**brachyenteron** is necessary for morphogenesis of the posterior gut but not for anteroposterior axial elongation from the posterior growth zone in the intermediate-germband cricket *Gryllus bimaculatus*

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In the long-germband insect *Drosophila*, all body segments and posterior terminal structures, including the posterior gut and anal pads, are specified at the blastoderm stage. In short- and intermediate-germband insects, however, posterior segments are sequentially produced from the posterior growth zone, a process resembling somitogenesis in vertebrates, and invagination of the posterior gut starts after anteroposterior (AP) axial elongation from the growth zone. The mechanisms underlying posterior segmentation and terminal patterning in these insects are poorly understood. In order to elucidate these mechanisms, we have investigated the roles of the *Brachury/brachyenteron* (*Bralbyn*) homolog in the intermediate-germband cricket *Gryllus bimaculatus*. Loss-of-function analysis by RNA interference (RNAi) revealed that *Gryllus byn* (*Gb*’*byn*) is not required for AP axial elongation or normal segment formation, but is required for specification of the posterior gut. We also analyzed *Gryllus caudal* (*Gb*’*cad*) RNAi embryos using in situ hybridization with a *Gb*’*byn* probe, and found that *Gb*’*cad* is required for internalization of the posterior gut primordium, in addition to AP axial elongation. These results suggest that the functions of *byn* and *cad* in posterior terminal patterning are highly conserved in *Gryllus* and *Drosophila* despite their divergent posterior patterning. Moreover, because it is thought that the progressive growth of the AP axis from the growth zone, controlled by a genetic program involving *Cdx/cad* and *Bralbyn*, might be ancestral to bilaterians, our data suggest that the function of *Bralbyn* in this process might have been lost in insects.

**KEY WORDS:** *Gryllus bimaculatus*, Intermediate-germband insect, *brachyenteron*, *caudal*, Posterior patterning, RNA interference

**INTRODUCTION**

Molecular mechanisms of anteroposterior (AP) patterning and segmentation are best understood in *Drosophila melanogaster*, and this knowledge provides a basis for investigating the evolution of these processes in the phylum Arthropoda. In the long-germband insect *Drosophila*, all body segments and posterior terminal structures, including the posterior gut and anal pads, are specified almost simultaneously at the blastoderm stage. This is in contrast to short- and intermediate-germband embryos, anterior segments are specified almost simultaneously at the blastoderm stage, whereas posterior segments are sequentially produced from the posterior growth zone, and invagination of the posterior gut starts after AP axial elongation from the growth zone. Thus, because this major difference in the developmental process of posterior patterning is observed among insects, changes in the mechanisms underlying this process are assumed to be key events in the evolutionary transition from short- and intermediate- to long-germband embryogenesis. However, we still do not know how the transition occurred at the molecular level, because the posterior patterning mechanisms in short- and intermediate-germband insects remain poorly understood.

Recent functional studies using RNA interference (RNAi) in short- and intermediate-germband insects have demonstrated that several transcription factors, including Caudal (*Cad*), Even-skipped (Eve) and Hunchback (*Hb*), and cellular signaling pathways, including Wingless (*Wg*)/Armadillo (*Arm*) and Torso signaling, are involved in elongation and/or segmentation from the posterior growth zone (*Copf* et al., 2004; Liu and Kaufman, 2004; Liu and Kaufman, 2005; Mito et al., 2005; Miyawaki et al., 2004; Schoppmeier and Schröder, 2005; Shinmyo et al., 2005). This has led to two tentative conclusions regarding the evolution of genetic mechanisms directing posterior patterning. First, because a number of homologs of these factors are involved in posterior terminal patterning in *Drosophila*, the terminal system found in *Drosophila* may be involved in AP axial specification from the growth zone. Second, because homologs of some of these factors are also involved in AP axial elongation from the primitive streak and tail bud in vertebrates, there may be common mechanisms for AP axial formation between arthropods and vertebrates. These hypotheses prompted us to investigate the role of the *Brachury/brachyenteron* (*Bralbyn*) gene, which is involved in morphogenesis of the posterior gut in *Drosophila* and AP axial elongation in vertebrates, in short- and intermediate-germband insects.
**RESULTS**

**Cloning of the Gryllus brachyenteron homolog**

In order to clone Gb byn, we designed degenerate primers to the conserved T domain of byn homologs from other organisms. We then performed RT-PCR using these primers and isolated a short Gb byn clone. This fragment allowed us to design specific primers for 5' and 3' RACE and, thereby, to isolate fragments of the gene. The Gb byn gene is predicted to encode 357 amino acids, with a highly conserved T domain. The alignment of T domains of Gb' Byn and Bra/Byn proteins of other animals is shown in Fig. 1.

**Expression pattern of Gb' byn during early embryogenesis**

Embryogenesis in Gryllus has been described previously (Miyawaki et al., 2004; Niwa et al., 1997; Zhang et al., 2005). Briefly, in terms of segmentation, the germ anlage is formed in the ventral side of the posterior quarter of the egg by stage 3.0. Anterior segmentation occurs almost simultaneously by stage 4.0, at least to the level of the segment polarity genes, because Gryllus wingless (Gb' wg) is expressed in five vertical stripes corresponding to the mandibular segments can be tracked by the appearance of Gb' wg stripes, which appear one by one in the third thoracic segment at stage 4.3, and then in abdominal segment 1 at stage 4.4. At stage 7.5, the posterior-most stripe appears in abdominal segment 10.

We observed the expression pattern of Gb' byn during early embryogenesis by whole-mount in situ hybridization (Fig. 2). We were unable to detect any Gb' byn expression prior to stage 3.0 (data not shown). Gb' byn transcripts were first detected as two spots in the posterior terminal region of the embryo at stage 3.8 (Fig. 2A). The Gb' byn expression appeared more strongly at stage 4.3 (Fig. 2B). During germ band elongation, Gb' byn continued to be expressed in the terminal region of the embryo within the ectoderm, where the...
hindgut primordium is presumably located (Fig. 2C-E). The Gb'byn-expressing cells of the terminal region started to sink inward just after the completion of germband elongation and segmentation (Fig. 2F,G).

**Expression patterns of Gb'byn and Gb'cad during late embryogenesis**

Next, we observed the expression pattern of Gb'byn during late embryogenesis, and compared this with the expression of Gb'cad (Fig. 3). At stage 8, when the proctodeum has extended and is positioned parallel to the body axis, Gb'byn was expressed in the developing hindgut (Fig. 3A,B). This Gb'byn expression was maintained during late embryogenesis (Fig. 3C,D). There was no expression of Gb'byn in the mesoderm surrounding the hindgut, posterior midgut and Malpighian tubules.

We have previously reported the expression pattern of Gb'cad during early embryogenesis (Shinmyo et al., 2005). Here, we focused on its expression during late embryogenesis. At stage 9, Gb'cad was expressed in a region adjacent to the hindgut (Fig. 3E). At stage 11, the expression domain was subdivided into two, corresponding to the Malpighian tubules and developing posterior midgut (Fig. 3F). In addition, Gb'cad was expressed throughout late embryogenesis in the cerci and in the region surrounding the orifice of the hindgut (Fig. 3E,F). Double staining indicated that the spatial patterns of Gb'cad and Gb'byn expression were almost complementary to one another in the posterior gut, i.e. in the posterior midgut, Malpighian tubules and hindgut (Fig. 3G,H).

Gb'byn RNAi nymphs exhibited severe defects in the posterior gut

To examine the function of Gb'byn during Gryllus embryogenesis, we used RNAi to deplete Gb'byn transcripts and produce knockdown phenocopies. Two RNAi methods have been established in Gryllus: embryonic RNAi (eRNAi) (Miyawaki et al., 2004), which involves microinjection of dsRNA into the early eggs; and parental RNAi (pRNAi) (Mito et al., 2005), which involves injection of dsRNA into adult virgin females to yield knockdown phenocopies. We confirmed that no qualitative phenocopy differences were produced when using eRNAi or pRNAi, and mainly used pRNAi for our analyses because it does not produce any injection artefacts. As with the wild type, eggs from the Gb'byn RNAi-injected females developed and hatched nymphs 12-13 days after egg laying. No obvious difference was observed in the cuticle patterns of wild-type and Gb'byn RNAi nymphs (Fig. 4A,B). However, most Gb'byn RNAi nymphs (95%, n=118 out of 124) exhibited inhibited growth in the first instar and died before reaching the second instar. To investigate the effects of Gb'byn depletion on gut formation, we compared the morphology of the alimentary canal in first-instar Gb'byn RNAi nymphs (Fig. 4D,E) with that of the wild type (Fig. 4C). The alimentary canal of the wild-type nymph consists of the foregut, including crop and proventriculus, the midgut, including gastric caecum and Malpighian tubules, and the hindgut, including the small and large intestines and rectum sac (Fig. 4C). The majority of Gb'byn RNAi nymphs exhibited severe morphological defects in the posterior gut (95%, n=38 out of 40; Fig. 4D,E), whereas the crop and proventriculus in the foregut, and the...
GB byn is necessary for differentiation of the posterior midgut and hindgut, and for elongation of the Malpighian tubules

To further investigate the effects of GB byn depletion on posterior terminal patterning, we examined the expression patterns of GB wg, Gryllus hedgehog (GB hh) and GB cad during GB byn RNAi embryogenesis. First, we confirmed that GB byn expression in the terminal region was reduced in the GB byn RNAi embryos at stage 5.2 (Fig. 5A,B). In late stages, GB byn expression in the hindgut (Fig. 5C) provides a useful marker for characterizing defects. In GB byn RNAi embryos, the GB byn expression domain was greatly reduced in the hindgut (92%, n=23 out of 25, Fig. 5D). Although this indicated a severe reduction of the hindgut, there was still a hindgut remnant that expressed GB byn in all GB byn RNAi embryos. This suggested that the hindgut primordium invaginated normally in GB byn RNAi embryos, but that subsequent development of the hindgut did not occur normally, thereby implying a requirement for GB byn in hindgut development post-invagination. However, we cannot rule out the possibility that GB byn is required for the hindgut invagination itself, because almost all GB byn RNAi embryos might show hypomorphic phenocopies, as judged by the fact that weak GB byn expression was detected in almost all GB byn RNAi embryos (Fig. 5B). In this case, hindgut development after invagination would be more sensitive to GB byn reduction than development before invagination.

Although GB hh expression is observed in the terminal region during germband elongation (Miyawaki et al., 2004), overlapping with GB byn expression, GB hh expression patterns were unaffected in the GB byn RNAi embryos (data not shown). During invagination of the proctodeum in wild-type embryos, GB hh is expressed in the developing hindgut (Inoue et al., 2002). At stage 11-12, the expression domain became subdivided into three regions: strong expression in the small intestine and rectum sac, and weak expression in the large intestine (Fig. 5E) (Inoue et al., 2002). In the GB byn RNAi embryos, abnormal expression of GB hh was observed in the hindgut remnant, probably as a combined pattern of the small intestine and rectum sac expression domains, with reductions in both (100%, n=25; Fig. 5F). This indicates a dramatic defect in the large intestine. In addition, GB hh expression in the Malpighian tubules was also disrupted in the GB byn RNAi embryos (Fig. 5F).

GB wg is expressed in the posterior growth zone during germband elongation (Miyawaki et al., 2004). This expression pattern was unaffected in the GB byn RNAi embryos (data not shown). In the wild-type embryos at stage 11-12, GB wg expression was detected in two regions, the anterior region of the small intestine and the posterior rectum of the hindgut (Fig. 5G) (Inoue et al., 2002). In the GB byn RNAi embryos, GB wg expression was detected in both anterior and posterior regions of the severely reduced hindgut, with reduced expression domains (92%, n=23 out of 25; Fig. 5H). This result indicates a dramatic defect in the large intestine of GB byn RNAi embryos, as well as relatively mild defects in the small intestine and rectum, consistent with the pattern of GB hh expression in the GB byn RNAi embryos.

GB cad is expressed in the posterior growth zone during germband elongation (Shinnyo et al., 2005). This expression pattern was unaffected in GB byn RNAi embryos (data not shown). In wild-type embryos at stage 9, GB cad was expressed in the region surrounding the orifice of the hindgut and in the region adjacent to the hindgut (Fig. 3E, Fig. 5I). GB cad expression in both domains was greatly reduced in the GB byn RNAi embryos (100%, n=10; Fig. 5J). Additional domains of GB cad expression in the cerci were not affected in the GB byn RNAi embryos (Fig. 5, compare I with J). At stages 11-12, GB cad expression was detected in the region surrounding the orifice of the hindgut, the Malpighian tubules and the
posterior midgut in wild-type embryos (Fig. 3F, Fig. 5K). Gb\textit{cad} expression in the region surrounding the orifice of the hindgut and posterior midgut was greatly reduced in the Gb\textit{byn} RNAi embryos (88%, n=22 out of 25; Fig. 5L), indicating a reduction in these structures. We also found that Gb\textit{cad} was weakly expressed in the very small remnant of the Malpighian tubules seen in all Gb\textit{byn} RNAi embryos (100%, n=25; Fig. 5L), indicating that the primordium of the Malpighian tubules was formed in Gb\textit{byn} RNAi embryos. This suggests that the disruption of the Malpighian tubules observed in the Gb\textit{byn} RNAi nymphs (Fig. 4D,E) resulted from an inhibition of tubule elongation. This interpretation is supported by the fact that the shortened Malpighian tubules were formed in most Gb\textit{byn} RNAi nymphs (Fig. 4D). Thus, in Gb\textit{byn} RNAi embryos, the expression patterns of the marker genes for the posterior gut suggest that Gb\textit{byn} is necessary for differentiation of the posterior midgut and hindgut, and for elongation of the Malpighian tubules.

Gb\textit{cad} is necessary for internalization of the hindgut primordium

In Drosophila, \textit{cad} is essential for invagination and maintenance of the hindgut primordium (Wu and Lengyel, 1998). Although it has been shown that \textit{cad} is required for the formation of all trunk segments in short- and intermediate-germband insects (Copf et al., 2004; Shinmyo et al., 2005), the role of \textit{cad} in posterior terminal patterning in these insects has not been investigated. To determine Gb\textit{cad} function in Gryllus, we generated embryos depleted of Gb\textit{cad} by pRNAi, and examined the expression patterns of Gb\textit{byn} during Gb\textit{cad} RNAi embryogenesis. First, we confirmed that most Gb\textit{cad} RNAi embryos obtained by pRNAi exhibited severe defects in the trunk segments (Fig. 6A-C), as described previously in Gb\textit{cad} eRNAi experiments (Shinmyo et al., 2005). In wild-type embryos at stage 4, Gb\textit{byn} was expressed in the posterior terminal region (Fig. 6D), and this remained unaffected in Gb\textit{cad} RNAi embryos (100%, n=10; Fig. 6E). This suggests that Gb\textit{cad} is not involved in establishing the hindgut primordium. In wild-type embryos, Gb\textit{byn}-expressing cells in the terminal region sink inwards at stage 7.5 and are completely internalized by stage 9 (Fig. 3C, Fig. 6F). In the Gb\textit{cad} RNAi embryos, the Gb\textit{byn}-expressing cells failed to invaginate at stage 9, remaining on the outside of the embryo (Fig. 6G,H).

We also examined the expression pattern of Gb\textit{hh}, which is also used as a marker gene for the hindgut (Fig. 6I). In the Gb\textit{cad} RNAi embryos, Gb\textit{hh} expression was observed in the external hindgut remnant (Fig. 6I,J). These observations indicate that the invagination of the hindgut primordium did not occur in the Gb\textit{cad} RNAi embryos, suggesting that Gb\textit{cad} is not necessary to establish the hindgut primordium, but is required for internalization of the primordium. However, we cannot rule out the possibility that the hindgut primordium, in which Gb\textit{byn} is normally expressed, is not correctly specified in the Gb\textit{cad} RNAi embryos. In this case, Gb\textit{cad} and Gb\textit{byn} would be activated independently in the hindgut primordium, and both genes would be necessary for the establishment of the hindgut primordium. Further expression analyses of hindgut markers in Gb\textit{cad} RNAi embryos will be required to determine Gb\textit{cad} function in specification of the hindgut primordium.

DISCUSSION

We have isolated the Gb\textit{byn} gene from the intermediate-germband cricket Gryllus bimaculatus and investigated its developmental function using RNAi. We found that Gb\textit{byn} is not required for AP axial elongation or normal segment formation, but is required for the specification of the posterior gut. We also found that Gb\textit{cad} is required for internalization of the hindgut primordium, in addition to AP axial elongation. Here, we discuss the functions of Gb\textit{cad} and Gb\textit{byn} in Gryllus embryogenesis, and compare them with their functions in other bilaterians.

Specification of the hindgut primordium appears to occur independently of posterior segment specification in Gryllus

In Drosophila, all segments and posterior terminal structures are specified by the blastoderm stage. By contrast, in Gryllus, posterior segments are specified in an anterior to posterior direction through
The interpretation may also be supported by the observation that Gb’byn expression appears normal in early Gb’cad RNAi embryos, which presumably lack all trunk segments at late stages (Fig. 6E).

It is important to note that tailless, which acts upstream of byn in Drosophila terminal patterning, is already expressed at the blastoderm stage at the posterior pole of Tribolium embryos. This suggests that there is a group of cells within the posterior growth zone that is determined at the blastoderm stage to produce the terminal structures in Tribolium (Schröder et al., 2000). This conceivably might also apply to Gryllus embryogenesis.

**Roles of Cdx/cad and Bra/byn in posterior gut patterning**

We found that the posterior gut, consisting of the posterior midgut, Malpighian tubules and hindgut, was severely reduced in Gb’byn RNAi nymphs (Fig. 4, Fig. 7A). Furthermore, detailed analysis of the expression patterns of tissue-specific markers revealed that Gb’byn is necessary for differentiation of the midgut and hindgut, and for elongation of the Malpighian tubules (Fig. 5, Fig. 7A). In Drosophila byn mutants, the posterior gut is severely reduced as a consequence of massive apoptosis in the gut primordia (Kispeet et al., 1994; Singer et al., 1996). It remains unclear whether apoptosis contributes to the reduced posterior gut in Gb’byn RNAi embryos because of a technical problem associated with the TUNEL staining. However, the similarities in phenotype suggest that byn function during embryogenesis is highly conserved between long- and intermediate-germband insects. Bra is not reported to be involved in gut formation in vertebrates, but it is expressed in the posterior gut endoderm of hemichordates (Peterson et al., 1999) and echinoderms (Gross and McClay, 2001; Shoguchi et al., 1999). Although the posterior gut endoderm of these animals is substantially different from the hindgut ectoderm of insects, these similarities suggest that the involvement of Bra/byn in specification of the posterior gut
Fig. 6. Effect of Gb’cad RNAi on posterior terminal patterning. (A-C) Wild-type (A) and Gb’cad RNAi (B, C) embryos at 12 days after egg laying. In the most severe cases (64%, n=178 out of 280), Gb’cad RNAi embryos completely lack gnathum, thorax and abdomen, whereas the anterior head is formed normally (B). In other cases (31%, n=87 out of 280), the normal anterior head and part of the trunk segments are formed (C). (D-H) Expression of Gb’by in wild-type (D, F) and Gb’cad RNAi (E, G, H) embryos at stages 4 (D, E) and 9 (F-H). At stage 4, Gb’by expression in the terminal regions was normal in all Gb’cad RNAi embryos (D, E). In wild-type embryos at stage 9, Gb’by was expressed in the internalized hindgut (F). In the Gb’cad RNAi embryos at this stage, the Gb’by-expressing cells failed to invaginate, remaining on the outside of the embryo (G, H). (I-K) Expression of Gb’hh in wild-type (I) and Gb’cad RNAi (J, K) embryos at stage 9. In wild-type embryos, Gb’hh expression was observed in the internalized hindgut, whereas, in the Gb’cad RNAi embryos, Gb’h expression was observed in the external remnant.

Fig. 7. Schematics of the profile of Gb’by and function. (A) A comparison of extrapolated expression patterns of Gb’h, Gb’wg, and Gb’cad in the gut of wild-type (left) and Gb’by RNAi (right) nymphs. In Gb’by RNAi nymphs, Gb’h expression is observed in the severely reduced hindgut as a probable combined pattern from the small intestine and rectum sac, and in the small remnant of the Malpighian tubules. Gb’wg expression seems to be observed in the small intestine and posterior rectum of the severely reduced hindgut of Gb’by RNAi nymphs. Gb’cad expression in the Gb’by RNAi nymph seems to be observed in the small remnant of the Malpighian tubules, and is almost completely eliminated in the region surrounding the orifice of the rectum and posterior midgut. The hatched regions indicate overlapping expression patterns. pv, proventriculus; gca, gastric caecum; mg, midgut; ht, Malpighian tubules; si, small intestine; li, large intestine; rsc, rectum sac; rec, rectum. (B) Comparison of the expression of Gb’by, Gb’wg, and Gb’cad in early embryos of Gryllus with those of Drosophila and mouse. In early Gryllus embryos, Gb’wg and Gb’cad expression is detected in the posterior growth zone (Miyawaki et al., 2004; Shinmyo et al., 2005), whereas Gb’by expression is restricted in the posterior terminal region (see Fig. 2). In Drosophila, all three genes are expressed in the posterior terminal region at the cellular blastoderm stage (Hoch and Pankratz, 1996; Kispert et al., 1994; Singer et al., 1996; Wu and Lengyel, 1998). In mouse, all three genes are expressed in the primitive streak of early embryos (Kispert and Herrmann, 1994; Liu et al., 1999; Meyer and Gruss, 1993).

might be ancestral to bilaterians. A similar presumption might also extend to the role of Cdx2/cad in gut development. In Gryllus, Gb’cad is expressed in the Malpighian tubules and posterior midgut endoderm during late embryogenesis (Fig. 2). Our RNAi analysis shows that Gb’cad is necessary for internalization of the posterior gut primordium (Fig. 6). In Drosophila, Dm’cad is known to be expressed in the Malpighian tubules and posterior midgut endoderm of older embryos (Macdonald and Struhl, 1986; Mlodzik et al., 1985), and to be essential for internalization and maintenance of the posterior gut primordium (Wu and Lengyel, 1998). Thus, the expression pattern and function of cad in posterior gut development are highly conserved between Gryllus and Drosophila. In vertebrates, Cdx genes are expressed in the gut endoderm during late embryogenesis (reviewed by Freund et al., 1998), and Cdx2 mutant mice develop intestinal tumors (Chawengsaksophak et al., 1997). In Caenorhabditis elegans, the cad homolog pal-1 is expressed zygotically in mesoderm cells of the posterior gut (Edgar et al., 2001). On the basis of these data, we hypothesize that the involvement of Cdx/cad and Bra/byn in the specification of the posterior gut might be an ancestral feature of bilaterians.
It should be noted that morphogenesis of the Malpighian tubules and posterior midgut is blocked in Gb\textsuperscript{byn} RNAi nymphs, and that Gb\textsuperscript{cad} expression in these tissues is reduced in Gb\textsuperscript{byn} RNAi embryos, even though Gb\textsuperscript{byn} expression is not detected in these tissues in Gryllus embryos. There are two possible explanations for this phenomenon. First, it might be that very low levels of Gb\textsuperscript{byn} expression, which are below detectable levels, are sufficient for the development of these tissues. In this case, Gb\textsuperscript{byn} would be required for the formation of the Malpighian tubules and posterior midgut through the direct or indirect regulation of Gb\textsuperscript{cad} expression in these structures. Second, morphogenesis of the Malpighian tubules and posterior midgut depends upon signaling from the contiguous hindgut, where Gb\textsuperscript{byn} is expressed. In this case, the reduction in Gb\textsuperscript{cad} expression in the Malpighian tubules and posterior midgut of the Gb\textsuperscript{byn} RNAi embryos would result from the inhibition of hindgut development. A similar phenomenon is also observed in Drosophila byn mutants (Singer et al., 1996), suggesting conservation in the mechanisms of terminal patterning.

**Evolution of Cdx/cad and Bralbyn function in AP axial elongation**

The progressive growth of AP axial structures from a posterior region is observed in such diverse animals as chordates, short- and intermediate-germband arthropods, annelids and molluscs. In short- and intermediate-germband arthropods, posterior segments are sequentially produced from the posterior growth zone, where cad is expressed (Copf et al., 2003; Dearden and Akam, 2001; Schulz et al., 1998; Shinmyo et al., 2005) (Fig. 7B) and required for AP axial elongation from the growth zone (Copf et al., 2004; Shinmyo et al., 2005). cad expression in the growth zone is likely to be regulated by Wg/Arm signaling in Gryllus embryos (Shinmyo et al., 2005) (Fig. 7B). Segmentation in short- and intermediate-germband arthropods resembles somitogenesis in vertebrates, in which somites are generated progressively from a posteriorly located presomitic zone (reviewed by Peel et al., 2005). In addition, the Cdx genes, which are regulated by Wnt signaling, are expressed in the nascent mesoderm of the primitive streak (Ikeya and Takada, 2001; Marom et al., 1997; Meyer and Gruss, 1993) (Fig. 7B), and are involved in axial elongation and somitogenesis (Epstein et al., 1997; Subramanian et al., 1995; van den Akker et al., 2002). These similarities suggest that the molecular mechanisms underlying these processes are conserved between short- and intermediate-germband arthropods and vertebrates. Recently, it has been shown that even-skipped (eve) is expressed in the posterior growth zone and is required for AP axial elongation in the intermediate-germband insect Oncopeltus fasciatus (Liu and Kaufman, 2005). This fact may also suggest conserved mechanisms for these processes because, in vertebrates, Evx1 (the eve homolog) is known to be expressed in the primitive streak and tail bud, although its function has not been investigated (Dush and Martin, 1992). These data suggest that AP axial formation from the posterior growth zone is ancestral to bilaterians. A similar hypothesis has been proposed, based on a comparison of Bra expression patterns in molluscs and vertebrates. In vertebrates, Bra is also expressed in the nascent mesoderm of the primitive streak and tail bud (Kispert and Herrmann, 1994; Knezevic et al., 1997; Wilkinson et al., 1990) (Fig. 7B), and is necessary for AP axial formation (Wilson and Beddington, 1997). Because Bra expression in the posterior pole of the AP axis, up to the end of molluscs larval development, is similar to that in vertebrates, Lartillot et al. (Lartillot et al., 2002) have proposed that Bra might have a conserved role in the regulation of AP patterning among bilaterians, through maintenance of the posterior growth zone. This hypothesis implies that the role of Bra/byn in AP axial elongation might be ancestral to bilaterians. Importantly, we found that Gb\textsuperscript{byn} is expressed exclusively in the posterior terminal region (Fig. 7B), and is not involved in AP axial elongation from the growth zone. Therefore, if the hypothesis is correct, our results suggest that the function of Bra/byn in AP axial elongation might have been lost in insects. More data from a wider range of protostomes will be required to confirm this.

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