Development 116, 447-456 (1992)
Printed in Great Britain © The Company of Biologists Limited 1992

Induction of *labial* expression in the *Drosophila* endoderm: response elements for *dpp* signalling and for autoregulation

GABI TREMML[1,*] and MARIANN BIENZ[2]

[1]*Zoologisches Institut, Universität Zürich, Winterthurerstr. 190, 8057 Zürich, Switzerland*
[2]*MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, UK*

*Present address: Howard Hughes Medical Institute, Columbia University, 701 West 168 Street, New York, NY 10032, USA

Summary

Extracellular signal proteins induce the homeotic gene *labial* (*lab*) to high levels of localised expression in the endoderm of *Drosophila* embryos. We aimed to identify *cis*-regulatory elements within the *lab* gene that respond to this induction by analysing the activity of stably integrated reporter gene constructs. Dissection of *lab* 5′ flanking sequences reveals two types of response elements. One of these mediates *lab* dependent activity, providing evidence that *lab* induction in the endoderm is autoregulatory. The other element, to a large extent independent of *lab* function, responds to *decapentaplegic* (*dpp*), a signal molecule related to mammalian TGF-β. Our evidence suggests that *lab* induction in the endoderm reflects coordinate action of two distinct factors one of which may be *lab* protein itself, and another whose localised activity or expression in the midgut depends on the *dpp* signal.

Key words: transcriptional induction, homeotic gene, autoregulation, growth factor-like signals, endoderm.

Introduction

A large part of the early *Drosophila* embryo is subdivided into parasegments (Martinez-Arias and Lawrence, 1985) at the blastoderm stage, a process under the control of segmentation gene products (Nüsslein-Volhard and Wieschaus, 1980). Parasegments (ps) are indistinguishable from one another at this stage, yet blastoderm cells are determined to follow distinct developmental pathways (Chan and Gehring, 1971). Unique states of determination are conferred by the products of homeotic genes which control the morphogenesis of external and internal segment-specific structures in the embryo, in the larva and in the adult (Lewis, 1963, 1978; Wakimoto and Kaufman, 1981; Teugels and Ghysen, 1985; Hooper, 1986; Tremml and Bienz, 1989a). Homeotic genes are activated in the blastoderm embryo in distinct domains along the anteroposterior axis (reviewed by Akam, 1987), and their continued activity in these domains is required throughout development (Morata and García-Bellido, 1976).

Domains of homeotic gene expression are specified by cues indicating position along the anteroposterior axis. In the ectoderm where most homeotic genes are expressed, and presumably in the mesoderm, such cues are provided intracellularly by gap genes (White and Lehmann, 1986; Harding and Levine, 1988; Irish et al., 1989; Reinitz and Levine, 1990; Tremml and Bienz, 1989b). Recent evidence shows that the product of one of these, *hunchback* protein, specifies expression boundaries of the homeotic gene *Ultra-bithorax* (*Ubx*) by directly acting as a transcriptional repressor (Qian et al., 1991; Zhang et al., 1991; Zhang and Bienz, 1992). The localised distribution of *hunchback* protein in the blastoderm embryo in turn is directly determined by a diffusible maternal morphogen (Driever et al., 1989; Struhl et al., 1989).

Only one of the homeotic genes, *labial* (*lab*), is also expressed in the endoderm (Diederich et al., 1989). The endoderm does not derive from the segmented part of the blastoderm embryo, but instead originates from two separate primordia located near the blastoderm poles; these subsequently grow towards each other and fuse to form a continuous cell sheet stretching along most of the anteroposterior axis (Poulson, 1950; Hartenstein et al., 1985; Campos-Ortega and Hartenstein, 1985). Therefore, it is unlikely that the positional cues specifying the *lab* expression domain (which is restricted along the anteroposterior axis) are provided intracellularly by gap gene products. Instead, *lab* expression in this germ layer is induced in response to an extracellular signal, the product of the *decapentaplegic* (*dpp*) gene (Immerglück et al., 1990; Panganiban et al., 1990; Reuter et al., 1990). The *dpp* product belongs to the group of TGF-β-like proteins (Padgett et al., 1987) some of which are known to induce expression of homeobox-containing genes in frog eggs (Ruiz i Altaba and Melton, 1989; Rosa, 1989; Cho et al., 1991; Taira et al., 1992). In addition, *lab* expression in the midgut is affected by another extracellular signal, the *wingless* (*wg*) protein (Immerglück et al., 1990) whose mammalian coun-

terpart is the *int-1* protein (Rijsewijk et al., 1987; Cabrera et al., 1987). *lab* induction in the midgut epithelium appears to be regulated at the level of transcript accumulation (Mlodzik et al., 1988), and we have argued that *dpp*-mediated induction, but perhaps not *wg*-mediated induction, may act at the level of transcription. Both *dpp* and *wg* expression in the visceral mesoderm are dependent, perhaps directly, on the localised expression and function of homeotic genes in the visceral mesoderm (Immerglück et al., 1990).

Of the molecules mediating TGF-β- and *int-1*-like signals through the membrane and into the nucleus, very few have been identified. Genes encoding putative signal-transducing membrane receptors for mammalian TGF-β-like molecules have been cloned (Mathews and Vale, 1991; Attisano et al., 1992; Lin et al., 1992), however little is known about their natural cytoplasmic and nuclear target proteins (reviewed by Massagué, 1990). There is some evidence that TGF-β may act through the transcription factors NF-1 (Rossi et al., 1988), AP-1 (Kim et al., 1990) and CREB (Kramer et al., 1991) in mammalian cells.

As a first step towards isolating nuclear factors mediating the *dpp* signal, we aimed to define *cis*-acting regulatory sequences within the *lab* gene which respond to *dpp* and perhaps to *wg* induction. We undertook a dissection of the DNA sequences upstream of the *lab* transcription start site and found a small fragment which, after linkeage to a β-galactosidase (β-gal) reporter gene, is sufficient to direct *lab*-like expression in the midgut epithelium of stably transformed embryos. We present evidence that this *lab* fragment contains *dpp* response elements as well as target sequences for *lab* autoregulation.

Materials and methods

Fly strains

The following mutant strains were used: lab^{vd1} (Diederich et al., 1989), dpp^{s4} (St. Johnston et al., 1990), wg^{cx4} (Baker, 1987), $abd\text{-}A^{M1}$ (Casanova et al., 1987), Pc^{3} (Lewis, 1978).

Strains were constructed which are homozygous for insertions on the second chromosome of the −6.3, of the HZ1.2, of the HZ550 or of the HZ255 construct and which also carry the lab^{vd1} mutation balanced with a TM3 chromosome containing a *hb*/βgal transposon (obtained from G. Struhl). Homozygous *abd-A* and *Pc* mutant embryos were identified by their phenotype in the gut, homozygous *wg* mutants by their abnormal morphology (Bienz and Tremml, 1988; Immerglück et al., 1990).

Plasmids

A 6.6. kb *Bam*HI fragment from the lambda phage 23.2 (Mlodzik et al., 1988) was subcloned into a bluescript vector. From this subclone, a 6.3 kb *Bam*HI/*Sac*II fragment (*Sac*II site at +325; Diederich et al., 1989; blunt-ended) was inserted into pL, a modified bluescript vector (obtained from T. Gutjahr; in pL, the original *Not*I site was destroyed, and a new *Not*I site was introduced into the original *Eco*RV site), cut with *Bam*HI and *Eco*RI (*Eco*RI site filled in). This step created a fusion gene (pL1) in which the first 29 amino acids of the *lab* protein (Diederich et al., 1989) were fused to β-gal protein coding sequences. For the −6.3 construct, a 6.3 kb *Xba*I/*Not*I fragment from the fusion plasmid pL1 was inserted into a Carnegie transformation vector (CZ.2; obtained from T. Gutjahr; CZ.2 is essentially the same as the Carnegie 20 vector used in Bienz et al., (1988), but contains a modified polylinker) cut with *Xba*I and *Not*I. For the −3.8 construct, a 3.8 kb *Not*I/*Hin*dIII fragment (*Hin*dIII site filled-in) from pL1 was inserted into CZ.2 cut with *Not*I and *Xba*I (*Xba*I site filled-in). For the −2.6 kb construct, a 2.6 *Not*I/*Eco*RI fragment (*Eco*RI site filled-in) was inserted into CZ.2 cut with *Not*I and *Xba*I (*Xba*I site filled-in).

For the HZ1.2 construct, a 1.2 kb *Hin*dIII/*Eco*RV fragment from pL1 was inserted, via a subcloning step in bluescript cut with *Hin*dIII and *Sal*I (*Sal*I site filled-in), as an *Xba*I/*Kpn*I fragment into the cloning cassette of HZ50PL (Hiromi and Gehring, 1987). To generate the HZ250, the HZ550 and the HZ400 constructs, the 1.2 kb bluescript subclone was cut with *Xba*I/*Cla*I (*Cla*I site filled-in), with *Cla*I (filled-in) or with *Kpn*I/*Cla*I (*Cla*I site filled-in), respectively, and the corresponding 250 bp, 550 bp or 400 bp fragments were then inserted into HZ50PL cut with *Not*I and *Xba*I (*Not*I site filled-in), with *Xba*I (filled-in; orientation of insert as in the *lab* gene) or with *Kpn*I and *Xba*I (*Xba*I site filled-in). For the HZ80 and the HZ255 constructs, the 1.2 kb bluescript subclone was cut with *Nar*I and *Asp*I or with *Nar*I and *Xba*I, respectively, all sites were filled-in and in both cases religated; from these religated plasmids, an 80 bp *Asp*I/*Cla*I fragment (*Cla*I site filled-in) or a 255 bp *Sac*II/*Cla*I fragment (*Cla*I site filled-in) was inserted into HZ50PL cut with *Asp*718 and *Xba*I (*Xba*I site filled-in) or with *Sac*II and *Asp*718 (*Asp*718 site filled-in), respectively. For the HZ220 construct, a 220 bp *Bst*XI fragment (blunt-ended) was inserted, via a subcloning step into a pGem vector cut with *Eco*RV, as a *Not*I/*Eag*I fragment (*Eag*I site filled-in) into HZ50PL cut with *Not*I and *Xba*I (*Xba*I site filled-in).

Isolation of transformants and β-gal staining analysis

5 independent transformant lines of each construct were isolated, and β-gal staining of transformed embryos was done as previously described (Bienz et al., 1988).

Results

cis-*regulatory* lab *sequences conferring midgut expression*

We have described the pattern of *lab* protein expression in the midgut epithelium (Immerglück et al., 1990). *lab* protein, though present at low levels in both anterior and posterior midgut primordia (Diederich et al., 1989), is not concentrated in cell nuclei at first. After fusion of the two primordia at late stage 12 (stages according to Campos-Ortega and Hartenstein, 1985), the levels of *lab* protein rise sharply and, at the same time, *lab* protein becomes localised in cell nuclei of the forming midgut epithelium (first visible in the most posterior region within the *lab* domain). At later stages, *lab* protein is exclusively found in the large midgut cell nuclei. *lab* protein levels remain graded within the band of *lab* expression, with highest levels most posteriorly. Thus, the posterior boundary of *lab* expression is very sharp. The band of *lab* expression in the midgut coextends approximately with *Ubx* expression in ps7 of the adhering visceral mesoderm, although the posterior *lab* boundary lies more anteriorly than the posterior *Ubx* expression boundary. The latter precisely coincides with the second midgut constriction (Bienz and Tremml, 1988; Tremml and Bienz, 1989a). Hence, there is a narrow albeit clearly visible gap, two or three cells wide, between *lab* protein expression and the second midgut constriction.

We first attempted to reconstruct *lab* expression in the midgut epithelium by fusing various pieces of *lab* upstream

sequences to a bacterial β-gal gene (with the fusion point just downstream of the *lab* translation start codon; see Materials and Methods). Flies were transformed with these constructs (Fig. 1), and embryos were analysed for β-gal staining. We found that a construct containing 6.3 kb of 5′ flanking DNA (with respect to the *lab* transcription start site; Diederich et al., 1989), confers a β-gal staining pattern (Fig. 2A,B) closely resembling endogenous *lab* expression. In particular, we observe strong β-gal staining in the midgut epithelium in a band whose extent is very similar if not identical with *lab* expression, as judged by double-staining with *Ubx* antibody (not shown) and by comparison with morphological landmarks such as the midgut constrictions. This staining first appears in a thin stripe, one or two cells wide, during early stage 13, i.e. after the contiguous midgut epithelium has formed completely (Fig. 2A), thus slightly lagging behind endogenous *lab* induction. This stripe of β-gal staining soon widens, and staining intensity gradually increases throughout embryonic development. Though β-gal staining is excluded from cell nuclei, we can see it to be strongest posteriorly within the β-gal band (Fig. 2B, 3A), mimicking graded expression of endogenous *lab* protein. As in *lab* protein expression, we see a gap, 2-3 cells wide, between the sharp posterior boundary of β-gal staining and the second midgut constriction (Fig. 3C). There is no β-gal staining in the midgut primordia, but there is strong staining in the head which we have not analysed any further (but cf. Chouinard and Kaufman, 1991).

We next tested a −3.8 kb and a −2.6 kb fusion construct (Fig. 1) for β-gal expression in the midgut. We found that the −3.8 kb construct is capable of directing a very similar pattern of β-gal expression in the midgut as the −6.3 kb construct (not shown), whereas the −2.6 kb construct does not support any β-gal expression in the midgut of transformed embryos (although we see some β-gal staining in the head; Fig. 2G). Thus, the 1.2 kb fragment located between −3.8 kb and −2.6 kb upstream of the *lab* transcription start site is necessary for expression in the midgut.

We joined this 1.2 kb fragment to an hsp70 TATA-box and a β-gal gene (HZ50PL; Hiromi and Gehring, 1987) to test whether this fragment is sufficient to direct a *lab*-like pattern of β-gal expression in the midgut. We found this to be the case as transformants of this construct (called HZ1.2) show β-gal expression in the midgut in a pattern (Fig. 2C,D) which is essentially the same as the one described above (Fig. 2A,B). In particular, β-gal staining also first appears at stage 13 as a thin stripe (Fig. 2C), is graded within the band of expression and has a sharp posterior boundary (Fig. 2D), with a gap between this boundary and the second midgut constriction. Notably, we do not see any β-gal expression elsewhere in the embryo. We conclude that the 1.2 kb fragment is both required and sufficient to direct a *lab*-like β-gal expression pattern in the midgut.

We split this 1.2 kb fragment into three subfragments to narrow down the *cis*-regulatory element(s) conferring midgut expression. Only one of the these fragments (Fig. 1) supported any β-gal expression in transformed embryos. This one, called HZ550, conferred a pattern of very strong β-gal staining in the midgut (Fig. 2E,F) which, at first sight, appeared to be the same as the one described. On closer inspection, however, we find two important differences. Firstly, β-gal staining is less graded and not as asymmetrical as the β-gal staining patterns described above; staining is almost uniformly strong throughout the HZ550 expression band, though somewhat decreased towards both

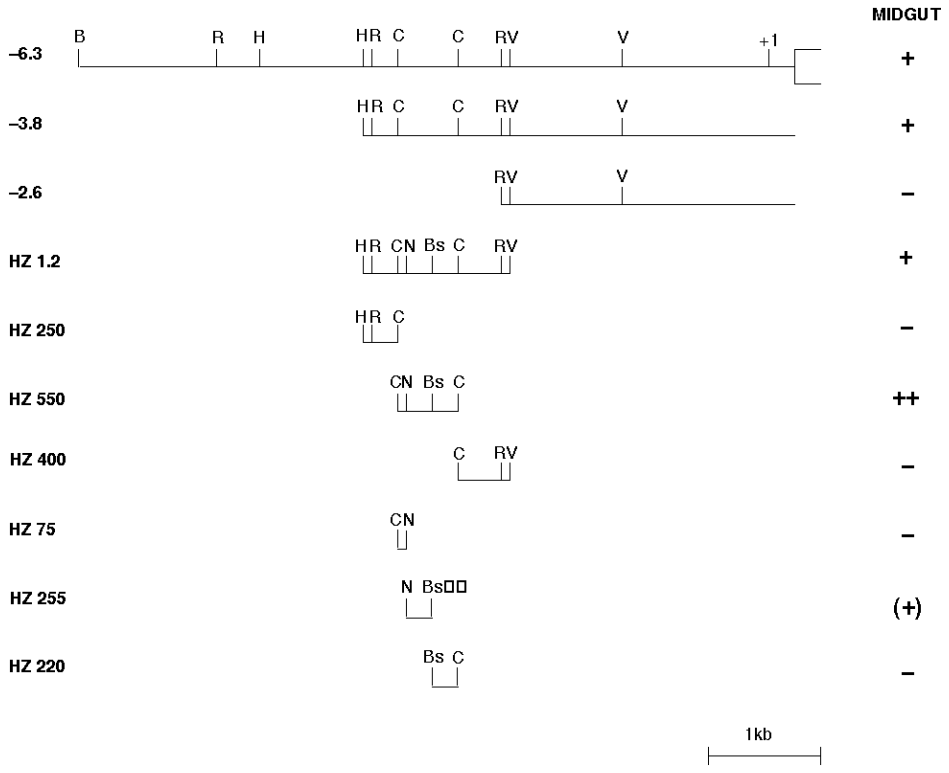

Fig. 1. β-gal constructs containing *lab* upstream sequences. Top three lines, β-gal constructs containing various stretches of *lab* 5′ flanking sequence fused to β-gal (open box) immediately downstream of the *lab* AUG (*lab* transcription start site marked with +1; see Diederich et al., 1989). Below, various fragments of *lab* upstream sequence used for insertion into HZ50PL (Hiromi and Gehring, 1987). B, *Bam*HI; Bs, *Bst*XI; C, *Cla*I; H, *Hin*dIII; N, *Nar*I; R, *Eco*RI; V, *Eco*RV; all sequences to scale. Activities (+) of the corresponding β-gal constructs (names at the left) in the midgut of transformants is given at the right (−, no expression).

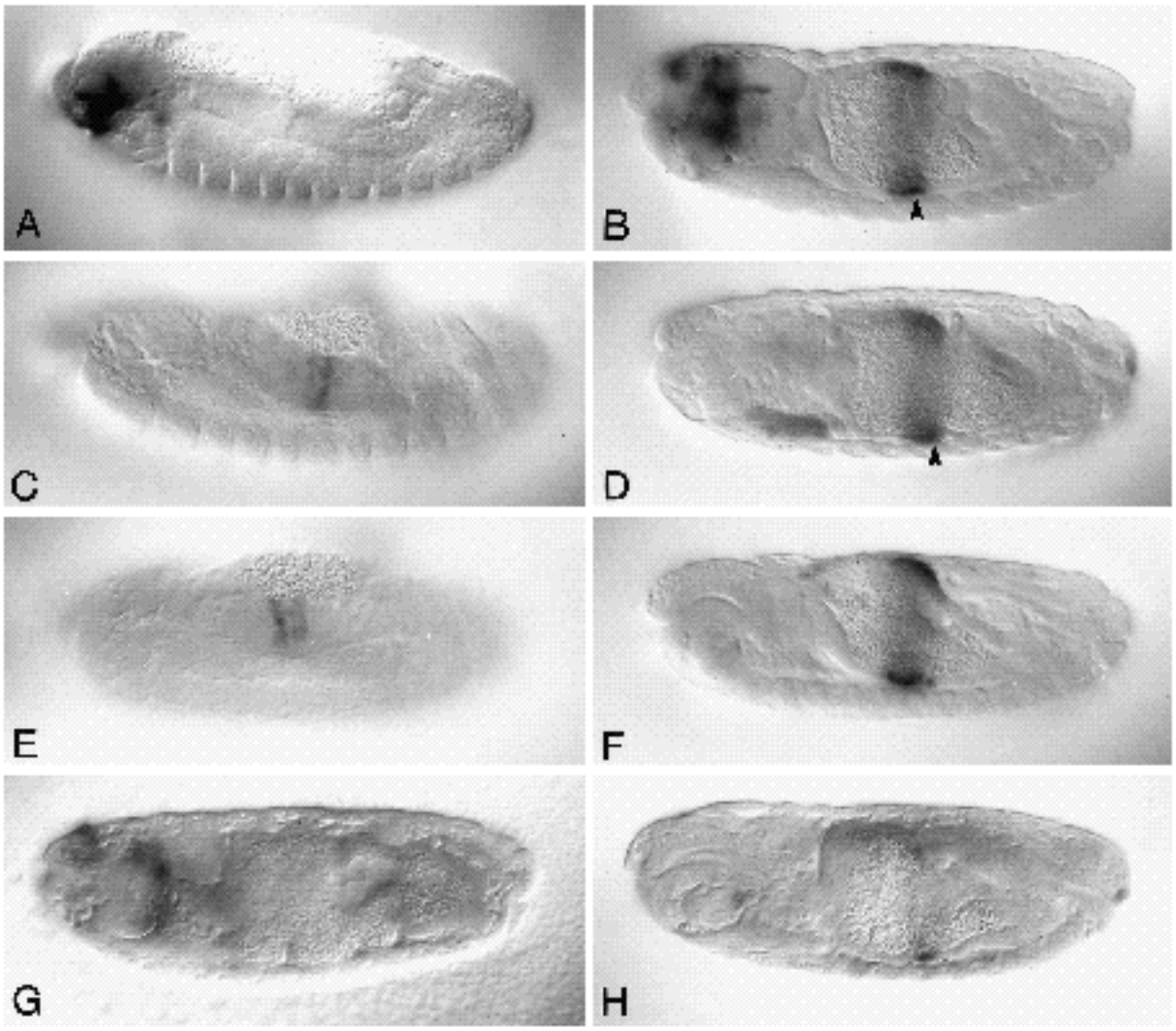

Fig. 2. β-gal staining patterns in transformed embryos. β-gal staining appears during stage 13 in the midgut epithelium of −6.3 (A), HZ1.2 (C) and HZ550 transformants (E) and increases subsequently (B, D, F, early stage 15). Note the graded β-gal expression in the *lab* expression domain of −6.3 (B) and HZ1.2 transformants (D; arrowheads in B and D point to cells near the incipient second midgut constriction that express highest levels of β-gal protein) which is not apparent in HZ550 transformants (F). Weak β-gal staining is visible at stage 15 in the midgut of HZ255 transformants (H). −2.6 transformants do not show any β-gal staining in the midgut (G, stage 15 embryo). Anterior to the left.

margins (Fig. 3B). Secondly, there is no gap between β-gal staining and the second midgut constriction (Fig. 3D): the row of 2 or 3 cells just preceding this constriction shows moderately strong β-gal staining in HZ550 transformants. Thus, there is "ectopic" β-gal expression in these transformants in cells of the midgut which do not express any *lab* protein. We observe some weak β-gal staining in the epidermis of HZ550 transformants which is not related to *lab* expression in any way.

Further dissectional analysis showed that there are sequences within a minimal 255 bp fragment which are capable of directing a low level of β-gal expression in the midgut (Fig. 2H). In this case, the β-gal stripe is narrower than any of the ones described and β-gal staining appears to coincide with those cells that express highest levels of endogenous *lab* protein (Fig. 3E). Neither of the other two subfragments conferred any expression. We conclude that the minimal 255 bp fragment is sufficient to some extent to direct a *lab*-like pattern in the midgut. In addition, sequences outside this 255 bp fragment, though not sufficient, are needed for high levels of β-gal expression in the midgut.

Sequences mediating positive autoregulation in the midgut

Most of the β-gal expression patterns conferred by *lab* upstream sequences closely mimic *lab* protein expression, and we therefore asked whether these patterns are dependent on endogenous *lab* function. We established fly strains homozygous for a −6.3, a HZ1.2, a HZ550 or a HZ255 transposon which also contain a *lab* loss-of-function mutation balanced with a chromosome bearing a β-gal marker transposon (see Materials and Methods). Among the off-

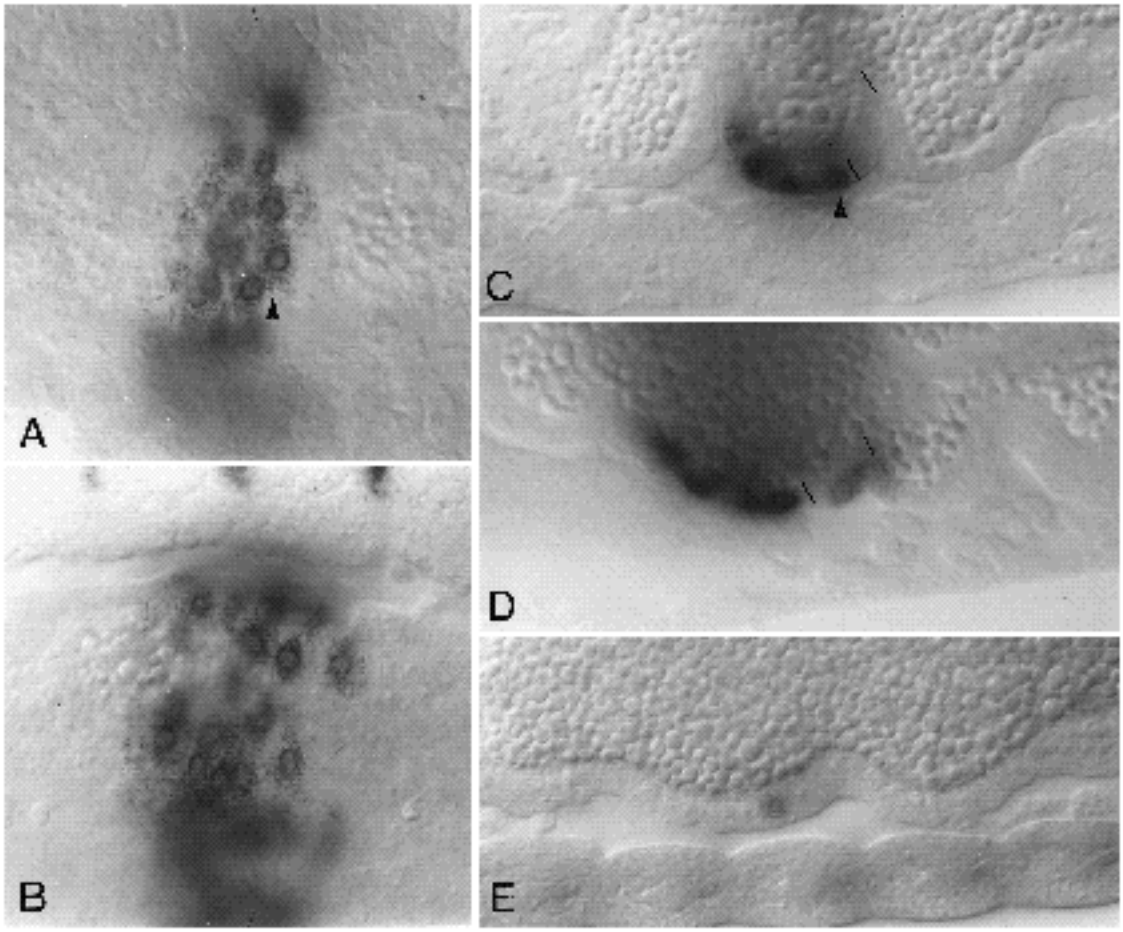

Fig. 3. High magnification views of β-gal expression in the midgut. β-gal expression in the midgut epithelium (A, B, superficial views at stage 14; C-E, optical sagittal sections at stage 15) shows anteroposterior gradient of staining in −6.3 (A, C; staining strongest in most posterior cells, indicated by arrowhead), but not in HZ550 transformants (B, D; staining strongest in the middle of the *lab* domain). Note also the gap (indicated by two bars in C) between the sharp posterior boundary of β-gal staining in −6.3 transformants and the second midgut constriction; in HZ550 transformants, cells within this gap (indicated by two bars in D) express low β-gal levels. (E) HZ255 transformant (stage 15); position of a cell expressing highest β-gal levels with respect to the second midgut constriction. Anterior to the left.

spring embryos from these strains, homozygous *lab*⁻ embryos can be identified by their lack of β-gal staining in anterior regions of the embryo due to the β-gal marker transposon.

In both −6.3 and HZ1.2 strains, we found that, prior to late stage 15, β-gal staining in the midgut is completely eliminated in all embryos that lack β-gal staining in the head (the *lab*⁻ homozygotes; Fig. 4B,E). After formation of the midgut constrictions during late stage 15, we begin to see low levels of β-gal staining appearing between the first and the second constriction, approximately where *lab* protein is expressed in the wild type (Fig. 4C,F). Its intensity increases slightly during the last stages of embryogenesis (not shown). We conclude that most of the midgut expression conferred by the two constructs is due to activation, directly or indirectly, by endogenous *lab* protein.

We found a somewhat different result with the third strain containing the HZ550 construct. Initially, after the formation of the contiguous midgut epithelium, we see no β-gal staining in homozygous *lab*⁻ embryos (although there is staining in the wild type at this stage). However, very soon after, β-gal expression appears in the midgut (Fig. 4H) and reaches moderately strong levels by the time the midgut constrictions form (Fig. 4I). The anteroposterior limits of β-gal staining appear to be the same in the mutants as in the wild type, and the only difference we can detect between the two genetic backgrounds is a reduction in β-gal staining intensity in the *lab*⁻ embryos throughout embryonic development (Fig. 4I compared to Fig. 4G). Finally, in the case of HZ255 transformants, β-gal staining is the same in all respects (first appearance, intensity and spatial distribution) in wild-type and *lab*⁻ embryos (not shown). We conclude that β-gal expression mediated by these two constructs is only very slightly (HZ550) or not at all (HZ255) dependent on *lab* function.

Requirement for dpp *function*

Induction of high and graded levels of *lab* protein in the midgut epithelium requires the function of two control genes, *wg* and *dpp* (Immerglück et al., 1990). In *wg* mutant

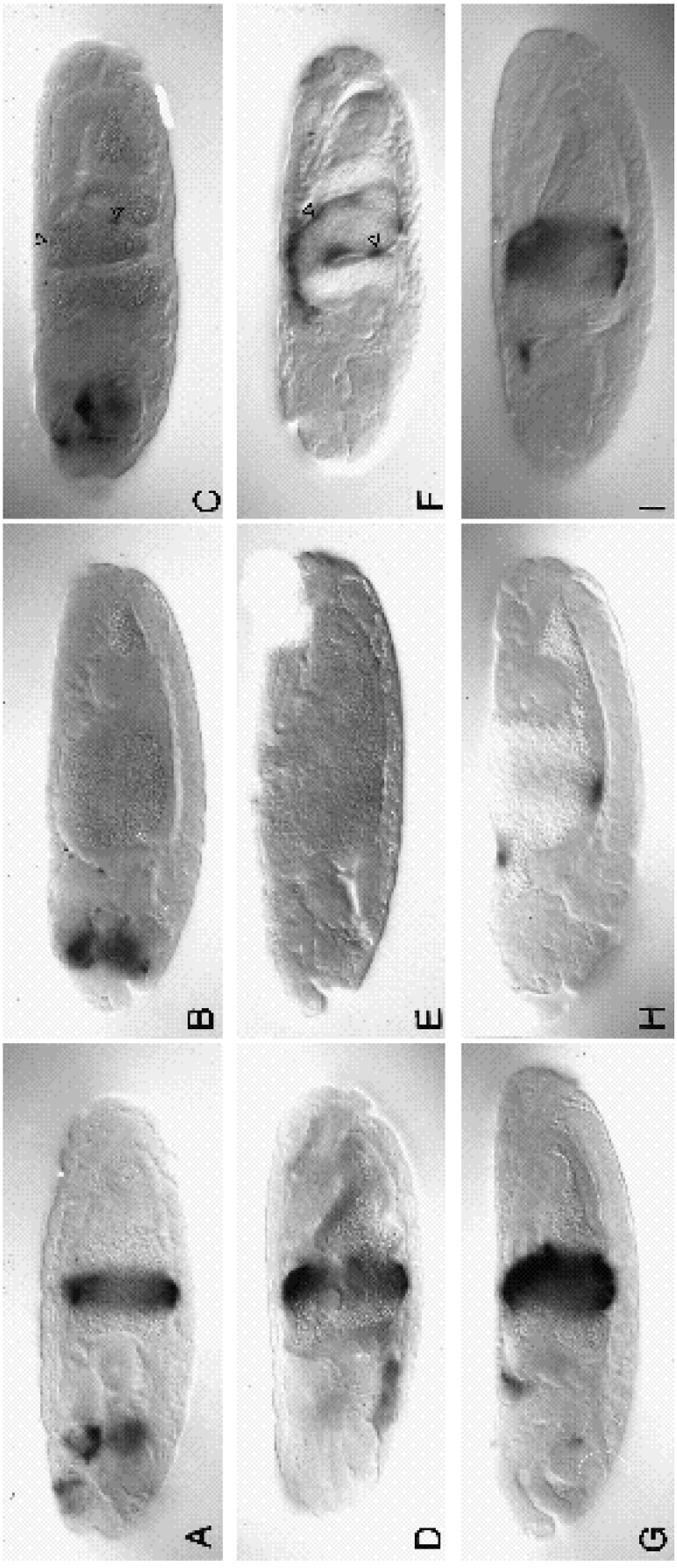

Fig. 4. β-gal expression patterns in *lab* mutants. Wild-type (A, D, G; all early stage 15) or *lab*⁻ embryos at stage 14 (B, E, H), and at early (I) or late stage 15 (C, F), stained for β-gal activity. β-gal staining in –6.3 (A-C) and in HZ1.2 transformants (D-F) is not detectable till very late stages in *lab* mutants (weakly staining cells marked by open triangles in C and F). In HZ550 transformants (G-I), β-gal staining is somewhat reduced in *lab* mutants (compare midgut staining in I and G). Anterior to the left.

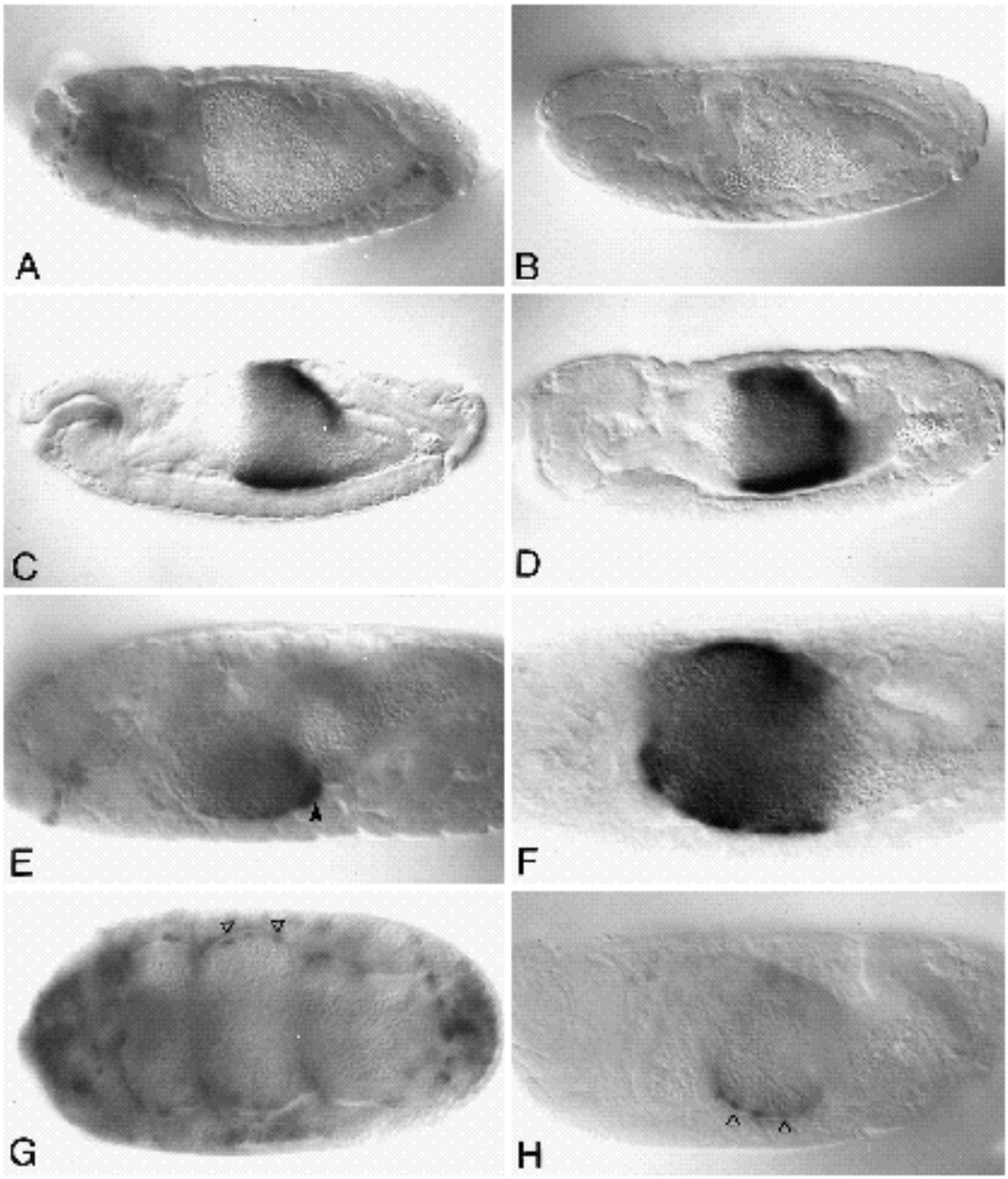

Fig. 5. β-gal expression in other mutants. −6.3 (A, C, E, G) or HZ550 transformants (B, D, F, H) during stage 14 (A-D) or stage 15 (E-H). β-gal staining is undetectable in the midgut of *dpp* mutants (A, B), is derepressed posteriorly in *abd-A* (C, D) or anteriorly in *Pc* mutants (E, F), and is reduced considerably in *wg* mutants (G, H; open triangles point to some of the cells expressing low levels of β-gal protein). Note that β-gal staining is graded only in E (arrowhead indicates cells with strongest β-gal staining). Anterior to the left.

embryos, *lab* protein is partly cytoplasmic, and overall levels of *lab* protein are somewhat reduced, implying a stimulatory role of *wg*. These *wg* effects are particularly apparent in those cells that, in the wild type, express highest levels of *lab* protein (the most posterior ones); as a result, graded expression of *lab* protein appears to be lost. There also seems to be an inhibitory effect of *wg* on *lab* expression: *lab* expression in the midgut is derepressed posteriorly in *wg* mutants (see Fig. 4d in Immerglück et al., 1990) and, thus, the posterior *lab* expression boundary is dependent on *wg* function. The effect of *dpp* on *lab* expression in the midgut is more straightforward: in *dpp* mutant embryos, *lab* expression is not induced at all in the midgut. This led us to suggest that the *dpp* signal is primarily responsible for *lab* induction in the midgut.

We asked whether β-gal expression in the midgut mediated by the different constructs requires the function of *wg* and *dpp*. We expected to see at the minimum some effect in these mutants, as β-gal staining in each case is at least partly dependent on *lab* function (see above). Nevertheless, we reckoned that it might be possible to assess whether such effects reflect a requirement for either of these genes

which is not mediated by *lab* protein: if there was a *lab*-independent requirement, the effect in the corresponding mutant should be more severe than the effect in *lab* mutants, a difference that should be particularly obvious in HZ550 or in HZ255 transformants. We therefore analysed β-gal staining in transformants bearing various different constructs in *wg*⁻ or *dpp*⁻ backgrounds (for crosses and identification of homozygous mutant embryos, see Materials and Methods).

dpp⁻ embryos show no β-gal staining whatsoever, irrespective of the stage or the type of construct they bear (Fig. 5A,B). Notably, this is also true for very advanced HZ550 transformant embryos which, in the absence of *lab* function, show substantial levels of β-gal staining (Fig. 5B, compare to Fig. 4I). Thus, there is a pronounced difference between the effects of *lab* versus *dpp* mutation. This result is most striking in the case of HZ550 transformants whose β-gal expression levels, though the highest among all transformants, are least dependent on endogenous *lab* function. We conclude that β-gal expression in the midgut of all transformants is completely dependent on *dpp* function. The result implies that the relative contributions of *dpp* and *lab* function to the β-gal patterns in the different transformants are not the same: the direct impact of *dpp* function on β-gal expression patterns is slight in −6.3 and HZ1.2 transformants, but strongly manifests itself in HZ550 transformants. The pattern in HZ255 transformants appears to be entirely due to *dpp* function.

We also tested whether β-gal expression mediated by the −6.3 or the HZ550 construct could be induced ectopically in *abdominal-A* (*abd-A*) and in *Polycomb* (*Pc*) mutant embryos, thus under conditions where *dpp* expression in the visceral mesoderm is derepressed (cf. Immerglück et al., 1990). Indeed, in both types of mutant embryos, we found ectopic β-gal staining, posteriorly (*abd-A*⁻ embryos; Fig. 5C,D) or anteriorly (*Pc*⁻ embryos; Fig. 5E,F). Note that, in *Pc*⁻ embryos, the levels of β-gal staining are highest in the most posterior cells in −6.3 (Fig. 5E), although not in HZ550 transformants (Fig. 5F), again underscoring the fact that the HZ550 β-gal pattern follows the pattern of *lab* protein expression to a lesser extent than the −6.3 βgal pattern does. There is no graded β-gal staining in any of the *abd-A*⁻ embryos (Fig. 5C,D) which lack *wg* function (Immerglück et al., 1990). We conclude that both types of construct are capable of mediating β-gal expression in regions of the midgut in which *lab* protein is normally not expressed. Though this could of course reflect endogenous *lab* protein which is derepressed under these conditions (Immerglück et al., 1990), it seems possible that some of the ectopic β-gal staining in HZ550 transformants could be directly due to derepressed *dpp* function.

Finally, we asked whether lack of *wg* function affects any of the β-gal expression patterns. We did not expect the HZ550 pattern to change much in a *wg*⁻ background as this pattern, in the wild type, does not show the features that we attribute to *wg* function (see above). However, we did expect changes in the −6.3 and HZ1.2 patterns, and the question in these cases was whether the changes are more pronounced than the ones attributable to the mutant *lab* protein pattern.

We found that each of the β-gal staining patterns in the midgut is much fainter in *wg*⁻ embryos (Fig. 5G,H) than in the wild type. We never see an anteroposterior gradient of β-gal staining. Furthermore, β-gal staining appears to be derepressed posteriorly as it reaches into the third midgut constriction which normally marks the boundary between ps9 and 10 (Tremml and Bienz, 1989a). All these effects, including the ones on the HZ550 β-gal pattern, mimic the effect of *wg* mutation on *lab* protein expression (see above). It is therefore likely that the effects of *wg* mutation on our β-gal expression patterns are indirect.

Discussion

We have shown that a 1.2 kb fragment of *lab* upstream sequences is necessary and sufficient to confer a *lab*-like expression pattern in the midgut epithelium. This fragment contains target sequences for *lab*-mediated activation, and we shall discuss a potential role of *lab* protein in activating its own expression in the midgut. But first, we shall summarise the evidence that this fragment also contains *dpp*-response sequences which map to a small region (255 bp) within a 550 bp subfragment. Both *lab*- and *dpp*-mediated activation occurs at the transcriptional level.

A dpp *response element*

Of all mutations tested, *dpp* mutation is the only one that shows a more severe effect on β-gal expression patterns than *lab* mutation. Therefore, while the effects of other mutations could be mediated entirely by *lab* protein (see below), at least one component of the *dpp* mutational effects has to be independent of *lab* function. *lab*-independent *dpp* effects are prominent in the case of the 550 bp: all of HZ550-mediated β-gal staining in the midgut requires *dpp* function, but very little of it is due to *lab* function. Finally, the very weak β-gal expression mediated by the 255 bp subfragment probably reflects residual *dpp*-mediated activation which is entirely independent of *lab* function.

Further evidence argues for a *lab*-independent role of *dpp* in activating the 550 bp subfragment. Firstly, the HZ550 construct mediates β-gal expression in the cells immediately preceding the second midgut constriction, i.e. in cells that do not express *lab* protein. This “ectopic” β-gal expression is totally *dpp*-dependent (but not dependent e.g. on *wg* function) and, thus, probably reflects activation by a *dpp*-dependent factor. Secondly, β-gal staining within the HZ550 expression band is not graded, but strong in most cells, apart from those near the margins. Either, cells in the anterior portion of the *lab* domain express higher levels of β-gal than *lab* protein, or cells in the posterior portion express lower levels of β-gal than of *lab* protein. Whatever the case, this indicates weak responsiveness of the 550 bp fragment to *lab* protein, but strong responsiveness to another factor. The latter is completely dependent on *dpp* function.

Taken together, there is strong evidence for a factor distinct from and independent of *lab* protein whose activity is controlled by *dpp*. This factor evidently acts through the 550 bp subfragment to confer most, though not all HZ550-

derived β-gal expression; its target sequences within this fragment probably map to a 255 bp sequence stretch. However, the same factor does not seem active enough on its own to direct much β-gal expression through the large 1.2 kb fragment, perhaps due to increased distance of its binding sites from the TATA-box. Alternatively, its activity or binding to DNA may be prevented by another protein which occupies *lab* target sites in the absence of *lab* function (see below).

It is possible that the putative *dpp*-dependent factor is present in the whole of the endoderm and perhaps in other cells, but that it is only active in those cells that receive a high enough dose of the *dpp* signal. Receptors for TGF-β-like signals in mammalian cells are transmembrane serine/threonine kinases (Mathews and Vale, 1991; Attisano et al., 1992; Lin et al., 1992), and candidates for transcription factors activated by these signals in mammalian cells have been identified (Rossi et al., 1988; Kim et al., 1990; Kramer et al., 1991). Analysis of proteins binding to the 255 bp sequence may lead to the identification of such a factor in *Drosophila*. Alternatively, it is possible that the *dpp* signal locally induces the expression of this putative factor. Indeed, a number of genes other than *lab* are induced by *dpp* in the midgut, one of which is the DFRA gene (our unpublished results), the *Drosophila* homolog of c-*fos* (Perkins et al., 1990). Another gene induced by *dpp* in the visceral mesoderm is *wg* (Immerglück et al., 1990); however, it is very unlikely that *dpp*-induced activity of the putative factor is entirely mediated by *wg* as the effects of *dpp* mutation on β-gal expression in our transformants are more severe than those of *wg* mutation.

lab *autoregulation in the midgut*

As mentioned above, the 1.2 kb *lab* fragment confers high levels of *lab*-dependent β-gal expression in the midgut. The same fragment was shown to mediate *lab*-dependent activation in the head (Chouinard and Kaufman, 1991). As these sequences respond to *lab* protein in different germ layers and at different stages of embryogenesis, it appears likely, though not proved, that the effect of *lab* protein is direct.

Which step does *lab* protein autoregulate? As *dpp* expression decays during late embryonic stages (Panganiban et al., 1990), one might expect *lab* protein to have a self-maintenance function from these late stages onwards. While this may be possible, it cannot be the sole function of *lab* autoregulation: β-gal staining in *lab* mutants does not slowly decay, but instead slowly appears and slightly intensifies during late embryonic stages. Evidently, the strongest effects of *lab* mutation in all transformants are seen at early stages, during or just after induction. We therefore conclude that, in the absence of *lab* function, *dpp*-mediated induction is hardly working (in −6.3 and HZ1.2 transformants) or working with reduced efficiency (in HZ550 and HZ255 transformants). Clearly, *lab* protein functions during or prior to induction.

Induction in the midgut mediated by two independent factors

Previous genetic studies showed that *lab* expression in the midgut is entirely dependent on *dpp* function and somewhat dependent on *wg* function whose expression in the visceral mesoderm in turn is dependent on *dpp* function (Immerglück et al., 1990). Our present results argue for a role of at least two independent factors, a *dpp*-dependent factor and a *lab*-dependent factor, during induction of *lab* expression in the forming midgut epithelium. One might ask whether there is also evidence for an independent role of *wg* during induction.

The β-gal pattern in HZ550 transformants shows several features which are distinct from the patterns in −6.3 and HZ1.2 transformants. These features suggest that the HZ550 construct does not respond to *wg* function, whereas the larger ones apparently do. However, responsiveness to *wg* function parallels the responsiveness of these constructs to *lab* function. Indeed, all effects of *wg* mutation on β-gal patterns can be explained by assuming that these effects are entirely mediated through *lab* protein. It is therefore still an open question whether *wg* acts independently of *lab* and whether it acts at the transcriptional level.

In summary, our results suggest that *lab* induction in the midgut epithelium is conferred by the coordinate action of (at least) two distinct factors. The *dpp*-dependent factor functions very inefficiently in the absence of *lab*, and the *lab*-dependent factor does not seem to function at all on its own, given that most of the predicted *lab* target sites are located within DNA sequences which are completely inactive on their own. It is conceivable that the DNA-binding activity of the *lab*-dependent factor relies on cooperativity provided by the *dpp*-dependent factor. Alternatively, the *lab*-dependent factor itself is modified and thereby activated by the *dpp* signal. Biochemical analysis will be needed to distinguish between these possibilities.

We thank Markus Affolter for discussions, Walter Gehring for the *labial* genomic clones and Ferdi Thüringer for comments on the manuscript. This work was supported by the Swiss National Science Foundation (grant nr. 31-26198.89 to M. B.).

References

Akam, **M**. (1987). The molecular basis for metameric pattern in the *Drosophila* embryo. *Development* **101**, 1-22.

Attisano, **L**., **Wrana**, **J**. **L**., **Cheifetz**, **S**. **and Massagué**, **J**. (1992). Novel activin receptors: distinct genes and alternative mRNA splicing generate a repertoire of serine/threonine kinase receptors. *Cell* **68**, 97-108.

Baker, **N**. (1987). Molecular cloning of sequences from *wingless*, a segment polarity gene of *Drosophila*: the spatial distribution of transcripts in embryos. *EMBO J.* **6**, 1765-1774.

Bienz, **M**., **Saari**, **G**., **Tremml**, **G**., **Müller**, **J**., **Züst**, **B**. **andLawrence**, **P**. **A**. (1988). Differential regulation of *Ultrabithorax* in two germ layers of *Drosophila*. *Cell* **53**, 567-576.

Bienz, **M**. **andTremml**, **G**. (1988). Domain of *Ultrabithorax* expression in *Drosophila* visceral mesoderm from autoregulation and exclusion. *Nature* **333**, 576-578.

Cabrera, **C**. **V**., **Alonso**, **M**. **C**., **Johnston**, **P**., **Phillips**, **R**. **G**. **and Lawrence**, **P**. **A**. (1987). Phenocopies induced with antisense RNA identify the *wingless* gene. *Cell* **50**, 659-663.

Campos-Ortega, **J**. **A**. **and Hartenstein**, **V**. (1985). *The Embryonic Development of* Drosophila melanogaster. Berlin: Springer Verlag.

Casanova, **J**., **Sánchez-Herrero**, **E**., **Busturia**, **A**. **andMorata**, **G**. (1987). Double and triple mutant combinations of the bithorax complex in *Drosophila*. *EMBO J.* **6**, 3101-3109.

Chan, **L**.-**N**. **andGehring**, **W**. **J**. (1971). Determination of blastoderm cells in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **68**, 2217-2221.

Cho, **K**. **W**. **Y**., **Blumberg**, **B**., **Steinbeisser**, **H**. **and De Robertis**, **E**. **M**.

(1991). Molecular nature of Spemann's organizer: the role of the Xenopus homeobox gene *goosecoid*. *Cell* **67**, 1111-1120.

Chouinard, S. and Kaufman, T. C. (1991). Control of expression of the homeotic *labial* (*lab*) locus of *Drosophila melanogaster*: evidence for both positive and negative autogenous regulation. *Development* **113**, 1267-1280.

Diederich, R. J., Merrill, V. K. L., Pultz, M. A. and Kaufman, T. C. (1989). Isolation, structure, and expression of *labial*, a homeotic gene of the Antennapedia complex involved in *Drosophila* head development. *Genes Dev.* **3**, 399-414.

Driever, W., Thoma, G. andNüsslein-Volhard, C. (1989). Determination of spatial domains of zygotic gene expression in the *Drosophila* embryo by the affinity of binding sites for the bicoid morphogen. *Nature* **340**, 363-367.

Harding, K. and Levine, M. (1988). Gap genes define the limits of *Antennapedia* and *bithorax* gene expression during early development of *Drosophila*. *EMBO J.* **7**, 205-214.

Hartenstein, V., Technau, G. M. andCampos-Ortega, J. A. (1985). Fate mapping in wild-type *Drosophila melanogaster*. III. A fate map of the blastoderm. *Wilhelm Roux Arch. Dev. Biol.* **194**, 213-216.

Hiromi, Y. and Gehring, W. J. (1987). Regulation and function of the *Drosophila* segmentation gene *fushi tarazu*. *Cell* **50**, 963-974.

Hooper, J. E. (1986). Homeotic gene function in the muscles of *Drosophila* larvae. *EMBO J.* **5**, 2321-2329.

Immerglück, K., Lawrence, P. A. andBienz, M. (1990). Induction across germ layers in *Drosophila* mediated by a genetic cascade. *Cell* **62**, 261-268.

Irish, V. F., Martinez-Arias, A. andAkam, M. (1989). Spatial regulation of the *Antennapedia* and *Ultrabithorax* homeotic genes during *Drosophila* early development. *EMBO J.* **8**, 1527-1538.

Kim, S.-J., Angel, P., Lafyatis, R., Hattori, K., Kim, K. Y., Sporn, M. B., Karin, M. and Roberts, A. B. (1990). Autoinduction of TGF-β1 is mediated by the AP-1 complex. *Mol. Cell. Biol.* **10**, 1492-1497.

Kramer, I. M., Koornneef, I., de Laat, S. W. and van den Eijnden-van Raaij, A. J. M. (1991). TGF-β1 induces phosphorylation of the cyclic AMP responsive element binding protein in ML-CCI64 cells. *EMBO J.* **10**, 1083-1089.

Lewis, E. B. (1963). Genes and developmental pathways. *Am. Zool.* **3**, 53-56.

Lewis, E. B. (1978). A gene complex controlling segmentation in *Drosophila*. *Nature* **276**, 565-570.

Lin, H. Y., Wang, X.-F., Ng-Eaton, E., Weinberg, R. A. andLodish, H. F. (1992). Expression cloning of the TGF-β type II receptor, a functional transmembrane serine/threonine kinase. *Cell* **68**, 775-785.

Martinez-Arias, A. and Lawrence, P. A. (1985). Parasegments and compartments in the *Drosophila* embryo. *Nature* **313**, 639-642.

Massagué, J. (1990). The transforming growth factor-β family. *Ann. Rev. Cell Biol.* **6**, 597-641.

Mathews, L. S. andVale, W. W. (1991). Expression cloning of an activin receptor, a predicted transmembrane serine kinase. *Cell* **65**, 973-982.

Mlodzik, M., Fjose, A. and Gehring, W. J. (1988). Molecular structure and spatial expression of a homeobox gene from the *labial* region of the Antennapedia-complex. *EMBO J.* **7**, 2569-2578.

Morata, G. and García-Bellido, A. (1976). Developmental analysis of some mutants of the bithorax system of *Drosophila*. *Wilhelm Roux Arch. Dev. Biol.* **179**, 125-143.

Nüsslein-Volhard, C. and Wieschaus, E. (1980). Mutations affecting segment number and polarity in *Drosophila*. *Nature* **287**, 795-801.

Padgett, R. W., St. Johnston, R. D. and Gelbart, W. M. (1987). A transcript from a *Drosophila* pattern gene predicts a protein homologous to the transforming growth factor-β gene family. *Nature* **325**, 81-84.

Panganiban, G. E. F., Reuter, R., Scott, M. P. and Hoffmann, F. M. (1990). A *Drosophila* growth factor homolog, *decapentaplegic*, regulates homeotic gene expression within and across germ layers during midgut morphogenesis. *Development* **110**, 141-150.

Perkins, K. K., Admon, A., Patel, N. and Tjian, R. (1990). The *Drosophila* Fos-related AP-1 protein is a developmentally regulated transcription factor. *Genes Dev.* **4**, 822-834.

Poulson, D. F. (1950). Histogenesis, organogenesis, and differentiation in the embryo of *Drosophila melanogaster Meigen*. In *The Biology of* Drosophila, (ed. M. Demerec), pp.168-274, New York: Hafner.

Qian, S., Capovilla, M. andPirotta, V. (1991). The *bx* region enhancer, a distant *cis*-control element of the *Drosophila Ubx* gene and its regulation by *hunchback* and other segmentation genes. *EMBO J.* **10**, 1415-1425.

Reinitz, J. and Levine, M. (1990). Control of the initiation of homeotic gene expression by the gap genes *giant* and *tailless*. *Dev. Biol.* **140**, 57-72.

Reuter, R., Panganiban, G. E. F., Hoffmann, F. M. and Scott, M. P. (1990). Homeotic genes regulate the expression of putative growth factors in the visceral mesoderm of *Drosophila* embryos. *Development* **110**, 1031-1040.

Rijsewijk, F., Schuerman, M., Wagenaar, E., Parren, P., Weigel, D. and Nusse, R. (1987). The *Drosophila* homolog of the mouse mammary oncogene *int-1* is identical to the segment polarity gene *wingless*. *Cell* **50**, 649-657.

Rosa, F. M. (1989). *Mix.1*, a homeobox mRNA inducible by mesoderm inducers, is expressed mostly in the presumptive endodermal cells of *Xenopus* embryos. *Cell* **57**, 965-974.

Rossi, P., Karsenty, G., Roberts, A. B., Roche, N. S., Sporn, M. B. and de Crombrugghe, B. (1988). A nuclear factor 1 binding site mediates the transcriptional activation of a type I collagen promoter by transforming growth factor-β. *Cell* **52**, 405-414.

Ruiz i Altaba, A. and Melton, B. A. (1989). Interaction between peptide growth factors and homeobox genes in the establishment of anteroposterior polarity in frog embryos. *Nature* **341**, 33-38.

St. Johnston, R. D., Hoffmann, F. M., Blackman, R. K., Segal, D., Grimaila, R., Padgett, R. W., Irick, H. A. and Gelbart, W. M. (1990). *Genes Dev.* **4**, 1114-1127.

Struhl, G., Struhl, K. and Macdonald, P. M. (1989). The gradient morphogen *bicoid* is a concentration-dependent transcriptional activator. *Cell* **57**, 1259-1273.

Taira, M., Jamrich, M., Good, P. J. and Dawid, I. B. (1992). The LIM domain-containing homeo box gene *Xlim-1* is expressed specifically in the organizer region of *Xenopus* gastrula embryos. *Genes Dev.* **6**, 356-366.

Teugels, E. andGhysen, A. (1985). Domains of action of bithorax genes in *Drosophila* central nervous system. *Nature* **314**, 558-561.

Tremml, G. and Bienz, M. (1989a). Homeotic gene expression in the visceral mesoderm of *Drosophila* embryos. *EMBO J.* **8**, 2677-2685.

Tremml, G. andBienz, M. (1989b). An essential function of *even-skipped* for homeotic gene expression in the *Drosophila* visceral mesoderm. *EMBO J.* **8,** 2687-2693.

Wakimoto, B. T. and Kaufman, T. C. (1981). Analysis of larval segmentation in lethal genotypes associated with the *Antennapedia* gene complex in *Drosophila*. *Dev. Biol.* **81**, 51-64.

White, R. A. H. and Lehmann, R. (1986). A gap gene, *hunchback*, regulates the spatial expression of *Ultrabithorax*. *Cell* **47,** 311-321.

Zhang, C.-C. and Bienz, M. (1992). Segmental determination in *Drosophila* conferred by *hunchback*, a direct repressor of the homeotic gene *Ultrabithorax*. *Proc. Natl. Acad. Sci. USA*, in press.

Zhang, C.-C., Müller, J., Hoch, M., Jäckle, H. and Bienz, M. (1991). Target sequences for *hunchback* in a control region conferring *Ultrabithorax* expression boundaries. *Development* **113**, 1171-1179.

(*Accepted 8 July 1992*)