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# *Trans*- and *cis*-acting requirements for blastodermal expression of the head gap gene *buttonhead*

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**Abstract**

The *Drosophila* gene *buttonhead* (*btd*) encodes a zinc-finger protein related to the human transcription factor Sp1. *btd* is expressed in the syncytial blastoderm embryo in a stripe covering the anlagen of the antennal, intercalary and mandibular head segments. *btd* has been characterized as a head gap gene, since these segments are deleted in *btd* mutant embryos. We report here that the *cis*-acting elements required for *btd* head stripe expression are contained in a 1 kb DNA fragment, located about 3 kb upstream of the promoter. The four maternal coordinate systems are necessary for correct *btd* head stripe expression, likely by acting through the 1 kb *cis*-acting control region. Expression of the *btd* head stripe depends on the anterior morphogen encoded by the gene *bicoid* (*bcd*). *bcd*-dependent activation also involves the activity of the morphogens of the posterior and dorsoventral systems, hunchback and dorsal, respectively, which act together to control the spatial limits of the expression domain. Finally, the terminal system takes part in the regulation of *btd* head stripe expression by enhancing activation at low levels of activity and repression at high levels of activity.

*Keywords: Drosophila*; Gap gene; *buttonhead*; Gene regulation; Head development

## 1. Introduction

Much of our understanding of the biological process of pattern formation has derived from the combined molecular and genetic analysis of early *Drosophila* development. These studies have defined elaborate cascades of gene interactions which sequentially subdivide the embryo into an array of specialized segments and different types of tissues (reviewed in Ingham, 1988). These cascades are initiated by maternally deposited morphogens which define the spatially restricted domains of zygotic gene expression within a single-layer epithelium, the blastoderm (reviewed in St Johnston and Nüsslein-Volhard, 1992). A single maternal system specifies cell fate along the dorsoventral axis of the embryo by controlling the nuclear localization of the transcription factor encoded by *dorsal* (*dl*). Along the anteroposterior axis, three maternal systems, termed the anterior, the posterior and the terminal system, establish the basic body pattern by asymmetrically distributing the activity of the transcription factors encoded by *bcd*, *hunchback* (*hb*) and the postulated gene *Y*, respectively (St Johnston and Nüsslein-Volhard, 1992). Their activities regulate the localized expression of the zygotic gap genes in distinct domains along the anterior-posterior axis (reviewed in Pankratz and Jäckle, 1993). In the prospective trunk, gap gene activities in turn establish the repetitive patterns of pair rule gene expression, which define the metameric patterns of segment polarity gene expression (Pankratz and Jäckle, 1990; Pankratz et al., 1990; Small et al., 1991; Stanojevic et al., 1991). In addition, the spatial domains of the homeotic selector genes of the Antennapedia and Bithorax complexes are defined to eventually assign segment identity (reviewed in Akam 1987; McGinnis and Krumlauf, 1992).

In contrast to the trunk, which is defined by an array of distinct segments, head segmentation is morphologically obscured. However, four cephalic segments (labral, ocu-

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lar, antennal and intercalary) and the three gnathal segments (mandibular, maxillary and labial) can be assigned on the basis of the metameric patterns of segment polarity gene expression and by internal head sensory organs (Cohen and Jürgens, 1990; Schmidt-Ott and Technau, 1992; Schmidt-Ott et al., 1994). While the maxillary and labial segments are specified in a manner analogous to the trunk segments, neither pair rule genes (Macdonald et al., 1986; Cohen and Jürgens, 1990; Lardelli and Ish-Horowicz, 1993) nor homeotic selector genes are known to play a role for the establishment of the other head segments (reviewed in Cohen and Jürgens, 1991; McGinnis and Krumlauf, 1992; Jürgens and Hartenstein, 1993). Consequently, an alternative mechanism must be invoked to account for segmentation of the anterior head region (Cohen and Jürgens, 1990).

Head and trunk segmentation differs also with respect to the expression patterns of gap genes. While the gap genes acting in the trunk region, such as *hb*, *Krüppel* (*Kr*) or *knirps* (*kni*), are expressed in adjacent stripe domains with relatively small overlaps (reviewed in Pankratz and Jäckle, 1993), the corresponding expression domains of the head gap genes *orthodenticle* (*otd*), *empty spiracles* (*ems*) and *btd* are broadly overlapping (Dalton et al., 1989; Finkelstein and Perrimon, 1990; Walldorf and Gehring, 1992; Wimmer et al., 1993). Phenotypic analyses of mutations in the head gap genes indicate that each of them is required for the formation of a contiguous block of two or three head segments. The domains of action of the three genes overlap, but their posterior margins are out of phase by one segment (Cohen and Jürgens, 1990; Schmidt-Ott et al., 1994). Taken together with the lack of pair rule-like genes acting in the head region, the phasing of the segmental deletions in the head gap gene mutants suggests that these genes might be responsible for segmentation of the head (Cohen and Jürgens, 1990). In addition, it had been proposed that the head gap genes might act in a combinatorial manner to specify the identity of segments. In this view the head gap genes also represent a functional equivalent of the homeotic selector genes which act in the trunk region (Cohen and Jürgens, 1990).

While *otd* and *ems* encode homeodomain-containing transcription factors (Dalton et al., 1989; Finkelstein et al., 1990; Walldorf and Gehring, 1992), *btd* encodes a zinc-finger protein related to the human transcription factor Sp1 (Wimmer et al., 1993). *btd* transcripts are first expressed in a stripe domain in the syncytial blastoderm embryo. This domain covers the anlagen of the antennal, intercalary and mandibular segments, which fail to develop in *btd* mutant embryos (Wimmer et al., 1993).

Here we show that *btd* head stripe expression requires the activity of the four maternal coordinate systems. Activation of blastodermal *btd* expression is strictly dependent on the anterior morphogen encoded by the gene *bcd*. The morphogens of the posterior and dorsoventral systems, hunchback and dorsal, are required for the correct spatial expression of *btd*, probably by acting as co-activators. Our data also suggest that the terminal system affects *btd* expression in a complex manner; it differentially enhances and represses activation depending on the level of activity. The *cis*-acting control region of the *btd* locus which mediates expression in the head stripe domain is contained within a 1 kb DNA fragment. It contains potential in vitro binding sites for bicoid, hunchback, dorsal and the terminal gap gene product tailless, suggesting that the control of *btd* head stripe expression involves their direct interaction with the 1 kb *cis*-acting control region.

## 2. Results

### 2.1. Maternal control of buttonhead expression

*btd* expression is first detected in the syncytial blastoderm forming a head stripe between 65–77% egg length (Fig. 1c; 100% is the anterior tip of the egg). During cellularization, the head stripe expression domain narrows dorsally and forms a wedge shape. At the cellular blastoderm stage, a dorsal 'head spot' appears in a more anterior region of the embryo (Fig. 1h). The head stripe expression domain covers the anlagen of the segments affected in *btd* mutants, whereas the *btd* head spot expression domain cannot be correlated with phenotypic effects observed in *btd* mutants (Wimmer et al., 1993).

Embryos derived from females homozygous mutant for the gene *bcd* lack head and thoracic segments (Frohnhöfer and Nüsslein-Volhard, 1986). In such embryos, the blastoderm expression domains of *btd* are absent. Thus bicoid is required for the initial transcriptional activation of *btd*. We next asked whether *bcd* activation and the spatial limits of *btd* expression depend on the level of *bcd* activity. For this, we examined the *btd* expression patterns in embryos from females with increasing copy numbers of the *bcd* gene (1, 2, 4 or 6), which result in proportional variations of the bicoid morphogen gradient in the embryos (Driever and Nüsslein-Volhard, 1988b). The *btd* head stripe and the head spot were shifted proportionally towards posterior when the bicoid concentration in the embryo was increased (Fig. 1). This observation suggests that *btd* activation depends on critical threshold levels of bicoid concentration which affects both the anterior and the posterior borders of the head stripe expression domain.

Embryos lacking *hb* activity fail to develop thorax and head structures (Lehmann and Nüsslein-Volhard, 1987). *hb* activity is maternally controlled by the posterior system and zygotically by the anterior system (Tautz, 1988). In order to eliminate *hb* activity from embryos, we used a molecular genetic system which generates embryos lacking both maternal and zygotic *hb* activities (Fig. 2; Simpson-Brose et al., 1994). In such embryos, the expression of *btd* is restricted to a small ventral spot in the anterior region of the embryo (Fig. 2c). This spot fades during the

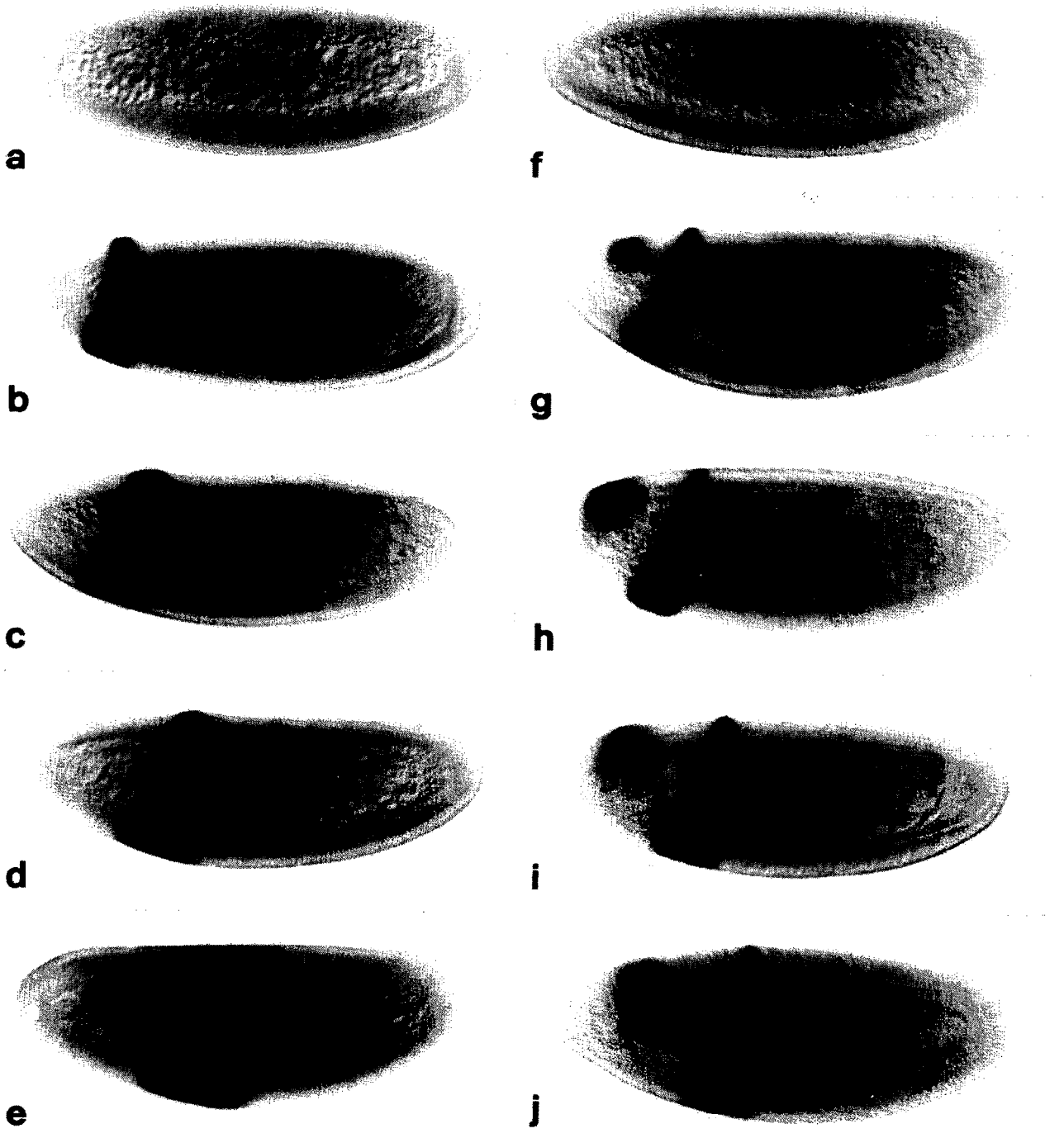


Fig. 1. *bcd* dependence of the blastodermal expression pattern of *btd*. In situ hybridization with *btd* cDNA 5–2. (a–e) Syncytial blastoderm. (f–j) Cellularisation or cellular blastoderm. (a,f) Embryos derived from mothers homozygous for $bcd^{E1}$. (b,g) Embryos derived from mothers heterozygous for $bcd^{E1}$: one copy of *bcd* in maternal genome. (c,h) Wildtype embryos: two copies of *bcd* in maternal genome. (d,i) Embryos derived from a multicopy *bcd* stock: four copies of *bcd* in maternal genome. (e,j) Six copies of *bcd* in maternal genome. The activity of the maternal organiser gene *bcd* is necessary for the activation of the blastodermal *btd* expression (*btd* head stripe and dorsal spot). The anterior as well as the posterior borders of the *btd* head stripe are depending on bicoid concentration.

cellular blastoderm stage (Fig. 2f) when *btd* continues with strong expression in the head stripe region of wildtype embryos. These observations indicate that in addition to bicoid, *hb* activity is also strictly required for *btd* expression.

In embryos derived from females homozygous mutant for the gene *torso* (*tor*), a key component for the terminal system (Klingler et al., 1988), the *btd* head stripe expands slightly towards anterior and the head spot cannot be detected (Fig. 3a). The terminal system therefore has two

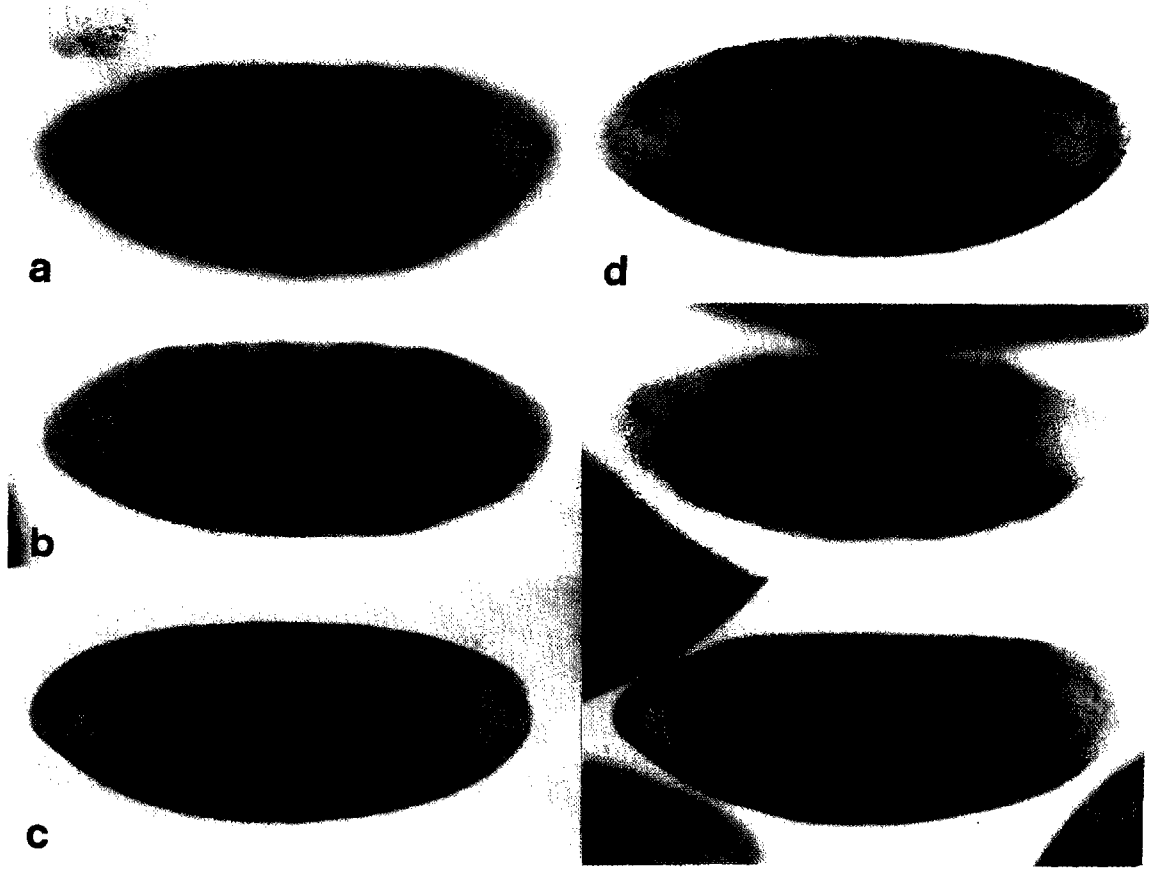


Fig. 2. Dependence of *btd* expression on *hb* activity. All embryos shown lack the maternal contribution of *hb* activity due to the BBNH system (Simpson-Brose et al., 1994). Embryos were double stained using a *btd* RNA probe (blue) and anti-*even skipped* antibodies (brown) to identify the zygotic *hb* genotype. (a–c) Early blastoderm stages. (d–f) Late blastoderm stages. (a,d) Embryos with two wildtype copies of zygotic *hb*: *btd* expression pattern slightly shifted towards the anterior probably due to the reduced *bcd* activity in BBNH-derived embryos. (b,e) Embryos with one wildtype copy of zygotic *hb*. (c,f) Embryos lacking *hb* activity. *btd* expression is reduced to a small transient ventral spot (c), which fades away before the late blastoderm stage (f).

distinct functions. It helps to establish the anterior limit of *btd* expression to form the head stripe domain, and it is needed for the activation of *btd* in the head spot domain. Ectopic activity of the terminal system, generated by the dominant gain-of-function allele $tor^{4021}$, shifts the *btd* head stripe domain towards posterior and enlarges the head spot (Fig. 3b–d). The posterior shift of the head stripe resembles the *btd* expression pattern in embryos derived from females containing four copies of *bcd* in the maternal genome (Fig. 1i). In some of these embryos the head stripe is dorsally and lateroventrally interrupted (Fig. 3c) while others lack the head stripe expression domain completely (Fig. 3d). The different effects of ectopic *tor* activity due to the gain-of-function allele $tor^{4021}$ may reflect variable *tor* activity. These results indicate that high activity of the terminal system is acting negatively on *btd* head stripe expression. However, since the expression of *btd* does not expand to the anterior tip in the absence of *tor* activity, the terminal system cannot be the only source of repression in the anterior-most region of wild type embryos.

Since both the head stripe expression domain and the head spot display dorsoventral asymmetries, we examined *btd* expression in embryos derived from females mutant for *dl*. Embryos lacking *dl* activity fail to develop ventral structures (Nüsslein-Volhard et al., 1987). In such embryos, *btd* is expressed in a thin head stripe domain which is shifted anteriorly (Fig. 3e). Its width resembles the extent of the head stripe on its dorsal side and no dorsoventral asymmetry can be observed. Similarly, the head spot shifts towards the anterior pole and expands symmetrically to the ventral side. In embryos with ubiquitous *dl* activity which derived from females containing a dominant gain-of-function allele of the gene *Toll* (Anderson et al., 1985), *btd* is expressed in a broad, symmetrical head stripe and the width of this expression domain corresponds to the ventral extent of the *btd* head stripe. The head spot is absent in such embryos (Fig. 3f). These results suggest that the dorsoventral asymmetry displayed by the *btd* head stripe in wild type embryos involves *dl*-dependent *btd* activation on the ventral side. Furthermore, these results suggest that the head spot is spatially re-

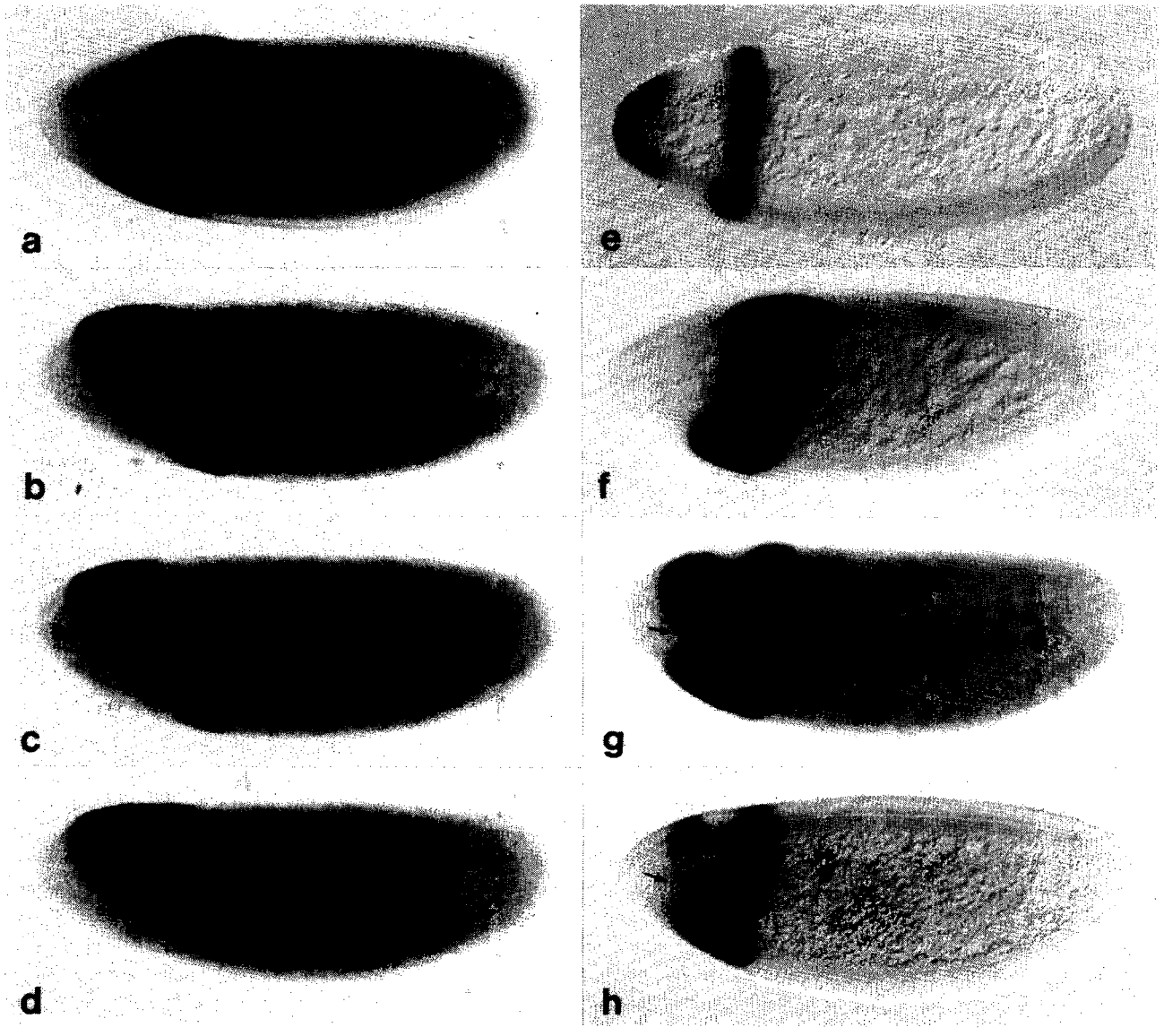


Fig. 3. Dependence of *btd* expression on the terminal and dorsoventral systems. In situ hybridization with *btd* cDNA 5–2. (a) Embryo derived from mother homozygous mutant for $tor^{PM}$: the *btd* head stripe expands towards anterior, but does not extend to the anterior pole. The dorsal spot is not formed. (b–d) Embryos derived from mothers carrying the gain of function allele $tor^{4021}$: expression in the *btd* head stripe varies, dorsal spot expression is increased. (b,c) *btd* head stripe is shifted towards posterior. (c) *btd* head stripe is dorsally and ventrolaterally repressed. (d) *btd* head stripe is not formed. (e) Embryo derived from mother homozygous mutant for $dl^{15}$; the *btd* head stripe is shifted towards the anterior and reduced to a thin band, the dorsal spot is shifted to the anterior pole and extends to the ventral side. (f) Embryo derived from mother carrying the gain of function allele $Toll^{9Q}$; *btd* head stripe is expressed as a wide band around the embryo, dorsal spot is not formed. (g) Embryo homozygous mutant for $tll^{g}$. (h) Embryo homozygous mutant for $hkb^{2}/tll^{g}$. (g,h) *btd* head stripe expands towards anterior, forming a kind of 'horn' laterally (arrow).

stricted through *dl* activity, preventing its activation on the ventral side of the embryo.

### 2.2. Zygotic control of buttonhead expression

To examine whether zygotic segmentation genes might regulate *btd* activation, we examined *btd* expression in embryos mutant for zygotic segmentation genes including *otd*, *ems*, *giant*, *hb* (zygotic), *Kr*, *runt*, *spalt* and *teashirt* and in embryos mutant for zygotic genes involved in dorsoventral patterning, such as *snail*, *twist* and *zerknüllt*. None of these genes show any influence on the pattern of *btd* expression (data not shown). Double mutants lacking maternal *tor* and zygotic *otd* activities show only a slight anterior expansion as observed with embryos lacking *tor* activity (see above). These results indicate that the absence of any of these zygotic gene activities does not by itself affect *btd* expression. However, since further combinations of double or triple mutations were not examined, redundant effects can not be excluded. These observations suggest that the regulatory input from the

maternal activators bicoid, hunchback and dorsal may be direct.

We next asked whether the zygotic gap genes of the terminal system, *tailless* (*tll*) and *huckebein* (*hkb*), mediate *tor*-dependent repression of *btd*. In *tll* mutant embryos or *tll/hkb* double mutant embryos the head stripe expands anteriorly (Fig. 3g,h). The expanded *btd* expression domain resembles roughly the pattern of embryos lacking *tor* activity. However, the expanded expression domain displays dorsoventral asymmetry and leads to the formation of a lateral 'horn' which points towards the anterior (Fig. 3h). This unusual expression pattern may reflect complex interactions of the different maternal systems in the head region which are beyond our current understanding. The head spot is not affected by mutations in the terminal gap genes. The genetic scenario concerning maternal factors required for the activation and for regulation of the spatial limits of the *btd* head stripe domain are summarized in Fig. 4.

### 2.3. *Cis-acting control region of the buttonhead locus*

A transgene containing a 10.5 kb genomic DNA fragment of the *btd* locus was previously shown to rescue *btd* mutants (Wimmer et al., 1993). This fragment therefore contains *cis*-acting control elements sufficient for the correct spatial and temporal expression of *btd*. We inserted the 5.2 kb genomic upstream region of the rescuing transgene, reaching from the EcoRV site to the second BamHI site (*btd* RV-2ndB; Fig. 5) into the promoterless P-element vector CHABΔSal (Wimmer et al., 1993). Reporter gene expression in embryos carrying this transgene construct show that the 5.2 kb genomic fragment contains the *cis*-acting control region sufficient to mediate head stripe expression and the *btd* promotor (Figs. 5, 6a,d).

To further delimit the control region, subfragments of the RV-2ndB DNA fragment were inserted into the P-element vector pCaSpeR/hs/43/AUG/β/gal (CHAB; Thummel and Pirotta, 1992). The resulting constructs use the hsp43 basal promoter. Fig. 5 shows a summary of the blastodermal reporter gene expression patterns directed by the fragments examined.

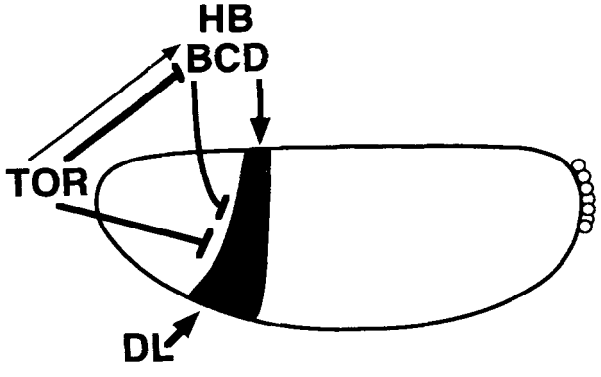


Fig. 4. Schematic representation of the maternal transacting factor requirement for blastodermal headstripe expression of the gene *btd*. Arrows represent activating activities, bars repressing activities of the indicated factors. For details, see text.

The 1080 bp Ss-Bg fragment (Fig. 5) was the shortest fragment directing the correct pattern of head stripe expression (Fig. 6b,e). Truncations from its 3′ region resulted in reporter gene expression in the head stripe (Ss-Ns, Fig. 5) which expanded posteriorly, without forming the normally sharp border of expression (Fig. 6c,f). This suggests that the 296 bp Ss-Ns fragment contains the sequences which are necessary for activation of gene expression. The 790 bp Ns-Bg fragment does not mediate reporter gene expression by itself (Fig. 5), but apparently contains elements necessary for setting the posterior border of the expression domain in the context of a larger fragment. DNA sequence analysis of the Ss-Bg fragment revealed several potential binding sites for bicoid, dorsal, hunchback and tailless (Fig. 7). The specific functions of these binding sites have not yet been examined.

## 3. Discussion

We provide evidence that *btd* expression is regulated by the four maternal organizer systems which control body pattern formation: the anterior morphogens bicoid and hunchback (Frohnhöfer and Nüsslein-Volhard, 1986; Hülskamp et al., 1990; Struhl et al., 1992; Simpson-Brose et al., 1994), the dorsal morphogen, and the terminal system (reviewed in St Johnston and Nüsslein-Volhard, 1992).

Bicoid forms a stable concentration gradient which is thought to specify positional information (Driever and Nüsslein-Volhard, 1988a,b) for the activation of zygotic target genes such as *hb* (Tautz et al., 1987; Schröder et al., 1988; Driever and Nüsslein-Volhard, 1989; Struhl et al., 1989). Since embryos lack more head segments in the absence of *bcd* activity than in the absence of zygotic *hb* activity, one or several additional zygotic segmentation gene(s) acting anteriorly to the domain of the *hb* activity were proposed (gene *X*; Driever et al., 1989). Both the expression patterns and the functional requirements of the head gap genes *otd*, *ems* and *btd* (Dalton et al., 1989; Cohen and Jürgens, 1990; Finkelstein and Perrimon, 1990; Walldorf and Gehring, 1992; Wimmer et al., 1993) are consistent with the argument that they function in the proposed gene *X*-like manner. However, none of them has been shown to be directly regulated by bicoid. Our analysis of the genetic requirement for the regulation of *btd* head stripe expression demonstrates that *bcd* is required for the activation of *btd* and that its regulatory input on *btd* expression is not mediated by the known zygotic target genes of bicoid. The concentration-dependent response and the presence of several potential *bcd* in vitro binding sites within the *cis*-regulatory region of the *btd* gene provide circumstantial evidence that bicoid is the direct activator of *btd* expression.

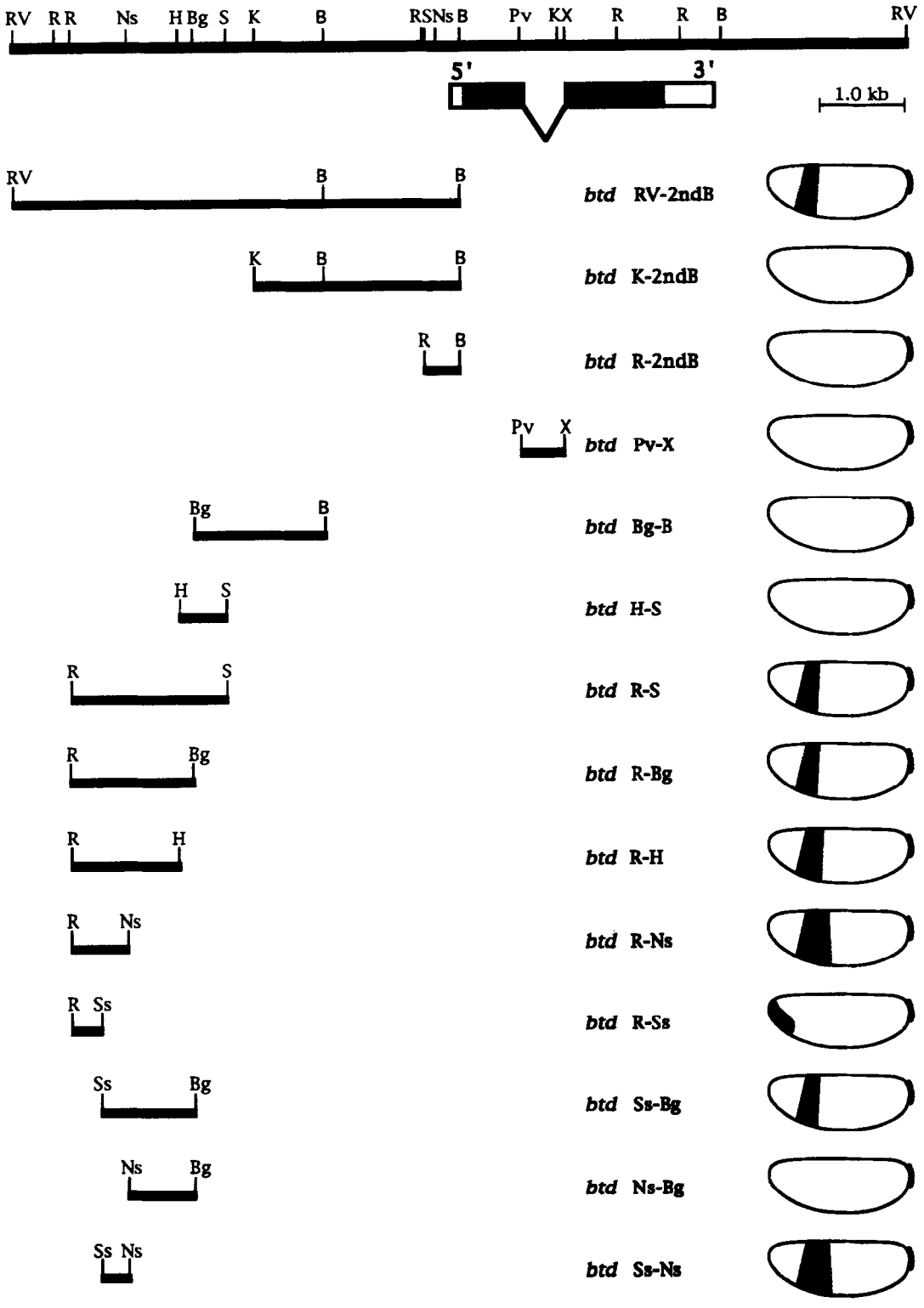


Fig. 5. Schematic representation of the analysis of *btd cis*-regulatory elements. The blastodermal expression patterns mediated by the different constructs are schematically depicted on the right. Restriction sites: B, BamHI; Bg, BglII; H, HindIII; K, KpnI; Ns, NsiI; Pv, PvuII; R, EcoRI; RV, EcoRV; S, SalI; Ss, SspI; X, XbaI.

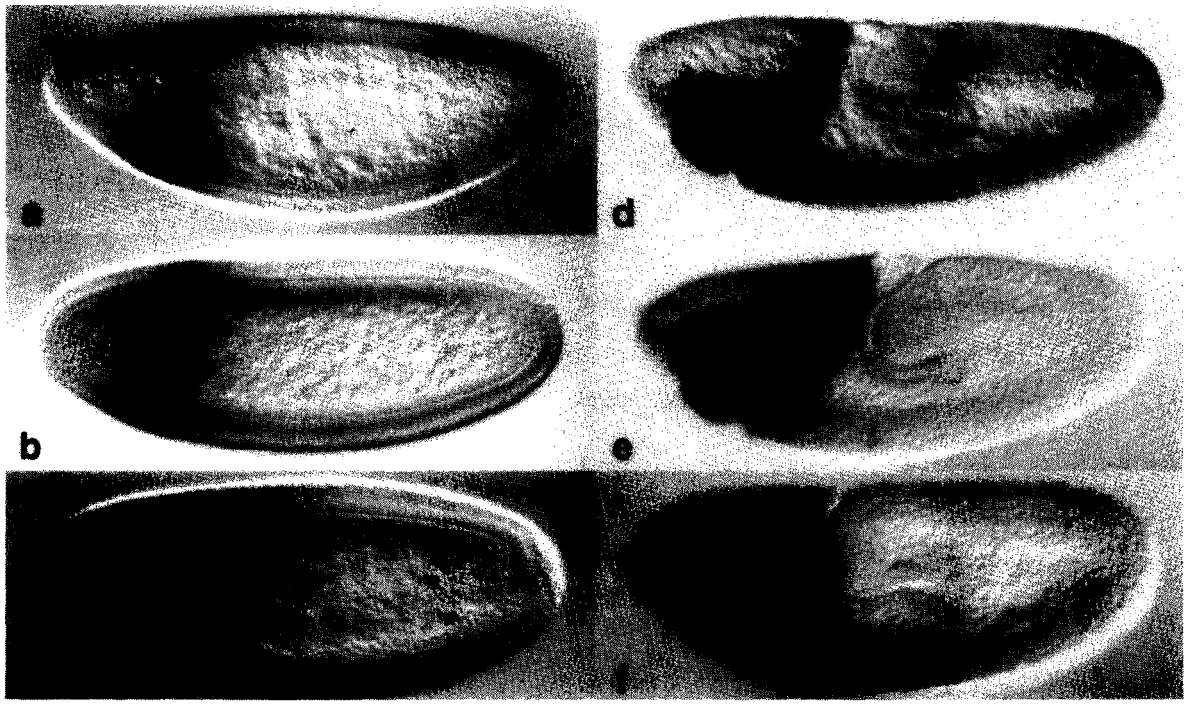


Fig. 6. Expression patterns of *btd* reporter gene constructs. Antibody stainings with anti-β-galactosidase antibodies. (a–c) Blastoderm stage. (d–f) Early germ band extension stage (difference in posterior border of expression more clearly detectable due to the perdurance of β-galactosidase). (a,d) *btd* RV-2ndB, mediates *btd* blastoderm expression pattern carrying *btd*-homologous promoter; (b,e) *btd* Ss-Bg, 1080 bp fragment mediates *btd* blastoderm expression in combination with heterologous promoter; (c,f) *btd* Ss-Ns, 296 bp fragment mediates activation in region of *btd* head stripe without sharp posterior border leading to a posterior expansion of the expression domain.

Bicoid is supposed to act as the primary morphogen in anterior pattern formation by binding to sites of different affinity in the promoters of different target genes (Driever et al., 1989; Struhl et al., 1989). However, high affinity bicoid binding sites alone are not sufficient to provide gene expression within the correct spatial limits of the *hb* expression domain, but rather mediate gene expression restricted to the peak levels of bicoid in the anterior pole region (Ronchi et al., 1993). The addition of hunchback binding sites to the high affinity bicoid binding sites results in a posteriorly expanded domain of gene expression. A promoter containing bicoid and hunchback binding sites fails to mediate gene expression in the absence of bicoid activity and hunchback binding sites alone fail to mediate gene activation as well. This suggests that the spatial limit of the zygotic *hb* expression domain is generated by a mechanism involving a synergistic interaction between hunchback and bicoid (Simpson-Brose et al., 1994). Thus, hunchback is required to sense the correct position along the anterior-posterior axis of the embryos above which zygotic *hb* expression is activated in response to bicoid. The results showing dependence of the *btd* head stripe expression on *bcd* and *hb* activities suggest that *btd* is regulated in a similar way. However, the molecular genetic system used to eliminate *hb* activity from the embryo might have additional effects, such as a shift of posteriorly active segmentation genes towards the anterior. Their activity may repress *btd* expression and the apparent lack of activation could in fact be due to repression by genes which are normally repressed by hunchback.

An interaction of bicoid and dorsal might be responsible for the control of the dorsoventral asymmetry of *btd* head stripe expression. Initially, the three head gap genes *otd*, *ems* and *btd* are expressed in symmetrically arranged circumferential head stripes. While *otd* and *ems* become ventrally repressed during cellularization (Dalton et al., 1989; Finkelstein and Perrimon, 1990; Walldorf and Gehring, 1992), the *btd* head stripe expression domain maintains its width ventrally, but narrows dorsally (Fig. 1c,h; Wimmer et al., 1993). Therefore, the regulatory input of dorsal on *otd* and *ems* must be different from its input on *btd* expression. In fact, dorsal has been shown to act as a repressor and as an activator of transcription depending on the context of the promotor sequences (reviewed in Ip and Levine, 1992). By interaction with DNA-bound co-repressors, dorsal can be turned into a repressor (Huang et al., 1993; Jiang et al., 1993; Kirov et al., 1993) which may explain the ventral retraction of the *otd* and *ems* expression domains.


Fig. 7. DNA sequence of the *btd cis*-acting control region (1800 nucleotides of the EcoRI-SalI fragment; see Fig. 5 for restriction sites). Potential in vitro binding sites for bicoid (Driever and Nüsslein-Volhard, 1989), dorsal (Lenardo and Baltimore, 1989), hunchback (Treisman and Desplan, 1989) and tailless (Hoch et al., 1992) are underlined and labelled by BCD, DL, HB and TLL, respectively. Potential high-affinity binding sites for bicoid are indicated in bold letters.

In the case of *btd* expression, dorsal does not act as a repressor, nor is *dl* activity sufficient to provide *btd* activation in the absence of *bcd* activity. Our data are therefore consistent with a model in which dorsal supports bicoid in activating *btd* expression similar to the proposed role of hunchback in setting a spatial limit for the activation in response to the bicoid gradient (see above). The requirement of combined bicoid and dorsal activities for gene activation has previously been shown for the anterior cap domains of the gene pair knirps and knirps-related (Rothe et al., 1994). Furthermore, it has been reported that the terminal and dorsoventral signalling pathways are able to combine their activities. In the pole regions of the embryo, dorsal looses its repressing function due to *tor* activity, while its function as transcriptional activator is not affected (Rusch and Levine, 1994). This pole-specific interplay between the two organizer systems causes a unique situation so that the zygotic target genes which are activated by dorsal on the ventral side and the target genes which are repressed by dorsal at the ventral side can be co-expressed in the pole regions.

The mode of regulation of the anteriorly acting gap genes *hb*, *otd*, *ems* and *btd* by the terminal system is different for *hb* and *otd* and for *ems* and *btd*, respectively. *otd* and *hb* are initially expressed in an anterior cap; their expression domains subsequently retract from the anterior pole in response to local torso receptor tyrosine kinase activity (Tautz, 1988; Finkelstein and Perrimon, 1990). The apparent repression at the anterior pole is probably not due to a *tor*-dependent repressor but rather depends on a *tor*-dependent inactivation of bicoid (Ronchi et al., 1993). In contrast, *ems* and *btd* expression is initiated in distinct stripes which expand only slightly in the absence of the terminal organizer activity (Fig. 3a; not shown for *ems*). Since the expansion occurs in *tll* mutant embryos, it is likely that tailless acts as a *tor*-dependent repressor of *btd* expression, consistent with the presence of potential binding sites for tailless within the *cis*-acting region of

*btd*. However, the comparatively weak anterior expansion of the expression pattern suggests that the major anterior repression is provided through the anterior system itself. Alterations of the bicoid gradient affect mainly the posterior limit of the *otd* expression domain (Finkelstein and Perrimon, 1990), whereas in the case of both *ems* and *btd*, the sizes of the head stripe domains are maintained but the stripes are shifted (Fig. 1; Dalton et al., 1989; Walldorf and Gehring, 1992). This suggests that the bicoid concentration may not only determine the position of the posterior border of expression by providing activator function but may also limit the expression domains anteriorly through repression.

The variable patterns of *btd* expression in embryos derived from females containing a gain-of-function allele of *tor* suggest that, although the terminal system may add only little to the regulation of the *btd* head stripe expression domain, its activity could nevertheless affect the expression pattern through bicoid severely. Repression of the head stripe expression as seen in a fraction of the embryos can be explained by the inactivation of bicoid function through ectopic *tor* activity (Ronchi et al., 1993). However, the shift of the *btd* head stripe domain to a position resembling the situation of increased bicoid concentration indicates that activated torso can also potentiate bicoid-dependent activation as has been proposed for *sloppy paired 1* gene expression in the head region of the embryo (Grossniklaus et al., 1994).

Our observations suggest that bicoid might require the presence of coactivators to account for the observed pattern of *btd* expression. bicoid and hunchback may synergistically interact to provide the posterior limit of the *btd* head stripe expression, as described for zygotic *hb* expression (Simpson-Brose et al., 1994). The terminal system is likely to provide two different inputs. At low levels of activity the terminal system supports bicoid by enhancing its activator function, while at high levels of activity in the anterior pole region it represses *btd* expression presumably through activation of *tll* and inactivation of *bcd* activity. The finding of bicoid, hunchback, tailless and dorsal binding sites within the *cis*-acting *btd* control region suggests inputs from these *trans*-acting transcription factors, although direct action of these factors remains to be shown.

## 4. Materials and methods

### *4.1. Fly stocks*

Oregon R was used as a wildtype strain. The different mutant fly lines we used are described in Lindsley and Zimm (1992): *bcd*$^{E1}$, *dl*$^{15}$, *ems*$^{9H}$, *ems*$^{9Q}$, *giant*[Df(1)-62g18], *hb*$^{7M48}$, *hkb*$^{2}$/*tll*$^{g}$, *Krüppel*, *otd*$^{YH}$, *otd*[Df(1)-KA14], *runt*$^{LB5}$, *snail*$^{IG}$, *spalt*$^{16}$, *teashirt*$^{8}$, *Toll*$^{9Q}$, *tll*$^{g}$, *tor*$^{4021}$, *tor*$^{PM}$, *twist*$^{ID96}$, *zerknüllt*$^{W36}$. An *otd*; *tor* (*otd*$^{YH}$/FM6; *tor*$^{PM}$/CyO) double mutant stock was generated by crossing to double balancer stocks. Embryos lacking maternal *hb* activity were generated using the BBNH system described in Simpson-Brose et al. (1994). Females containing additional copies of *bcd* were obtained from the strain bcd$^{+5}$bcd$^{+8}$/FM7; +/+ (Driever and Nüsslein-Volhard, 1988b).

### *4.2. Reporter gene constructs and generation of transgenic fly lines*

The constructs *btd* RV-2ndB, *btd* K-2ndB and *btd* R-2ndB were cloned into the promoterless vector CHABΔSal (Wimmer et al., 1993). The constructs *btd* Pv-X, *btd* Bg-B, *btd* H-S, *btd* R-S, *btd* R-Bg, *btd* R-H, *btd* R-Ns, *btd* R-Ss, *btd* Ss-Bg, *btd* Ns-Bg and *btd* Ss-Ns were cloned into CHAB (pCaSpeR/hs/43/AUG/β/gal; Thummel and Pirrotta, 1992). The restriction sites are indicated in the legend to Fig. 5 and a detailed description of the clonings is given in Wimmer (1995). To generate transgenic fly lines the constructs were injected together with the helper plasmid pΔ2–3 (Laski et al., 1986) into embryos of strain Df(1)/*w67c23,y* (Rubin and Spradling, 1982). For each construct, at least three independent fly lines were analysed.

### *4.3. mRNA and protein detection*

In situ hybridization to whole mount preparations of embryos was performed as described by Tautz and Pfeifle (1989). Antibody staining with anti-β-galactosidase antibodies (Cappel) to whole mount embryos was carried out as described by Macdonald and Struhl (1986) using the Vectastain ABC Elite horseradish peroxidase system. Double stainings (Fig. 2) were done as described in Simpson-Brose et al. (1994) using anti-even skipped antibodies and a *btd* RNA in situ probe. Embryos were identified by either the genotype of the mother, by double staining, or by alterations of the expression pattern in a quarter of the embryos.

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