problem, we asked whether the original A–P characters would be retained in a grafted tissue that was induced to make a new fissure as shown in Fig. 1A ($-1.5$ into $-1.0$). Prior to the graft, a donor tissue was labeled with DiI and DiO in the anterior and posterior portions, respectively (red and green circles, Fig. 5). We then examined expression of the anterior marker Lunatic fringe (L-fringe) and the posterior marker Delta1 by whole-mount in situ hybridization (Sato et al., 2002). The grafted side displayed a pattern of expression almost identical to the untreated control side ($n = 7$ for L-fringe; Figs. 5A, B; $n = 9$ for Delta1, Fig. 5G), suggesting that a majority of the donor $-1.5$-derived cells rearranged its A–P identity when relocated to the position $-1$. This observation was further confirmed by double staining for in situ hybridization with Delta1 probe and QCPN in the same histological section (Figs. 5D–F, $n = 4$). The control graft ($-1.5$ to $-1.5$) retained their A–P identity (Figs. 5H–K, $n = 5$). Furthermore, we allowed the manipulated embryos to develop until embryonic day 4 when dorsal root ganglia formed. The specific localization of the dorsal root ganglia is known to depend on the anterior character in each somitic segment (Kalcheim and Teillet, 1989; Sato et al., 2002). As shown in Fig. 5C, the posterior half of a segment and the anterior half of its posteriorly adjacent segment were occupied by transplanted quail cells ($n = 11$). In the former, even though the cells derived from the anterior half of a developing somitic unit, they did not support the formation of a dorsal root ganglion. In contrast, originally posterior half-cells of a somitic unit allowed the dorsal root ganglion to form. Thus, we detected the rearrangement of the A–P identity upon the morphological boundary formation by early molecular markers and also by their morphogenetic events. Taken together, we conclude that the cells in the anterior PSM are still plastic in rearranging their A–P characters. We propose that during normal somitogenesis, these cells are biased as to their A–P identities but not fully determined prior to the morphological segmentation. Subsequently, the fissure formation may act as an additional step on these cells to undergo determination and/or fine-tuning of the A–P characters (see also Discussion).

**Discussion**

In this study, we have demonstrated a novel activity that acts in a ventral-to-dorsal direction at the anterior end of PSM to form a fissure during somitogenesis. We previously...
reported that another activity, designated as the segmenter, acts in a posterior-to-anterior direction to make such a fissure (Sato et al., 2002). Therefore, at least two signals act unidirectionally to accomplish somitic fissure formation. Molecular studies have shown that this morphological segmentation is the culmination of a series of events that commence in the posterior PSM. These include a segmentation clock of self-generating periodicity (Giudicelli and Lewis, 2004; Pourquie, 2000, 2003, references therein), and also the determination of the next-forming boundary regulated primarily by the transcription factor MesP2 (Saga and Takeda, 2001). These molecular studies, however, have dealt with the mechanisms concerning the A–P axis, and very little attention has been paid to the events occurring along the D–V axis. Relatively later processes in somitogenesis are known to depend on the D–V axis of the embryo, in particular, for differentiation of the dermomyotome and sclerotome in the dorsal and ventral portions of a formed somite, respectively. These distinctions after a somite forms are determined by the surrounding tissues of the somite, including the epidermal ectoderm dorsally, and the notochord ventrally (Borycki and Emerson, 2000; Brand-Saberi and Christ, 2000, references therein). However, it remained unknown whether the PSM itself displays D–V polarity, and if so, if this polarity is relevant to the somitogenesis. In this study, we have shown that the PSM is polarized along the D–V axis prior to the somite formation, and also that this D–V polarity plays an important role in the formation of a somitic fissure.

The ventral border cells provide signals that act in a V-to-D direction

We have demonstrated that a small population of ventral cells at −1 exhibits an ability to induce an ectopic fissure in a non-segmenting region −1.5. Particularly, the ventral cells located posteriorly to the prospective boundary appear to be crucial, consistent with our previous report (Sato et al., 2002). Our findings demonstrated in the present study suggest that, during normal somitogenesis, the V-to-D acting signal at the prospective boundary plays an important role in directing the segmentation. An unexpected finding obtained in this study was that the ventral-derived signal acts solely in the dorsal direction but not ventrally, regardless of the position where the inductive signal was provided along the D–V axis of the anterior PSM. We have presented evidence that the unidirectional action of the signal is, at least in part, attributable to the polarity in the population of signal-receiving cells (dorsal PSM cells), since the inversion of the recipient cells abolished the inductive phenomena which would normally be elicited by the ventral cells (Fig. 2I). Thus, the PSM is polarized along the D–V axis. This polarization might be independent of the D–V polarity acquired after a somite forms, which is critical for the production of the dermomyotome and sclerotome, since a D–V inversion of the young somites does not affect differentiation of these tissues (Aoyama, 1993) but does impede the fissure formation (this study). In addition, we did not observe significant effects by the adjacent ectoderm or endoderm on the fissure formation whereas these neighboring tissues are known to affect the D–V polarity of the somite after segmentation. The D–V polarity relevant to the fissure formation might be established earlier during PSM formation.

We have also demonstrated that, whereas the ventral border cells are sufficient for the formation of an ectopic fissure, these cells are not necessarily the single source for the V-to-D signal during normal segmentation. This was revealed by the fact that, although blocking the signals of the ventral border cells abolished the formation of ectopic fissure in −1.5-derived tissue, a separation of the dorsal PSM from the ventral one of normal embryos did not affect

Footnotes:

Fig. 6. A model showing two inductive events that occur during the formation of a somitic fissure. At the beginning of a segmentation cycle (Step 1), multiple cells (pale red) that possess the ability to act on the dorsal neighboring cells (blue arrows) are distributed along the D–V axis in the region posterior to the presumptive boundary. Subsequently (Step 2), the D–V aligned posterior border cells (red) act simultaneously with the segmenter activity on the anterior border cells (black arrows; Sato et al., 2002) leading to the formation of a morphological segmentation boundary (Step 3). This model does not exclude the possibility that dorsally acting signal and segmenter act simultaneously. The fissure formation is concomitant with fine-tuning of the A–P regionalization within a formed somite.
the segmentation. With these findings, it is reasonable to predict that multiple sources of the dorsally acting signal are distributed along the D–V axis at the prospective boundary (most likely within the posterior border cells) during normal segmentation.

We propose a model in Fig. 6, showing a possible mechanism underlying the fissure formation regulated by the two distinct inductive signals that act unidirectionally along the D–V and the A–P axes, respectively. In this model, by the time of the onset of a new segmentation cycle, multiple posterior border cells (pale red) are distributed along the D–V axis, which have acquired the ability to send a signal (blue arrows) to their dorsal neighbors (Step 1), and subsequently all the posterior border cells become capable of sending the second signal, segmenter (black arrows), and they simultaneously act on the anteriorly adjacent cells (Step 2) (Sato et al., 2002). These actions ultimately lead to the separation between the anterior and posterior border cells to make a fissure (Step 3). In this model, the ventro-dorsally acting signal is considered as a segmenter-generating activity.

We speculate that the inductive action occurring at −1 along the D–V axis is employed to coordinate the posteriorly aligned border cells so that these cells can simultaneously act on their anterior cells to make a smooth fissure (Fig. 6). It is conceivable that MesP2, known to be required for segmentation with the specific expression at −1 (Saga and Takeda, 2001; Saga et al., 1997), roughly defines the −1 position, and the cells that become posterior border cells subsequently coordinate between themselves to finalize the gap formation. At present, however, we have no evidence to exclude the possibility that the dorsally acting cells act simultaneously on the anterior cells.

The molecular mechanisms underlying the unidirectional signaling along the D–V axis are yet to be determined; no molecule has been reported to exhibit a dorso-ventrally differential pattern of expression at the anterior PSM. It is conceivable that each of the anterior PSM cells is polarized along the D–V axis since the −1 ventral cells could act on the dorsal neighbors but not on the ventral ones regardless of the position where the ventral cells were placed along the D–V axis (Figs. 2A–F). We previously proposed a similar possibility when we reported the segmenter, which acts in a posterior-to-anterior direction: each of the segmenter-receiving cells might be polarized along the A–P axis (Sato et al., 2002). It is therefore possible that each of the cells located near the prospective boundary is polarized both along the A–P and the D–V axes. Interestingly, it has recently been reported that the cells undergoing convergent extension during early gastrulation in Xenopus interpret information along both the D–V axis (more precisely the M–L axis) and A–P axis (Ninomiya et al., 2004). In addition, together with our finding that the V-to-D signal appears to be mediated by intimate cell–cell interactions (Fig. 3B), the presumed polarization of individual PSM cells at −1 as proposed here is reminiscent of the planar cell polarity (PCP) phenomena studied extensively in Drosophila (Adler, 2002; Uemura and Shimada, 2003). Although PCP has so far not been reported for mesenchymal cells, it is worth noting that Wnt 11 (Tonegawa et al., 2003) and paraxial protocadherin (PAPC) (Rhee et al., 2003), the molecules implicated in PCP events (Heisenberg et al., 2000; Kim et al., 1998), are expressed in the anterior PSM.

Although it has previously been reported that the interactions along the M–L axis are crucial for the coordination of cyclic expression of L-fringe mRNA in posterior PSM (Freitas et al., 2001), we did not detect an importance of cell communication along the M–L axis for the morphological segmentation.

Plasticity of the A–P characters in a single somite during segmentation

Although the possibility that the A–P identity in a single somite is acquired at or before the segmentation was proposed previously (Keynes and Stern, 1984), most of recent papers have supported “before the segmentation” with little evidence shown for “at the segmentation” (Palmeirim et al., 1998; Saga and Takeda, 2001). Our findings in this study provide new evidence that the A–P characters can be rearranged upon morphological segmentation. Consistent with this, we previously observed that experimentally potentiated posterior border cells by Notch-activation could also rearrange the A–P identities when transplanted into the −1.5 region (Sato et al., 2002). Thus, we propose that, during normal segmentation, the A–P characters are largely established within a presumptive somite, and the fissure formation that subsequently occurs refines the characters so that the anterior and posterior halves become explicitly distinct between each other. This distinction is critical for morphogenesis later in development, including the definition of the migrating pathway of the neural crest cells, and ressegmentation of the sclerotomal cells.

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