The lines gene of Drosophila is required for specific functions of the Abdominal-B HOX protein

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SUMMARY

The Hox genes encode homeobox transcription factors that control the formation of segment specific structures in the anterior-posterior axis. HOX proteins regulate the transcription of downstream targets acting both as repressors and as activators. Due to the similarity of their homeoboxes it is likely that much of the specificity of HOX proteins is determined by interaction with transcriptional cofactors, but few HOX cofactor proteins have yet been described. Here I present genetic evidence showing that lines, a segment polarity gene of Drosophila, is required for the function of the Abdominal-B protein. In lines mutant embryos Abdominal-B protein expression is normal but incapable of promoting its normal functions: formation of the posterior spiracles and specification of an eighth abdominal denticle belt. These defects arise because in lines mutant embryos the Abdominal-B protein cannot activate its direct target empty spiracles or other downstream genes while it can function as a repressor of Ultrabithorax and abdominal-A. The lines gene seems to be required exclusively for Abdominal-B but not for the function of other Hox genes.

INTRODUCTION

The body of Drosophila is composed of a series of segments morphologically different from each other. During development segmentation is established by the segment polarity genes and the specification of the different morphologies of the segments along the anterior posterior axis is controlled by the Hox genes of the Bithorax and Antennapedia complexes (Kaufman et al., 1980; Lewis, 1978). Although Hox and segment polarity genes are activated by the same factors (gap and pair-rule genes) they are believed to function independently (Harding and Levine, 1988; Ingham and Martínez-Arias, 1986; Jack and McGinnis, 1990; Müller and Bienz, 1992; White and Lehmann, 1986; Zhang et al., 1991). Flies lacking all Hox genes form normal segments that are identical to each other; while flies lacking segment polarity genes are not segmented but they have morphological differences along the anterior posterior axis. Therefore, with the exception of the engrailed gene that modulates locally the expression of Ultrabithorax and abdominal-A (Macías et al., 1994; Mann, 1994), it is thought that the segment polarity genes are not required for the function of Hox genes.

The Hox proteins form a family of transcription factors present in all animal phyla (Carroll, 1995). They specify the morphological differences along the anterior-posterior axis by being expressed in different segments where they regulate specific subsets of downstream genes. HOX proteins bind through the homeodomain to their target’s cis-regulatory regions. It has been shown that, in vitro, many HOX proteins recognise the same 6 bp consensus sequence (reviewed by Mann and Chan, 1996). Because of this lack of specificity and because this sequence is present in almost all genes it has been proposed that, in vivo, transcriptional cofactors or particular posttranscriptional modifications are required for a particular HOX protein to recognise its specific downstream targets. One such cofactor is encoded by the extradenticle/pbx gene in Drosophila and vertebrates (Peifer and Wieschaus, 1990; Rauskolb et al., 1993). In vivo, UBX, ABD-A and DFD Hox proteins require the Extradenticle protein (EXD) (Peifer and Wieschaus, 1990; Pinsonneault et al., 1997). In vitro, EXD increases the binding affinity of UBX and ABD-A for their target sequences (Chan et al., 1994; Chang et al., 1995; Van Dijk and Murre, 1994).

Recently FTZ-F1 has been shown to be a cofactor for the Fushi-tarazu protein (FTZ) (Guichet et al., 1997; Yu et al., 1997). FTZ is a divergent member of the Hox family (Dawes et al., 1994) and the fact that it also requires a cofactor protein suggests that most Hox proteins need specific cofactors to increase their binding specificity.

No cofactors have yet been described for the ABD-B Hox protein. Here I report genetic results indicating that lines (lin), besides acting as a segment polarity gene in Drosophila (Bokor and DiNardo, 1996; Volk and VijayRaghavan, 1994), is also required for some functions of ABD-B. Lines is needed for ABD-B to activate its downstream targets cut, spalt and empty spiracles. In the absence of lines the ABD-B protein is expressed in a normal pattern and can repress Ubx and abd-A expression. lin function is also shown to be required mainly, if
not exclusively, for Abd-B but not for other Hox genes. It is proposed that this double role could be explained if lines was required for the posttranscriptional modification or as a co-factor of both ABD-B and of proteins involved in the segment polarity pathway.

MATERIALS AND METHODS

The linG1 and Df(2R)linG75 lines null alleles (Bokor and DiNardo, 1996) and bithorax complex single (Ubx<sup>1</sup>; abd-A<sup>M1</sup>; Abd-B<sup>M1</sup>; Abd-A<sup>B0</sup>; double Ubx<sup>abd-A</sup> (Df(3R)Ubx 109); or triple Ubx<sup>abd-A</sup>-Abd-B<sup>-</sup> (Ubx<sup>M12</sup>;abd-A<sup>M1</sup>;Abd-B<sup>B0</sup>) allelic combinations (Casanova et al., 1986) were used. Clones in the germ line indicate that lines has no maternal product (Peter Bokor and Steve DiNardo, personal communication). To avoid interference with any mutations associated with the alleles used, the experiments were performed with homozygous and double heterozygous alleles. Ectopic expression of the ABD-Bm protein was induced with the 69B-GAL4 driver and the UAS-Abd-B 1,1 responder lines (Castelli-Gair et al., 1994). The ABD-Bm isoform was used because it is the only isoform expressed in A8 in the regions that give rise to denticles and spiracles. The following antibodies were used: anti en (Patel et al., 1989); 1A2E9 anti Abd-B (Celniker et al., 1989); FP3.38 anti Ubx (White and Wilcox, 1984); anti abd-A (Macías et al., 1990); anti ct (Blochinger et al., 1990); anti sal (Kühnlein et al., 1994); anti Dll (Vachon et al., 1992) and anti Scr (Glicksman and Brower, 1988). The wg (Baker, 1987), dpp (St. Johnston and Gelbart, 1987), T48 (Strutt and White, 1994) and ems (Jones and McGinnis, 1993) cDNA probes were used for whole-mount in situ hybridisation.

RESULTS AND DISCUSSION

Mutations in the lines (lin) gene of Drosophila (Nüsslein-Volhard et al., 1984) cause defects in most ectodermal derivatives of the larva. Briefly, the polarity of the abdominal denticle belts is abnormal, the head skeleton is malformed, the anal pad is absent, the hind gut is abnormal, the eighth abdominal segment (A8) does not differentiate an abdominal denticle belt and the posterior spiracles are missing (Fig. 1).

Cuticle phenotype of lin mutant embryos

In the wild-type embryo the ventral denticle belts from A2 to A7 have a trapezoidal shape with the denticles organised in six rows. The first and fourth denticle rows point anteriorly while the rest point posteriorly. In lin mutant embryos the denticle belts loose their trapezoidal shape, all denticles point posteriorly and there are only four rows of denticles. In some cases a few ectopic denticles form in the naked area between denticle belts. The defects on the dorsal cuticle have already been described (Bokor and DiNardo, 1996).

The tail and A8 segment of lin embryos are highly abnormal (Fig. 1D). The A8 denticle belt is replaced by naked cuticle that occasionally forms a few denticles less pigmented than the normal ventral denticles. This abnormal A8 cuticle does not resemble that of any region of the wild-type or of the lin mutant embryo. The absence of anal pads and the abnormal hind gut suggest abnormal development of A11 (Jürgens and Hartenstein, 1993), however, other aspects of the tail development like the formation of an anal tuft (thought to represent the unsegmented telson; Jürgens and Hartenstein, 1993) are normal. In wild-type embryos each sensory organ can be distinguished by its shape and position with respect to certain landmarks and this can be used to identify the different tail segments. In lin embryos the sensory elements are formed at roughly correct positions but have an abnormal shape. This, and the fact that most tail landmarks are missing, makes the sensory organs in the tail hard to correlate unequivocally to
their wild-type counterparts. It is therefore difficult to tell what other segments are affected in lin mutants.

Because of the disruption of the polarity of the dorsal abdominal denticle belts it has been proposed that lin is a gene of the segment polarity class (Bokor and DiNardo, 1996). However other aspects of the lin phenotype like the abnormal head skeleton and tail suggest that lin could interact with the zygotic genes of the terminal system, while the absence of the posterior spiracles suggests interactions with Abd-B (Fig. 1).

**lin is dispensable for tail segmentation and Abd-B activation**

To determine more precisely the function of the lin gene I have focused on the development of A8, a segment controlled by the terminal genes and where Abd-B is the primary determinant of segment morphology. Many structures characteristic of A8 are missing in lin embryos but this is not due to abnormal embryonic segmentation. Staining with en (en) antibody, a marker for the posterior compartment of each segment, shows that the A8 segment forms normally (Fig. 2A,C). However, after the extended germ band stage, the expression of en does not evolve normally in A8. In the wild type, en is expressed in the cells that encircle the opening of the posterior spiracles (Kuhn et al., 1992), but in lin mutants en continues to be expressed in a straight band of cells, consistent with A8 not developing posterior spiracles (Fig. 2B,D). This defect in the evolution of en expression is also observed in Abd-B mutants (Kuhn et al., 1995).

As the development of the posterior spiracles and the evolution of en expression are controlled by Abd-B (Kuhn et al., 1995; Sánchez-Herrero et al., 1985), I have tested if lin is required for the expression of Abd-B. In the wild type, Abd-B is expressed at high levels posterior to A8. Anteriorly Abd-B is expressed in a dynamic pattern from A5 to A7 initially confined to the tracheal pits and later extending to other cells in these segments (Boulet et al., 1991; Celniker et al., 1990; DeLorenzi and Bienz, 1990). The expression of Abd-B in the abdomen of lin mutants is indistinguishable from that in the wild type at all stages (Fig. 3A-D), the only difference being the ectopic expression of Abd-B in the anlage of the anal pads (Fig. 3C,D). This result shows that lin is not required for the activation of Abd-B in the abdominal segments.

**lin and Abd-B are required for the activation of genes involved in posterior spiracle morphogenesis**

The Abd-B gene directs the formation of the posterior spiracles by controlling downstream target genes. One of these genes required for posterior spiracle development is empty spiracles (ems) (Dalton et al., 1989; Jürgens et al., 1984; Walldorf and Gehring, 1992). The ems gene encodes a transcription factor whose expression in the posterior spiracles is regulated by Abd-B. Jones and McGinnis (1993) immunopurified an ems enhancer element bound to Abd-B. This enhancer drives expression of a lacZ reporter in the posterior spiracles in an Abd-B dependent manner, proving that ems expression in the posterior spiracles is directly regulated by Abd-B. I have looked at the expression of ems in lin mutant embryos. In lin embryos the transcription of ems is not activated in the posterior spiracles (Fig. 4A,B), showing that lin is required for Abd-B to activate its direct downstream target.

Other putative ABD-B downstream targets are the cut (ct) and spalt (sal) genes. These genes encode transcription factors (Blochinger et al., 1990; Kühnlein et al., 1994) and, among other functions, they are required for the normal development of the posterior spiracles. The activation of ct and sal in the anlage of the posterior spiracles requires ABD-B function but is independent of each other and of ems, suggesting that they are all independently controlled by Abd-B (J.C-G unpublished). In lin mutants neither ct nor sal are activated in the anlage of the posterior spiracles (Fig. 4C-F). These results indicate that lin is not required for the activation of cut and spalt in the anlage of the posterior spiracles.
show that in lin mutant embryos ABD-B is incapable of activating some of its targets.

The Abd-B gene is also required for the repression of other Hox genes (Macias et al., 1990; Struhl and White, 1985). In lin mutants the expression of abd-A and Ubx is normal showing that the ABD-B protein is functional (Fig. 3E,F).

Interactions between lin and Abd-B

The above results show that in lin mutants the ABD-B protein is expressed but it is only partially functional: it can repress anterior Hox genes but it is incapable of activating its targets. The lack of differentiation of an A8 denticle belt in lin mutants could also be due to this partial lack of function. In lin mutants ABD-B proteins are not capable of specifying an abdominal denticle belt, while at the same time they repress other Hox genes that can specify abdominal denticle belts, resulting in the poorly differentiated A8 denticle belts observed in lin mutants. To test this hypothesis I have studied double mutant embryos for lin and Abd-B. In lin; Abd-B double mutant embryos Ubx and abd-A can now be expressed in A8 and an abdominal denticle belt develops (Fig. 5B). This result demonstrates that the lack of a denticle belt in A8 in lin embryos is due to the abnormal function of ABD-B, as mutations in Abd-B rescue that aspect of the lin phenotype.

The requirement of lin for ABD-B function is not a specific property of the A8 segment. In wild-type embryos ectopic ABD-B expression using the GAL4 targeting system results in the formation of ectopic posterior spiracles in segments anterior to A8 (Castelli-Gair et al., 1994). In contrast, ectopic ABD-B expression in lin mutants does not form ectopic posterior spiracles (Fig. 5E) showing that no matter where the ABD-B protein is expressed in the embryo it requires lines to be fully functional.

lin is not required for the function of all Hox genes

Some of the phenotypes of lin mutant embryos are similar to those of abd-A mutant embryos. In both lin and abd-A mutants all the denticles of the A2-A7 denticle belts point posteriorly and the belt lacks the trapezoidal shape. To test if these phenotypes are due to the segment polarity function of lin or to a lack of function of abd-A in the absence of lin, I have tested if in lin embryos the abd-A downstream targets Ubx,Dll and T48 are expressed correctly (Struhl and White, 1985; Strutt and White, 1994; Vachon et al., 1992). Neither the repression of Dll and Ubx nor the activation of T48 in the ectoderm are affected in lin embryos (not shown). Moreover, the abnormal denticle belts formed in lin mutants are not due to the incapacity of abd-A to make abdominal denticles because in
**Fig. 6.** Model showing the proposed interactions between Lines and ABD-B in the embryo. In the anterior abdominal segments UBX and ABD-A direct the formation of denticle belts and other structures without lin requirement (only A7 shown). In A8 the ABD-B protein has lin-dependent and lin-independent functions. The repression of Ubx and abd-A in A8 is a lin-independent function, while other ABD-B functions like denticle belt and spiracle formation are lin dependent. In a lin mutant embryo ABD-B can still repress anterior Hox genes while is incapable of directing morphogenetic functions. This produces a lack of HOX input in A8 resulting in the abnormal ‘undifferentiated’ segment. Double mutants for lin and Abd-B allow UBX and ABD-A proteins to be expressed in A8, restoring the HOX input in A8 and leading to the formation of a denticle belt.

I have tested if other Hox genes are capable of regulating their downstream targets in the absence of lin. In lin mutant embryos Ubx is capable of repressing Dll and Scr in the ectoderm and to activate dpp expression in the visceral mesoderm. Similarly, in lin embryos, Deformed is also capable of activating Dll expression in the maxilla where this gene is its downstream target (O’Hara et al., 1993). Therefore, it seems that the only Hox gene that requires lin to function is Abd-B.

The function of Lines

I have provided evidence that in lin mutant embryos ABD-B is expressed in its normal domain but it is not capable of activating its targets while it is capable of repressing anterior Hox genes. This results in embryos in which the A8 segment forms but does not differentiate any recognizable structures due to the lack of a functional HOX protein (Fig. 6). The fact that the lack of A8 denticle belt in lin embryos can be rescued by a mutation on the Abd-B gene is particularly interesting. This result proves that at least this aspect of the lin phenotype is due to the presence of an ABD-B protein that is only capable of performing some of its normal functions.

The effect of lin on ABD-B can be explained at the molecular level if lin is required for protein posttranscriptional modification or as a transcriptional cofactor of ABD-B. There is some evidence that the ABD-B protein is posttranslationally modified (Boulet et al., 1991). If LIN was mediating this process, it would imply that such posttranscriptional modification is functional in vivo. Alternatively if lin is a transcriptional cofactor of ABD-B, lin would be interacting with ABD-B in a similar way to that proposed for extradenticle with Ubx and abd-A (Chan et al., 1994; Rauskolb et al., 1993) or Ftz-F1 with Ftz (Guichet et al., 1997; Yu et al., 1997). It is interesting that exd does not have any effect on ABD-B protein binding or function (Peifer and Wieschaus, 1990; Van Dijk and Murre, 1994), and that lin is specific for ABD-B but not for other Hox genes tested. This suggests that different HOX proteins will use different cofactors and this accounts for their DNA binding specificity.

It has been suggested that extradenticle is required for Hox genes to act as activators (Pinsonneault et al., 1997). The experiments described here support this idea as they imply that Abd-B can act as a repressor and that lin allows it to function in a dual repressor/activator mode.

There are reasons to believe that lin has other functions independent of the Hox genes. First, there are regions in the embryo where lin is required but no Hox genes are expressed. In the hindgut lin is required for en expression (Fig. 2B,D) and in the anal pads for Abd-B repression. Second, other groups have presented evidence showing that lin acts as a segment polarity gene. In the dorsal epidermis of the abdominal segments, lin functions along with wingless (wg) and hedgehog to specify the patterning of the different cell types (Bokor and DiNardo, 1996). lin is also required in the dorsal epidermis for the normal expression of groovin (Volk and VijayRaghavan, 1994). In lin mutants each segment forms an ectopic stripe of groovin-expressing cells that, as the normal stripe, can act as a site for muscle attachment. Because, as shown above, the effects of lin in the patterning of the dorsal epidermis from A1 to A7 are not due to lack of Hox function, lin has a dual role both as a segment polarity gene and as a modulator of Abd-B function.

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