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

GABI TREMML and MARIANN BIENZ

1Zoologisches Institut, Universität Zürich, Winterthurerstr. 190, 8057 Zürich, Switzerland
2MRC 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 (Immergülück 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 lineage 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: labodl (Diederich et al., 1989), dpp+8 (St. Johnston et al., 1990), wgcd (Baker, 1987), abd-AAL (Casanova et al., 1987), Pc- (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 labodl mutation balanced with a TM3 chromosome containing a hs/β-gal 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; Immergülück et al., 1990).

Plasmids

A 6.6 kb BamHI fragment from the lambda phage 23.2 (Mlodzik et al., 1988) was subcloned into a bluscript vector. From this subclone, a 6.3 kb BamHI/SacII fragment (SacII site at +325; Diederich et al., 1989; blunt-ended) was inserted into pl, a modified bluscript vector (obtained from T. Gutjahr; in pl, the original NotI site was destroyed, and a new NotI site was introduced into the original EcoRV site), cut with BamHI and EcoRI (EcoRI 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 Xbal/NotI 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 XbaI and NotI. For the –3.8 construct, a 3.8 kb NotI/HindIII fragment (HindIII site filled-in) from pl1 was inserted into CZ.2 cut with NotI and Xbal (Xbal site filled-in). For the –2.6 kb construct, a 2.6 NotI/EcoRI fragment (EcoRI site filled-in) was inserted into CZ.2 cut with NotI and Xbal (Xbal site filled-in).

For the HZ1.2 construct, a 1.2 kb HindIII/EcoRV fragment from pl1 was inserted, via a subcloning step in bluscript cut with HindIII and SalI (SalI site filled-in), as an Xbal/Kpnl 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 bluscript subclone was cut with Xbal/Kpnl (Kpnl site filled-in), with Kpnl (filled-in) or with Kpnl/ClaI (ClaI site filled-in), respectively, and the corresponding 250 bp, 550 bp or 400 bp fragments were then inserted into HZ50PL cut with NotI and Xbal (NotI site filled-in), with Xbal (filled-in; orientation of insert as in the lab gene) or with Kpnl and Xbal (Xbal site filled-in). For the HZ80 and the HZ255 constructs, the 1.2 kb bluscript subclone was cut with NotI and Aspl or with NotI and Xbal, respectively, all sites were filled-in and in both cases religated; from these religated plasmids, an 80 bp Aspl/ClaI fragment (ClaI site filled-in) or a 255 bp SacII/ClaI fragment (ClaI site filled-in) was inserted into HZ50PL cut with Asp718 and Xbal (Xbal site filled-in) or with SacII and Asp718 (Asp718 site filled-in), respectively. For the HZ220 construct, a 220 bp BstXI fragment (blunt-ended) was inserted, via a subcloning step into a pGem vector cut with EcoRV, as a NotI/EagI fragment (EagI site filled-in) into HZ50PL cut with NotI and Xbal (Xbal 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 (Immergülück 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 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. BamHI; Bs, BstXI; C, ClaI; H, HindIII; N, NarI; R, EcoRI; V, EcoRV; 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).](image)
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 transfectants 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.

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.

Subfragments 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-
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 -G3 (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.
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 on β-gal expression 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 (Matthews 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 transfectants 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 transfectants 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 transfectants). 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 Thürringer for comments on the manuscript. This work was supported by the Swiss National Science Foundation (grant nr. 31-26198.89 to M. B.).

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

Induction of labial expression in the midgut 455


(Accepted 8 July 1992)