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The *bicoid* Protein Determines Position in the Drosophila Embryo in a Concentration-Dependent Manner

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Summary

The *bicoid* (*bcd*) protein in a Drosophila embryo is derived from an anteriorly localized mRNA and comes to be distributed in an exponential concentration gradient along the anteroposterior axis. To determine whether the levels of *bcd* protein are directly related to certain cell fates, we manipulated the density and distribution of *bcd* mRNA by genetic means, measured the resultant alterations in height and shape of the *bcd* protein gradient, and correlated the gradient with the fate map of the respective embryos. Increases or decreases in *bcd* protein levels in a given region of the embryo cause a corresponding posterior or anterior shift of anterior anlagen in the embryo. The *bcd* protein thus has the properties of a morphogen that autonomously determines positions in the anterior half of the embryo.

Introduction

The polarity and pattern of the Drosophila embryo are determined by a small number of maternal effect genes. By their phenotypes, three groups of genes may be distinguished that define the anteroposterior pattern in largely nonoverlapping domains: the anterior (head and thorax), the posterior (abdomen), and the terminal (acron and telson) regions (Nüsslein-Volhard et al., 1987). The genes *bicoid* (*bcd*), *exuperantia* (*exu*), and *swallow* (*swa*) are required for the anterior segmented pattern of head and thorax (Frohnhöfer and Nüsslein-Volhard, 1986, 1987; Schüpbach und Wieschaus, 1986; Stephenson and Mahowald, 1987). Several lines of evidence suggest that it is the *bcd* gene product that determines anterior pattern. *bcd* codes for an mRNA localized at the anterior tip of the oocyte and early embryo (Frigerio et al., 1986; Berleth et al., 1988). Variations in the copy number of the wild-type bcd^+ gene cause corresponding shifts of anterior pattern elements along the anteroposterior egg axis (Frohnhöfer and Nüsslein-Volhard, 1986, 1987; Berleth et al., 1988). Cytoplasmic transplantation experiments reveal a long-range organizing effect of bcd^+ activity on the anteroposterior pattern (Frohnhöfer et al., 1987). The amount of transplantable bcd^+ activity required to rescue bcd^- mutant embryos is dependent on the number of bcd^+ copies in the donor females, suggesting that the rescuing capacity of bcd^+ is directly releated to the level of *bcd* mRNA present in the donor embryos. We have demonstrated in the accompanying paper that the localized *bcd* mRNA serves as a source for a *bcd* protein gradient which is established in early embryogenesis. The gradient is of exponential shape and spans the anterior two-thirds of the egg's length (Driever and Nüsslein-Volhard, 1988).

To assess a correlation between position on the fate map and *bcd* protein concentration, we measured the *bcd* protein distribution (Driever and Nüsslein-Volhard, 1988) in embryos from females homozygous for mutations affecting anterior development, as well as in embryos from females with one to four copies of the bcd^+ gene. We observed a strong correlation between *bcd* protein concentration and the positions of anterior anlagen on the embryonic fate map. We conclude that the *bcd* protein has the properties of a morphogen that determines cell fate along the anteroposterior axis in a concentration-dependent manner.

Results

Fate Map Changes in Mutants Affecting the Anterior Pattern

To determine the relationship between *bcd* protein levels and cell fate, we analyzed maternal mutations affecting anterior pattern with respect to their influence on *bcd* protein distribution. The cuticle phenotypes of mutations affecting the anterior pattern are shown in Figures 1A–1E. The embryonic fate maps can be readily visualized in the expression pattern of the zygotic segmentation gene *even-skipped* (*eve*; Frasch and Levine, 1987; Figures 1F–1J).

In *bcd* embryos, the anlagen for the entire anterior embryonic half are lacking while the posterior pattern is enlarged and spread to the anterior (Figure 1G); the posteriormost *eve* stripe is duplicated at the anterior, reflecting the duplication of the telson observed in the differentiated *bcd* embryos (Figure 1B). In weak *bcd* mutants, only the anteriormost region is reduced in size while the residual pattern is spread toward the anterior.

In *exu* and *swa* embryos, the anterior defects are similar to those observed in weak *bcd* mutant embryos (Figures 1C and 1D). However, the region of the thoracic and segmented head anlagen (parasegments 1–5) is much enlarged while the posterior pattern (parasegments 6–13) is compressed (Figures 1H and 1I; see, for discussion, Frohnhöfer and Nüsslein-Volhard, 1987). *staufen* (*stau*; Schüpbach and Wieschaus, 1986; Lehmann and Nüsslein-Volhard, unpublished) embryos display a less severe reduction of the anteriormost region (Figures 1E and 1J). *stau* embryos have reduced levels of transplantable bcd^+ activity (Frohnhöfer, 1987). In addition to the anterior defects, *stau*, as a member of the posterior-group genes, affects the development of the abdomen.

bcd Protein Distribution and Pattern in Mutants of the Anterior Group

Whole mount mutant embryos were immunostained using anti-*bcd* polyclonal antibodies, and the immunostain intensity was measured along the anteroposterior axis as

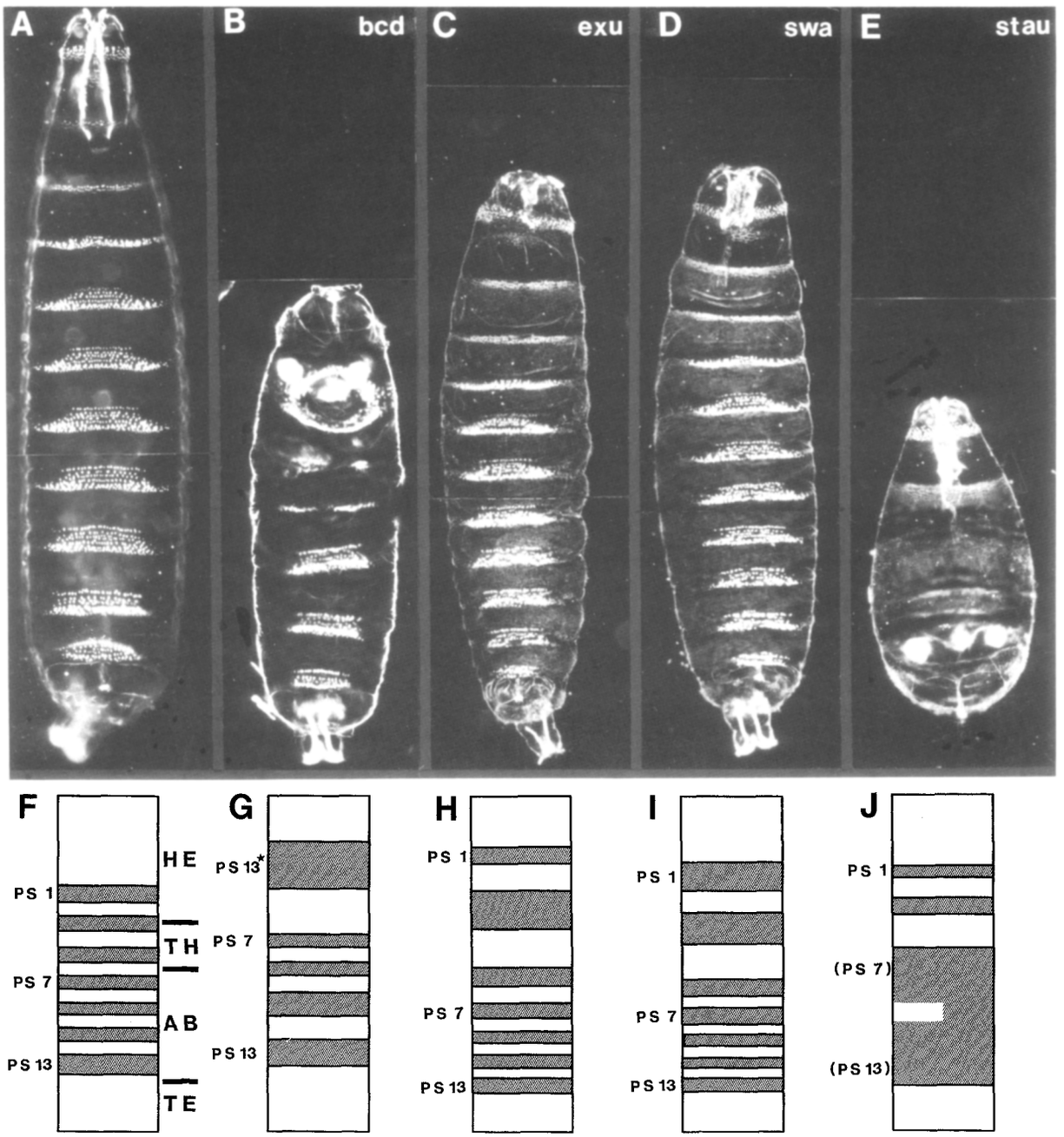

Figure 1. Maternal Effect Mutations Affecting the Anterior Pattern of the Drosophila Embryo

Cuticular patterns (A–E) and a schematic presentation of the expression pattern of *eve* as determined from whole mount staining of embryos at the blastoderm stage with anti-*eve* antibody (F–J) to illustrate changes in the fate map of mutant embryos. Anterior is at the top in all cases. (A, F) Wild type. (B) bcd^{E1}/bcd^{E1}, a strong *bcd* mutant. Head and thorax are replaced by a duplicated telson (PS13*), and the anterior abdomen is defective. (G) *eve* expression in $bcd^{E1}/Df(3R)LIN$. (C, H) exu^{PJ}/exu^{QR}. (D, I) Weak phenotype of swa^{14}/swa^{14}. (E, J) $stau^{D3}/stau^{D3}$ displays anterior as well as abdominal defects. PS1, PS7, and PS13 indicate parasegments 1, 7, and 13, respectively; HE, head; TH, thorax; AB, abdomen; TE, telson.

described by Driever and Nüsslein-Volhard (1988). For a quantitative evaluation and comparison of the *bcd* protein concentration, we included in each staining reaction embryos from females with the normal diploid gene dosage for *bcd*. To be able to distinguish these control embryos from the experimental embryos, the control embryos were mutant for *oskar* (*osk*) and thus lacked the pole cells (Lehmann and Nüsslein-Volhard, 1986). The *bcd* protein distribution in osk^- embryos is the same as in wild-type embryos, as shown in Figure 5B2.

In embryos at the syncytial blastoderm stage derived from both *exu* and *swa* females, *bcd* protein is distributed in a very shallow gradient at 40% to 100% egg length (Figures 2A, 2B, and 3A). Levels of *bcd* protein in *swa* mu-

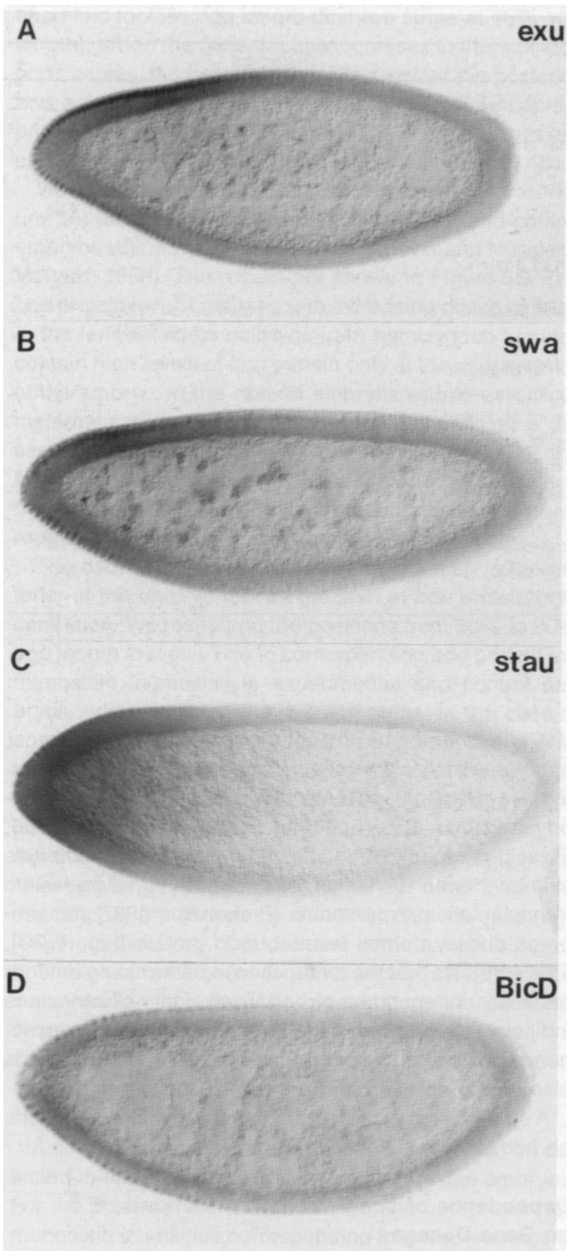

Figure 2. *bcd* Protein Distribution in Whole Mounts of Mutant Embryos

The maternal genotypes are exu^{PJ}/exu^{QR} (A), swa^{14}/swa^{14} (B), $stau^{D3}/Df(2R)PC4$ (C), and $BicD^{7134}/BicD^{IIIE48}$ (D).

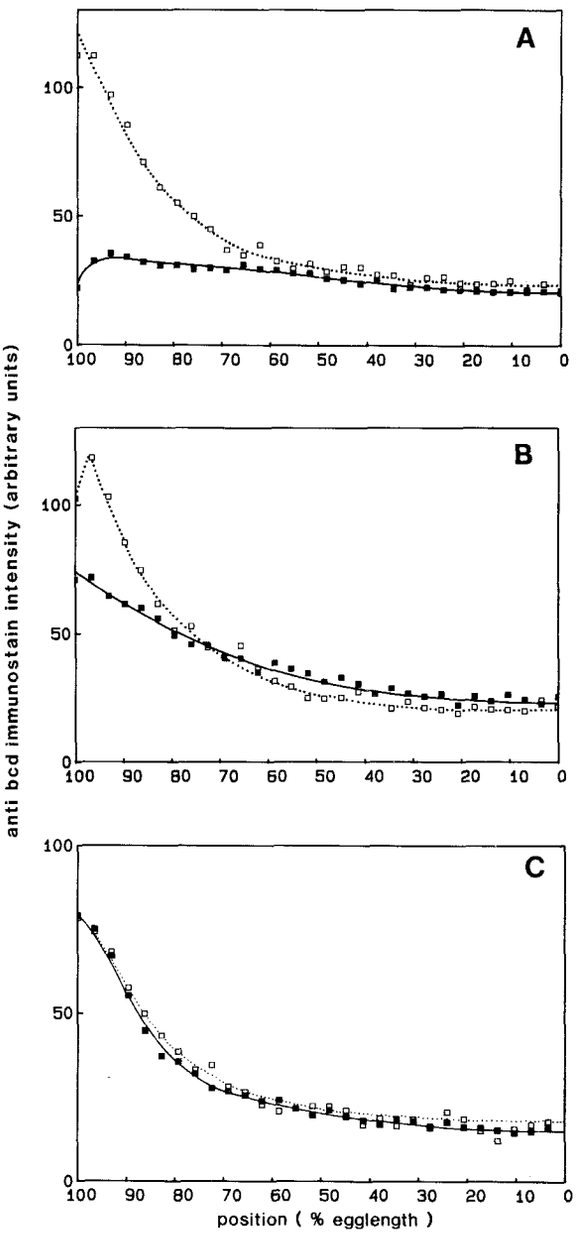

Figure 3. Anti-*bcd* Immunostain Intensities in Mutant Embryos

Quantification of immunostain intensity was as described in Driever and Nüsslein-Volhard (1988). Maternal genotypes are exu^{PJ}/exu^{QR} (A), $stau^{D3}/Df(2R)PC4$ (B), and tor^{WK}/tor^{HH} (C) (closed squares, heavy lines). Each graph includes measurements of control embryos (maternal genotype osk^{166}/osk^{166}) stained in the same batch, reflecting normal *bcd* staining intensity (open squares, dotted lines). Values posterior to 80% egg length reach background staining levels and vary from batch to batch (10 to 25 arbitrary units; for details see Driever and Nüsslein-Volhard, 1988).

tant embryos are slightly higher than in *exu* mutants (data not shown). Both *exu* and *swa* affect the localization of *bcd* mRNA at the anterior pole of the egg. In mutant embryos lower levels of *bcd* mRNA are distributed in a shallow concentration gradient throughout the embryo (Frohnhöfer and Nüsslein-Volhard, 1987; Berleth et al., 1988). Thus the *bcd* protein distribution in these two mutants closely parallels the *bcd* mRNA distribution. The lack of anterior anlagen in the mutants corresponds to the lack of higher levels of *bcd* protein determining the anterior third of the embryo in normal development. The expanded gnathal and thoracic regions observed in the fate maps of *exu* and *swa* embryos (Figures 1H and 1I) correspond to enlarged regions expressing low levels of *bcd* protein. *exu vasa* double mutants (not shown), which exhibit an even and low level of *bcd* mRNA along the anteroposterior axis (Berleth et al., 1988), display a homogeneously low level of *bcd* protein similar to thoracic levels in wild-type embryos.

Table 1. Effect of Maternal Mutations on Pattern and *bcd* Protein Gradient

Class	Mutant Gene	Pattern	Gradient
Anterior	*bicoid*	Head and thorax absent, telson duplicated anteriorly	Absent
	exuperantia	Anterior reduced, gnathal and thoracic region enlarged	Shallow and low
	swallow	Anterior reduced, gnathal and thoracic region enlarged	Shallow and low
	Bicaudal-D weak	Anterior defects	Shallow and very low
	Bicaudal-D strong	Mirror-image duplication of posterior structures	Absent
	exu vasa	Duplication of gnathal and thoracic structures	Even distribution of low protein concentration
Posterior	*oskar*	Anterior normal, deletion of abdomen	Normal
	vasa	Anterior normal, deletion of abdomen	Normal
	valois	Anterior normal, deletion of abdomen	Normal
	tudor	Anterior normal, deletion of abdomen	Normal
	pumilio	Anterior normal, deletion of abdomen	Normal
	nanos	Anterior normal, deletion of abdomen	Normal
	staufen	Anterior truncated, deletion of abdomen	Reduced maximum
Terminal	*torso*	Anterior and posterior truncated	Normal
	trunk	Anterior and posterior truncated	Normal
	torsolike	Anterior and posterior truncated	Normal

These embryos develop a phenotype with duplication of truncated head and thorax at the posterior (Schüpbach and Wieschaus, 1986).

Mutants at the locus *Bicaudal-D* (*BicD*; Mohler and Wieschaus, 1986) show mirror-image duplications of the posterior part of the embryo as a strong phenotype, and anterior defects similar to those of weak *bcd* mutants, as well as rare survivors, as weaker phenotypes. The phenotype seems to be caused by ectopic expression of posterior activity at the anterior (Lehmann and Nüsslein-Volhard, 1986, unpublished; Nüsslein-Volhard et al., 1987). *BicD* embryos without any detectable *bcd* protein in syncytial blastoderm can be found (not shown). This suggests that the lack of head and thoracic anlagen in stronger *BicD* mutant embryos is caused by the lack of *bcd* protein. Some embryos express *bcd* protein in the anterior of the embryo at a level corresponding approximately to the wild-type thoracic level (Figure 2D); these probably correspond to the weak embryonic *BicD* phenotype.

bcd Protein Distribution in Mutants of the Posterior and Terminal Group of Maternal Genes

As already mentioned, the *bcd* protein distribution is normal in embryos of maternal mutations affecting posterior development: *osk* (Figure 5B2), *vasa*, *tudor*, and *pumilio* (data not shown). The only exception is *stau*. Measurement of *bcd* protein distribution in *stau* embryos (Figures 2C and 3B) shows that the maximum level is reduced to less than 50% of the level in wild-type embryos. Compared with *exu* and *swa*, *stau* has the slightest and *exu* the most severe effect on anterior development (Figure 1), in agreement with the observed *bcd* protein concentrations.

torso (*tor*; Schüpbach and Wieschaus, 1986) is a member of the terminal-group genes (Nüsslein-Volhard et al., 1987). In *tor* embryos, the anteriormost and posteriormost structures (collectively termed acron and telson, respectively) are lacking and the anterior pattern is shifted anteriorly by about 3% (Schüpbach and Wieschaus, 1986; Frasch, personal communication). However, *tor* embryos exihibit normal levels of bcd^+ activity in cytoplasmic transplantation experiments (Frohnhöfer, 1987). *tor* embryos display normal levels of *bcd* protein (Figure 3C). This suggests that the *tor* function in determining anterior pattern is independent of *bcd*. Preliminary observations indicate that in other mutants of the terminal group of genes, *trunk* and *torsolike*, the *bcd* protein distribution is also normal.

A summary of the effects of various genetic conditions on *bcd* protein distribution is given in Table 1.

Dependence of *bcd* Protein Distribution on Gene Dosage

bcd is a dosage-sensitive gene. A deviation from the normal diploid dose of bcd^+ in the female causes a shift in the fate map of the early embryo along the anteroposterior axis (Frohnhöfer and Nüsslein-Volhard, 1986; Berleth et al., 1988). This shift can be monitored by the position of the head fold (cephalic furrow; Figures 4A1–4A4), the earliest morphological marker on the anteroposterior axis, which occurs during gastrulation normally at a position of about 65% egg length in the region of the anlagen of the posterior gnathal segments. For more posterior positions, the expression pattern of *eve* was determined to visualize alterations in fate maps of mutant embryos (Figures 4B1–4B4). The center of the first *eve* stripe is located at about 68% egg length in the wild-type embryo (Frasch et al., 1987).

A reduction in bcd^+ gene dosage from two to one copy in hemizygous females results in an anterior shift of the

head fold to 73% egg length (first *eve* stripe at 76% egg length). When the gene dosage increases to three or four bcd^+ copies, the head fold is shifted toward the posterior and appears at position 58% egg length (three copies) and 55% (four copies) (Figure 4A). The first *eve* stripes are at 63% and 59% egg length, respectively (Figure 4B).

We measured the intensity of the *bcd* immunostain (Figure 5A) along a medial line of experimental and control embryos stained in the same batch (Driever and Nüsslein-Volhard, 1988). The results are shown in Figure 5B. The *bcd* protein level increases with increasing doses of bcd^+ in the female. While embryos from hemizygous females contain high levels of *bcd* protein only at the very anterior of the embryo, in the case of embryos with three or four maternal copies of *bcd*, relatively intense staining is observed down to about 50% egg length. At the anterior, the *bcd* concentration exceeds that observed in wild-type embryos. The maximum of *bcd* protein concentration is roughly proportional to the number of gene copies.

The data show that the shift in the fate map in the anterior of the embryo follows the shift in *bcd* protein concentration. We measured the positions from 60% to 90% egg length that give rise to corresponding *bcd* protein immunostain intensities in experimental and control embryos, and calculated the average shifts. In the case of one bcd^+ copy, the corresponding positions are shifted anteriorward by 16% egg length compared to the wild type (shift of head fold and first *eve* stripe by 8% egg length each). In the case of three and four copies, the spread of the *bcd* protein posteriorly is by 12% (three copies) and 15% egg length (four copies), while the head folds (first *eve* stripes) are shifted by 6% (5%) and 9% (9%) egg length, respectively. The data are summarized in Figure 6. The overall shift between points with equal *bcd* protein concentration is somewhat greater than the observed change in the position of anterior markers. However, it is difficult to assess precisely the change in protein concentrations at the position of the head fold because of the shallow decline in stain intensity in this region.

A shift of the stripes of *eve* expression can also be detected in the posterior half of the experimental embryos. For the posterior half of the embryo, the *bcd* protein immunostain intensities corresponding to the different gene dosages do not differ detectably from immunostain intensities in the control embryos. However, the *bcd* protein concentration is significantly above background in the region of *eve* expression affected by the change in *bcd* gene dosage (Driever and Nüsslein-Volhard, 1988).

Discussion

The *bcd* protein is distributed in the embryo in a steep concentration gradient taking its origin from the mRNA source localized at the anterior pole. In this paper we compared local *bcd* protein concentrations and cell fate in embryos in which the shape of the gradient had been altered by manipulation of the maternal genome. We found a close correlation and conclude that, for the anterior pattern, position in the embryo is largely determined by local *bcd* protein concentration.

Autonomy of *bcd* Function

Analysis of *bcd* protein and pattern in maternal mutants such as *exu*, *swa*, and *stau* reveals that the *bcd* protein concentration is interpreted in more than one concentration range. Low levels of *bcd* protein, normally present at 50% to 60% egg length, prevail in the anterior half of *exu* and *swa* embryos. They determine enlarged gnathal and thoracic anlagen. Intermediate levels are required for the formation of the head, while for the anteriormost region, the acron, even higher protein concentrations are necessary as revealed by the phenotype of *stau* mutant embryos. For *exu*, *swa*, and *stau*, the alteration in *bcd* concentration is largely sufficient to explain the (anterior) mutant phenotypes. From transplantation experiments and phenotypic analysis (Frohnhöfer and Nüsslein-Volhard, 1986, 1987), we can further conclude that for the development of the abdomen the *bcd* concentration must be below a certain level, while high concentrations are inhibitory.

The analysis of maternal mutants also shows the autonomy of gradient interpretation. Medium and low protein levels are interpreted correctly regardless of whether high (*exu*) or very low (*exu vasa*) levels are present elsewhere in the embryo. This observed autonomy excludes the possibility that, for example, only the higher *bcd* concentration is relevant, which would initiate a cascade of inductive events that in turn would determine the polar pattern independent of local *bcd* concentrations. In addition, mechanisms involving a juxtaposition of regions of high and low *bcd* protein concentration are excluded for the determination of intermediate positions.

Determination of Position by the *bcd* Protein Concentration

Another line of evidence that the *bcd* protein is not only distributed in a graded fashion but that this distribution determines its biological function derives from the analysis of embryos from females with increased or decreased *bcd* gene dosage. For the anterior pattern we demonstrated that the shifts in the fate map as revealed by early landmarks follow the changes in *bcd* protein concentration.

The shifts of the anterior markers appear less pronounced than those of the respective protein concentrations (Figure 6). One explanation for this discrepancy may be that the markers chosen for study are in all likelihood not directly determined by the *bcd* protein concentration. Their exact positioning might depend in addition on complex interactions between the zygotic target genes of *bcd* (see below). A more important factor is the involvement of other maternal genes in the determination of anterior pattern. Genetic evidence shows that the anteriormost pattern requires, in addition to *bcd*, the activity of the *torso*-group genes (Nüsslein-Volhard et al., 1987). Since the *bcd* protein distribution in *tor* embryos is normal, the effect of *tor* on the anterior pattern is not, as in the case of *exu* and *swa*, mediated through *bcd*. The independent effect of *tor* on anterior pattern may explain the difference between *bcd* protein concentration and position observed in our gene dosage studies.

In embryos with three and four copies of the gene, con-

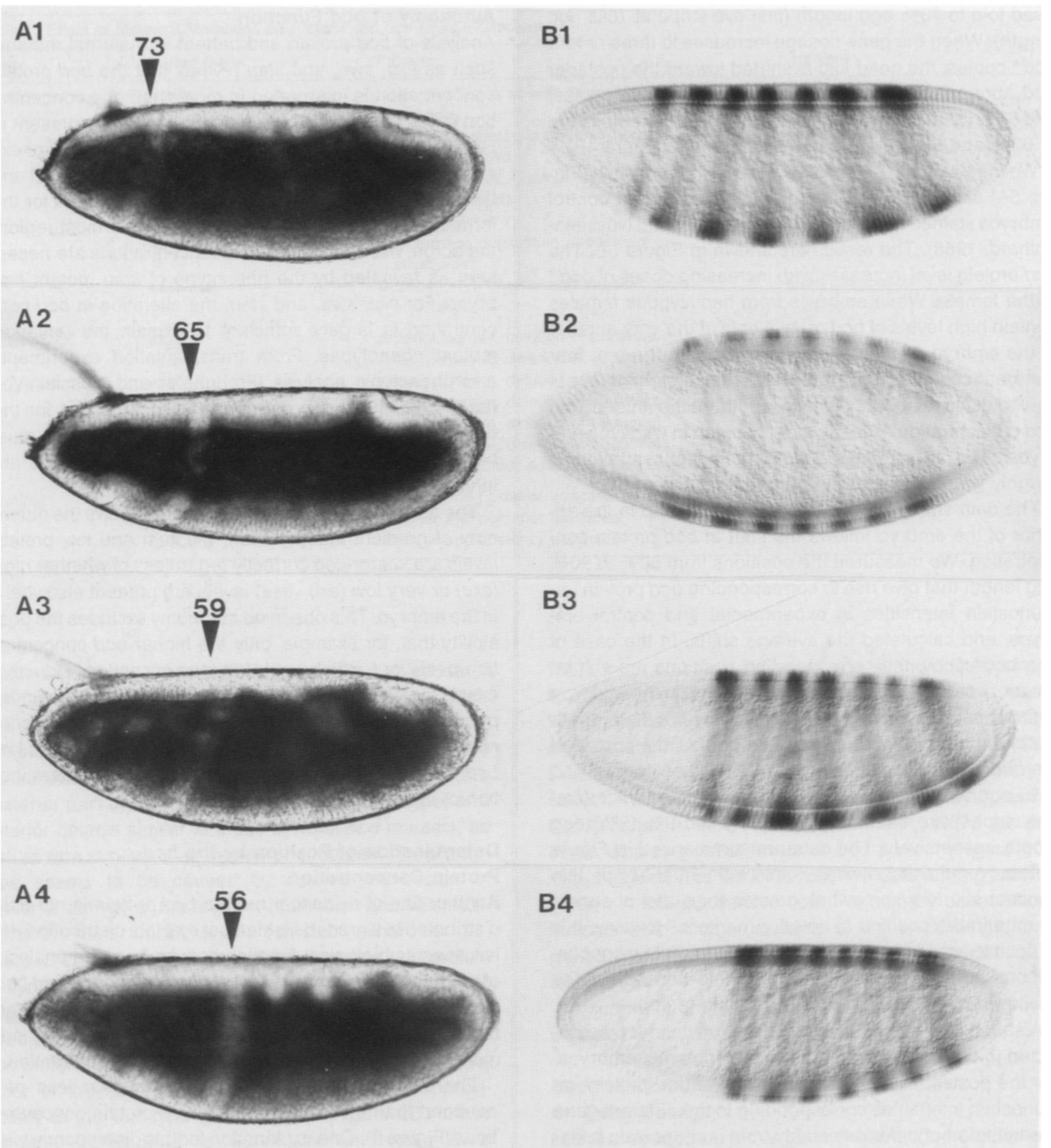

Figure 4. Dependence of the Fate Map on the Number of *bcd*$^{+}$ Copies in the Maternal Genome

(A1–A4) Living embryos at gastrulation (stage 7) from females with one to four copies of *bcd*$^{+}$. Arrows indicate the position of the head fold (% egg length). (B1–B4) Expression pattern of *eve* in embryos from females with one to four copies of *bcd*$^{+}$. Maternal genotypes: *Df(3R)LIN*/+ (A1 and B1), wild type (A2 and B2), *T(Y;3)MA9*^{p}/*T(Y;3)A109*^{d} (A3 and B3), and Dp *bcd*$^{+5}$/Dp *bcd*$^{+5}$; +/+ (A4 and B4).

centrations of *bcd* protein at the anteriormost egg region exceed those reached in control embryos, while in embryos from hemizygous females the highest *bcd* concentrations detected in control embryos are not reached at the anterior tip. All these abnormal genetic conditions give rise to normal hatching larvae. It appears that above a certain level, acron and head development is induced, but the *bcd* concentration is apparently not responsible for the further subdivision of this region. A higher than normal *bcd* concentration does not appear to be deleterious, and for survival it is not necessary to achieve wild-type levels at the anterior tip. As long as a certain range of *bcd* concentrations is provided, the shift in the fate map along the axis can be compensated for later in development. On the other hand, a slight further reduction of the maximum concentration does lead to the absence of anterior structures, lethality, and the development of more-posterior structures at anterior positions.

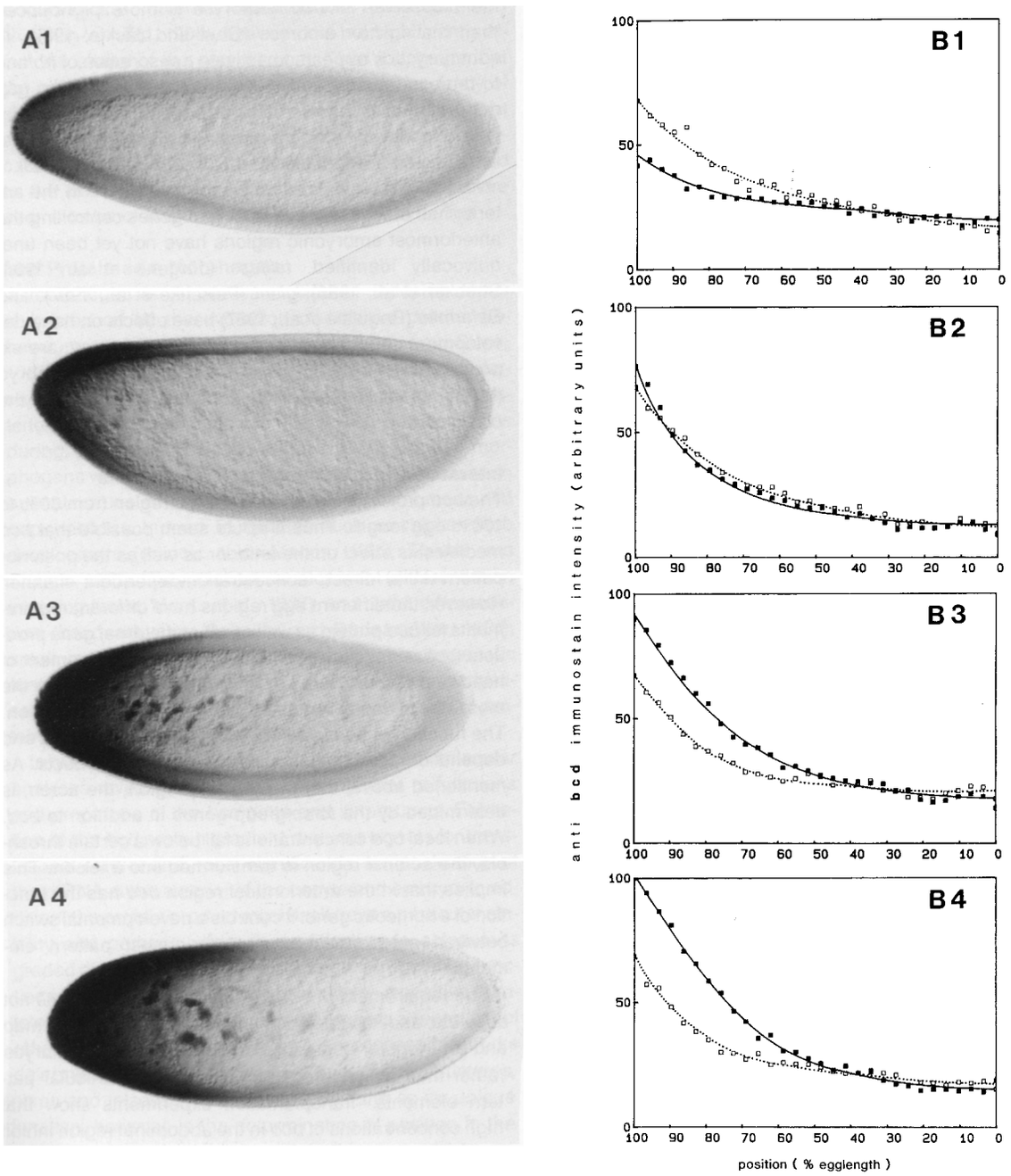

Figure 5. Dependence of *bcd* Protein Distribution on the Number of bcd^+ Copies in the Maternal Genome

(A1–A4) Anti-*bcd* immunostaining of whole mounts of embryos from females with one to four copies of bcd^+. (B1–B4) Relative immunostain intensities in embryos from females with one to four copies of bcd^+ (closed squares, heavy lines). Each graph includes measurements of control embryos with the wild-type number of *bcd* copies (maternal genotype osk^{166}, lacking pole cells) stained in the same batch (open squares, dotted lines). Values posterior to 80% egg length reach background levels, varying from batch to batch (see also Driever and Nüsslein-Volhard, 1988). Genotypes are as in Figure 4.

Gradient Interpretation

Our data do not permit us to determine how directly and with what precision cell fate is correlated with *bcd* protein concentration. It appears unlikely that minute concentration differences lead directly to qualitatively different responses of target cells. Rather, it appears that a small number of regions are determined by different concentration ranges of the *bcd* protein. Gradient interpretation probably involves a concentration-dependent activation of zygotic target genes of the gap class, in line with the segmentation model of Meinhardt (1986). This would subdivide the anterior of the embryo into a series of discrete,

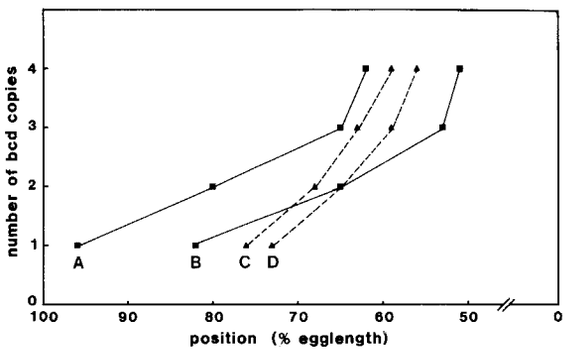

Figure 6. Comparison of Shifts in Fate Map and Staining Intensity with Increasing bcd^+ Gene Dosage

The heavy lines connect positions of equal anti-*bcd* immunostain intensity in embryos from females with one to four copies of bcd^+. Line A connects points with stain intensities equivalent to those at 80% egg length in the wild type; line B connects points with stain intensities equivalent to those at 65% egg length in the wild type. Dotted lines connect positions of markers (C, first *eve* stripe; D, head fold) in embryos from females with one to four copies of bcd^+.

nonoverlapping bands of cells differing with respect to the gap gene expressed (Meinhardt, 1986; Jäckle et al., 1986). According to such a model, the *bcd* gradient would set a rough framework which would be elaborated by the interaction of the gene products of the zygotic segmentation genes. High precision either in the shape of the gradient or in the differential response to *bcd* protein concentration is not required in this model.

The Gap Genes as Targets for *bcd*

One possible target for the *bcd* protein is the gap gene *hunchback* (*hb*; Nüsslein-Volhard and Wieschaus, 1980; Lehmann and Nüsslein-Volhard, 1987a). In *hb* mutant embryos the labial head segment and the thoracic segments are deleted, as in bcd^- embryos. In the wild-type embryo, zygotic *hb* expression extends from 55% to 100% egg length (anterior domain) and from 10% to 20% egg length (posterior domain) (Jäckle et al., 1986; Tautz et al., 1987). Molecular analysis of *hb* expression in bcd^- embryos confirmed that bcd^+ activity is required for the onset of zygotic *hb* expression in the anterior *hb* domain (Tautz, 1988). However, the posterior domain is not affected in *bcd* embryos; in fact, it is duplicated at the anterior. *Krüppel* (*Kr*), the gap gene controlling the region in the embryo posterior to that of *hb*, is expressed in a band at 50% to 60% egg length (Knipple et al., 1985; Gaul et al., 1987) and is not absolutely dependent on bcd^+ activity for its expression. In bcd^- embryos the domain of *Kr* expression is shifted toward the anterior and is enlarged, indicating that *bcd* negatively influences *Kr* expression (Gaul and Jäckle, 1987). This effect of *bcd* on *Kr* expression is in part mediated by the dependence of *hb* on *bcd*, because it has been shown that *hb* influences the anterior border of *Kr* (Jäckle et al., 1986). Since strong *bcd* alleles give rise to defects in regions posterior to the ones affected in hb^- embryos, the effect of *bcd* on *Kr* cannot only be via *hb*. Furthermore, the anterior shift of the *Kr* domain observed in bcd^- embryos is more pronounced than that in hb^- embryos (Gaul and Jäckle, 1987). In summary, *bcd* appears to activate transcription of *hb* and to repress transcription of *Kr*. It is possible that the gap gene controlling the region posterior to the *Kr* domain, *knirps*, is also negatively controlled by *bcd* (for further discussion see Frohnhöfer and Nüsslein-Volhard, 1986).

hb is probably not the only gap gene active in the anterior half of the embryo. Other gap genes controlling the anteriormost embryonic regions have not yet been unequivocally identified. *tailless* (Jürgens et al., 1984; Strecker et al., 1986), *giant* (Petschke et al., 1987), and *Deformed* (Regulski et al., 1987) have effects on head development and, at least in the case of *Deformed*, are expressed in unique regions in the anterior of the embryo (McGinnis et al., 1984). They appear likely targets for the *bcd* protein.

Interaction with Other Maternal Functions

The *bcd* protein is detected in a large region from 30% to 100% egg length. Thus it would seem possible that *bcd* mediates its effect on the anterior as well as the posterior pattern in a direct, concentration-dependent manner. However, the different egg regions have different requirements for *bcd* protein as well as other maternal gene products. *bcd* is absolutely necessary for the development of head and thorax, and we believe this region shows the most direct dependence on *bcd* protein concentration. The function of *bcd* in other regions may be different and depend on combinations with other gene products. As mentioned above, the anteriormost region, the acron, is determined by the *torso*-group genes in addition to *bcd*. When local *bcd* concentrations fall below a certain threshold, the acronal region is transformed into a telson. This implies that in the anteriormost region *bcd* has the function of a homeotic gene: it controls a developmental switch between anteriormost and posteriormost pattern elements.

The requirement of *bcd* for the posterior pattern is not absolute, and is reflected in an expansion of the fate map and an irregular segmentation pattern in bcd^- embryos rather than absence or transformation of particular pattern elements. Transplantation experiments show that high concentrations of *bcd* in the abdominal region inhibit abdomen formation completely (Frohnhöfer and Nüsslein-Volhard, 1986). Although *bcd* protein concentration is above the background level, our measurements fail to detect any significant changes in *bcd* protein concentration in the abdominal region when the gene dosage is varied. An indirect influence on abdominal segmentation may be mediated via interaction with the *caudal* gene function (Macdonald and Struhl, 1986, and personal communication; Mlodzik and Gehring, 1987; Driever and Nüsslein-Volhard, 1988).

In transplantation experiments an inhibition of anterior development by posterior pole plasm was observed (Lehmann and Nüsslein-Volhard, 1986; Frohnhöfer et al., 1986). Therefore it was of interest to see whether the shape of the *bcd* protein gradient was dependent on the posterior center. Elimination of the posterior activity by in-

troducing the maternal mutation *osk* (Lehmann and Nüsslein-Volhard, 1986), however, did not significantly affect the shape of the *bcd* gradient. Thus it appears that the *bcd* protein gradient is established independently of posterior activity. The decrease in *bcd* mRNA stability that can be observed when either posterior activity is mislocated at the anterior (e.g., in *BicD*) or *bcd* mRNA is spread to the posterior (in *exu*; Berleth et al., 1988) therefore seems to be of no function in the wild-type situation.

bcd Protein as a Morphogen

A morphogen is "a particular kind of inducing factor characterized by the evocation of different cellular behaviours at different concentrations" (see, e.g., Slack, 1987). Our data demonstrate that the quantitative differences in *bcd* protein concentration along the anteroposterior axis are transformed into qualitatively different states during early embryonic development. Historically, morphogens were expected to be small molecules because in animal tissues diffusion is limited by cell membranes and gap junctions. Indeed, in systems such as chicken limb development (retinoic acid; Maden, 1982; Thaller und Eichele, 1987), Dictyostelium development (differentiation inducing factor; Morris et al., 1987), and hydra differentiation (head activator; Schaller and Bodenmüller, 1981), small molecules are reported to fulfill morphogenetic functions. However, in the syncytial blastoderm in early insect embryos no cell boundaries limit the diffusion of proteins the size of the *bcd* molecule.

In summary, embryological, phenotypic, and molecular analyses reveal a suprisingly large number of functions and properties of the *bcd* gene and its products. Formation of the *bcd* protein gradient requires signals for translational control and, most significantly, for localization of the mRNA at the anterior of the oocyte and embryo. In addition, the protein product must itself have a particular stability and perhaps undergo modification to achieve its graded distribution and carry out its gene-regulating functions. We can infer that the protein acts as a positive regulator of transcription of some genes and as a repressor of other, zygotic target genes. It is further responsible for the determination of the quality of the terminal structures, acron and telson. The most important and so far unique function, however, is the determination of position in the anterior of the embryo in a concentration-dependent manner. The elucidation of the biochemical mechanisms underlying this as well as the other functions and properties of the *bcd* gene will be an exciting topic of future research.

Experimental Procedures

The wild-type stock was Oregon R. The *bcd* alleles and *Df(3R)LIN, bcd*$^{-}$, as well as the strain with three copies of the *bcd* gene (*C(1)RM,y; T(Y;3)MA9*^{p}*/T(Y;3)A109*^{d}) have been described (Frohnhöfer and Nüsslein-Volhard, 1986). The strain with four *bcd*$^{+}$ copies is homozygous for a *bcd*$^{+}$ duplication on the first chromosome derived from P element–mediated transformation (Berleth et al., 1988). The *exu* alleles *exu*^{PJ} and *exu*^{QR} were obtained from T. Schüpbach (Schüpbach and Wieschaus, 1986). The *swa* allele *swa*^{14} is *fs(1)1497* (Gans et al., 1975). The *vasa*^{PD} *exu*^{PJ} strain was obtained from T. Schüpbach. *BicD* alleles were *BicD*^{7134} and *BicD*^{IIIE} (Mohler and Wieschaus, 1986). Mutant alleles of the posterior-group genes were *osk*^{166} (Lehmann and Nüsslein-Volhard, 1986), *pum*^{680} (Lehmann and Nüsslein-Volhard, 1987b), *tud*^{WC}, *vls*^{PG}, *vls*^{PE}, and *vas*^{PD} (Schüpbach and Wieschaus, 1986). The *nos*^{L7} and *stau*^{D3} alleles were from Lehmann and Nüsslein-Volhard (unpublished); *Df(2L)A72* was from Ashburner. Mutant *tor* alleles were *tor*^{WK} and *tor*^{HH} (Schüpbach and Wieschaus, 1986). All mutant chromosomes carried suitable visible markers. Flies were grown and eggs collected under standard conditions (Nüsslein-Volhard et al., 1984). Staging of embryos was according to Campos-Ortega and Hartenstein (1985).

The procedures of immunological staining of whole mount embryos are described in the accompanying paper (Driever and Nüsslein-Volhard, 1988). The antibody against the *eve* protein was obtained from Frasch (Frasch et al., 1987). Evaluation of the *eve* staining patterns was according to the procedure described by Frohnhöfer and Nüsslein-Volhard (1987). The *bcd* protein concentration was measured along the anteroposterior egg axis in stage 4 embryos as described in Driever and Nüsslein-Volhard (1988).

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