Maternal genes required for the anterior localization of *bicoid* activity in the embryo of *Drosophila*

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The maternal gene *bicoid* (*bcd*) organizes anterior development by means of an activity localized at the anterior tip of the embryo. The mutant phenotypes of *swallow* (*swa*) and *exuperantia* (*exu*) share with weak *bcd* alleles the lack of anterior head structures. Cytoplasmic transplantations reveal a much reduced *bcd*⁺ activity at the anterior tip of *swa* and *exu* embryos. In contrast to weak *bcd* alleles, removal of anterior cytoplasm has very little effect on the *swa* or *exu* phenotype. We suggest that in embryos mutant for *exu* or *swa* the *bcd* activity is not restricted to the anterior tip but extends toward more posterior positions. These conclusions are supported by changes in the fate maps of *bcd*, *exu*, and *swa* mutant embryos and by the ectopic expression of anterior structures in certain double-mutant phenotypes.

[Key Words: *Drosophila*; *bicoid*; antero-posterior axis; fate map; maternal genes]

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The segmented pattern of the *Drosophila* embryo, like that of other insects (Sander 1976; Kalthoff 1979), is organized by two terminal centers located at the anterior and the posterior egg poles. They are both defined by localized and transplantable cytoplasmic activities and by the phenotypes and properties of maternal-effect genes (Frohnhöfer et al. 1986; Frohnhöfer and Nüsslein-Volhard 1986; Lehmann and Nüsslein-Volhard 1986). The posterior pole plasm harbors factors for pole cell determination as well as an activity that organizes polarity and segmentation of the abdomen. These properties are dependent on the expression of several maternal genes including *oskar*, *vasa*, *valois*, *staufen*, and *tudor* (Boswell and Mahowald 1985; Lehmann and Nüsslein-Volhard 1986; Schüpbach and Wieschaus 1986; R. Lehmann and C. Nüsslein-Volhard, in prep.).

Whereas genes affecting the posterior center have very similar phenotypes regarding their effect on the posterior pattern, the phenotypes of genes affecting the anterior pattern show only a superficial resemblance to each other. Of these genes, the *bicoid* (*bcd*) locus is unique with respect to its phenotype and organizing properties (Frohnhöfer and Nüsslein-Volhard 1986). In mutant *bcd* embryos, head and thorax are lacking and replaced by a duplicated posterior telson. *bcd*⁺ activity organizes the anterior pattern: Anterior cytoplasm from wild-type embryos transplanted into *bcd* embryos restores a near-normal pattern. When it is transplanted into more posterior positions, an anterior pattern (often accompanied by polarity reversals) can be induced at ectopic positions. *bcd*⁺ activity is localized in the anterior 15% of the egg (Frohnhöfer and Nüsslein-Volhard 1986) as is the *bcd* mRNA (Frigerio et al. 1986).

Two further groups of genes have been identified that affect anterior development. Mutations in these genes, however, remove only parts of the anterior head region. In this respect, they resemble weak *bcd* alleles. Mutants of the *torso* group eliminate both terminal parts of the longitudinal pattern, posteriorly the telson and anteriorly the labrum (Degelmann et al. 1986; Schüpbach and Wieschaus 1986). Significantly, mutations in the genes of the posterior center and in *bcd* do not eliminate development in the terminal regions, though in *bcd* mutant embryos, a telson develops anteriorly instead of the labral structures. Therefore, the *torso* group of genes appears to specify the terminal regions as a third entity of the longitudinal pattern. Depending on the presence of *bcd*⁺ activity, a labrum or a telson will arise from these terminal areas.

Embryos mutant for *exuperantia* (*exu*; Schüpbach and Wieschaus 1986) and *swallow* (*swa*; Gans et al. 1975; Mohler 1977; Stephenson and Mahowald 1987) have pattern defects in the head region that make them distinct from both the *torso* group genes and *bcd*. We have examined their effect on the *bcd*-dependent functions. We here present evidence that in *exu* and *swa* mutant embryos, the *bcd*⁺ activity is more evenly distributed than in wild-type embryos and suggest that both genes are involved in the anterior localization of *bcd*⁺ activity.

Results

The phenotypes in cuticular preparations: bicoid

Weak *bcd* alleles cause reduction and deletion of structures in the anterior head region. With increasing allelic

strength, all derivatives of the head and also the thorax are affected, whereas elements of the posterior telson are duplicated at the anterior. In addition, segmentation defects appear in the anterior abdomen. In strong alleles, head and thorax are lacking entirely.

A remarkable feature of the *bcd* hypomorphic series is the lack of a strict antero-posterior order of segments deleted with increasing allelic strength. Although the anterior-most head structure, the labrum, is the structure most sensitive to a reduction of *bcd* activity, the thoracic region may be already reduced in size when most of the other head elements are still present (although much smaller than normal). In addition to the anterior defects, most alleles show temperature-dependent segmentation defects in the abdomen. These consist of skipping or fusion of denticle bands, most frequently that of the abdominal segment 4 (A4) but also of A2 and A3. In severe cases the element of A2–A5 are involved, whereas the posterior-most abdominal segments A6–A8 remain normal. With few exceptions, the denticle band of A1 is at least partially preserved in *bcd* embryos. Its identity was confirmed by introducing *Ubx* into the progeny of *bcd* females. In homozygous *Ubx* larvae, only the first abdominal segment is transformed into thorax (Lewis 1978; Struhl 1984). Figure 1D shows a *Ubx/Ubx* embryo derived from a *bcd^{E1}* female with a partially transformed anterior denticle band.

In the hypomorphic alleles *bcd^{E3}* (Fig. 1B), *bcd^{E4}*, and *bcd¹¹¹*, there is a general correlation between the strength of the defects in head, thorax, and abdomen (though the abdominal defects depend inversely on temperature, see below). Two alleles, however, show peculiarities such that their position within the phenotypic series is ambiguous. *bcd^{E5}* causes only slight head defects (the labrum is frequently not eliminated), whereas comparatively strong segmentation defects occur in thorax and abdomen (Fig. 1C). Thus, small defects are spread over a large area (dispersed deletion profile). In contrast, in *bcd²⁻¹³* rather strong reductions of the anterior head region are not accompanied by any defects in the thorax or in the abdomen (localized deletion profile, Fig. 1A). In transheterozygous combinations, *bcd²⁻¹³* is enhanced more strongly by a deficiency than by any strong point mutation, whereas in combinations with other weak alleles, strong point mutations are indistinguishable from deficiencies.

exuperantia. The cuticular phenotype of embryos produced by *exu* females has been described by Schüpbach and Wieschaus (1986). Superficially, the *exu* phenotype resembles that of weak *bcd* alleles in that anterior head structures are deleted (Fig. 1E). On closer inspection, however, it deviates from the weak *bcd* phenotype. It has a very local deletion profile, and deletion of the anterior-most structures is never associated with defects in the thorax or anterior abdomen. In contrast, the remaining anterior regions appear enlarged rather than reduced in size: When only the labrum is eliminated (29°C), the antennal sense organ still has its normal size; when both labrum and antenna are eliminated (18°C), the maxillary structures are often increased in size. Moreover, the thoracic region in *exu* embryos is ex-

panded as judged by its absolute length ($289 \pm 8 \mu\text{m}$, $n = 15$, as compared to $220 \pm 8 \mu\text{m}$, $n = 10$, in newly hatched wild-type embryos) and an increased number of denticles in the prothorax.

Some duplication of posterior structures at the anterior tip is seen in *exu* embryos as well. This duplication rarely includes external cuticular structures but can be seen during gastrulation as a duplicated posterior midgut plate (Schüpbach and Wieschaus 1986). In contrast to *bcd*, occasional abdominal defects in *exu* are restricted to the posterior abdominal region. More frequently, segmentation is normal, but the overall size of the hind end is reduced (Fig. 1E).

swallow. *swa* and *exu* are fairly similar in phenotype, but in *swa* the head defects are somewhat weaker whereas the abdominal defects are stronger. At permissive conditions (29°C) *swa* embryos are hardly distinguishable from *exu* in phenotype (Fig. 1F). However, at low temperature (18°C) all *swa* embryos display abdominal segmentation defects, the majority retaining less than six denticle belts (*swa¹⁴/swa⁻*) (Fig. 1G,H). Furthermore, many embryos are already abnormal early during cleavage and fail to complete cellularization (Zalokar et al. 1975). Embryos may die before differentiation, and differentiated embryos frequently show defects all along the body. The head structures are completely absent occasionally, although this feature is likely to be caused by irregularities in cellularization rather than by a change in the patterning system.

For both *swa* and *exu*, the phenotypic variations with temperature (see below) are greater than interallelic differences. The two alleles of *swa* studied differ with respect to frequency and extent of abdominal defects; combinations with the deficiency indicate that neither allele is amorphic. The *exu* alleles are almost identical in strength. The *exu* phenotype was not clearly enhanced when the females were heterozygous for a synthetic deficiency constructed with aneuploids (see Experimental procedure) and a point mutant (*exu^{P1}*).

In summary, whereas all three genes affect the development of the anterior-most structures of the embryo, posterior structures are also affected, suggesting that the functions of these genes concern processes determining the pattern of a large region of the egg. The phenotypes of the genes *exu* and *swa* are quite similar to each other and distinct from that of *bicoid*.

Temperature dependence

The phenotypes of mutant alleles at all three loci are dependent on the temperature. In most hypomorphic *bcd* alleles, anterior defects are stronger when the embryos are raised at 29°C than at 18°C, whereas the abdominal phenotype is cold sensitive. The most striking change of phenotypic expression with temperature is found in the allele *bcd^{E3}*. At 29°C, an almost amorphic phenotype is developed, whereas at 18°C, the thorax is only slightly reduced and head structures are still prominent. Figure 2A describes the temperature-sensitive period of *bcd^{E3}* as determined by shifting eggs collected at various times after egg deposition, between 18°C and

Figure 1. Cuticular patterns of *bcd*, *exu*, and *swa* mutant embryos. The genotypes of the females producing the embryos shown were: (A) *bcd*²⁻¹³/*bcd*^{E1}, this embryo shows a local deletion profile with anterior defects only. (B) *bcd*^{E3}/*bcd*^{E3}, the typical hypomorphic *bcd* phenotype with defects in head and anterior abdomen (arrows). (C) *bcd*^{E3}/*bcd*^{E5}, dispersed deletion profile with strong pattern defects in thorax and anterior abdomen (arrows), whereas the head is rather complete as can be seen by the presence of a labrum (triangle). (D) *bcd*^{E1}/*bcd*^{E1}, the strong *bcd* phenotype. Head and thorax are replaced by a duplicated telson, and the anterior abdomen is defective. The embryo is homozygous for *Ubx*¹⁰⁹/*Ubx*^{79f}; its anterior-most denticle band seems to be a composite of denticles derived from both A1 and A2, the former of which are transformed by *Ubx* and show thoracic morphology (arrow). (E) *exu*^{P1}/*exu*^{P1}. (F–H) *swa*¹⁴/*DF(1)*/*F5*. All embryos show the 18°C phenotype, except for the *exu* and *swa* embryos in E and F (22°C).

Figure 2. Temperature-sensitive periods of the phenotypes of *bcd* (A), *exu* (B), and *swa* (C). The time scale in A indicates the developmental times in stages defined by Campos-Ortega and Hartenstein (1985) [(1,2) intravitelline cleavage; (3) pole cell formation; (4) syncytial blastoderm; (5) cellularization of blastoderm]. The vertical dotted lines in B and C indicate the time of oviposition. (○) Shift down; (●) shift up. Each point represents at least 30 embryos.

29°C. The phenotypic strength recorded as defects both in the abdomen (not shown) and in the anterior pattern of embryos developing after such shifts reveals a phenocritical period that begins around pole cell formation (stage 3) and lasts until the formation of cell membranes between the blastoderm nuclei during stage 5. This time coincides with the latest time at which phenotypic modifications can still be induced by transplantation of anterior cytoplasm (Frohnhofer and Nüsslein-Volhard 1986), shortly before the first phenotypic manifestations be-

come apparent during gastrulation. *exu* and *swa* are both cold sensitive. In *exu*, the anterior phenotype is enhanced, whereas in *swa*, the abdominal defects are strongly increased at 18°C. For both, the temperature-critical period is during oogenesis (Fig. 2B,C). Temperature changes after egg deposition had little if any effect on the *swa* or *exu* phenotypes. As cold sensitivity is observed in all alleles and allele combinations tested, it is possible that the temperature effect reflects a property of the process affected rather than the feature of a particular mutant gene product.

Fate maps

Observation of living mutant embryos during gastrulation reveals that the basic phenotypic features as observed in the differentiated embryos take their origin from an early alteration of cell fate in the embryos (Frohnhofer and Nüsslein-Volhard 1986; Schüpbach and Wieschaus 1986). To study the early fate maps, we determined the expression pattern of the pair rule gene *fushi tarazu* (*ftz*) in mutant embryos. We used a transformant line involving a construct linking the *ftz* promoter to the β -galactosidase gene from *E. coli* (Hiromi et al. 1985) as well as a polyclonal antibody against a *ftz*- β -galactosidase (β -gal) fusion protein (Carroll and Scott 1985). Both techniques yielded similar results. As the expression pattern of *ftz* can be seen earlier with the antibody than by staining for β -gal activity, the former data were used to construct fate maps for mutant embryos.

In the cellular blastoderm stage of wild-type embryos, the *ftz* gene is expressed in seven regularly spaced stripes positioned around the segmental boundaries of every other segment, from A8/9 at about 15% EL to the border between the labial and the maxillary segment located at about 65% EL just posterior to the cephalic furrow (Hafen et al. 1983; Carroll and Scott 1985; Martinez-Arias and Lawrence 1985). Whereas the posterior-most stripe is five cells wide in the blastoderm, all other stripes and interstripes extend to about three cell diameters in the antero-posterior axis; the *ftz* expression pattern at this early stage coincides approximately with the anlagen of the even-numbered parasegments PS2–PS14 (Figs. 3A, 4; Martinez-Arias and Lawrence 1985; Ingham and Martinez-Arias 1986).

In *bcd* embryos, the stripes are shifted to the anterior. This effect is observed even in embryos from *bcd* heterozygous females. With increasing allelic strength, the expansion of the *ftz* pattern anteriorly is enhanced (Figs. 3 and 4). The anterior two stripes (PS2 and PS4) become weak and finally disappear, whereas both the third stripe (marking the boundary between thorax and abdomen) and its distance to the fourth stripe is considerably enlarged (Fig. 3B,C, allele *bcd^{E4}*, *bcd^{E5}*). It is noteworthy that the allele-specific features observed in the cuticular phenotypes are also expressed in the stripe pattern: With a moderate anterior shift of the entire pattern, the PS6-stripe is already considerably enlarged in *bcd^{E5}* embryos, whereas in *bcd²⁻¹³* embryos the anterior shift is more pronounced and affects all stripes in a regular manner

(Fig. 3E). In strong alleles, a new stripe appears anteriorly that corresponds to a duplicated posterior stripe (PS14; Fig. 3D). In older embryos (containing the *ftz-β-gal* con-

struct and stained for *β-gal* activity), it borders the opening of the duplicated proctodeal invagination, which confirms its identity as a PS14-like stripe. Apart

Figure 3. Expression pattern of *ftz* in mutant embryos. An antibody against a *ftz-β-gal* fusion protein was bound to embryos from females of the genotypes wild type (A), *bcd^{E5}/Df(3R)LIN* (B), *bcd^{E4}/Df(3R)LIN* (C), *bcd³³⁻⁵/Df(3R)LIN* (D), *bcd²⁻¹³/Df(3R)LIN* (E), *exu^{PI}/exu^{QR}* (F), and *swa¹⁴/Df(1)JF5* (G), at 18°C and its distribution pattern revealed by processing with the biotin–avidin horseradish peroxidase staining system. The upper two panels indicate the positions of the parasegmental anlagen 2–14 and the segments of head, thorax, and abdomen in relation to the wild-type staining pattern.

from this, the total number of stripes in strong alleles never exceeds five, and by fusion of the anterior two stripes (PS6 and PS8) it may even be reduced to four (at lower temperature).

The final *bcd* phenotype thus reflects two distinct changes in the fate map: a reduction of anterior and an expansion of posterior anlagen. The reduction and deletion of gnathal and thoracic segments corresponds to a weakening and eventual disappearance of the anterior two *ftz* stripes. In the abdomen, no primordia seem to be eliminated, but the segmentation is distorted. Whereas the abdominal anlage is expanding, its anterior region fails to become separated into distinct realms of *ftz* expression, but no particular stripe is deleted.

The change of the *ftz* expression pattern in *exu* embryos is quite different from that in *bcd* embryos. Here it

is the posterior anlagen that are compressed and crowded whereas the anterior two stripes are much enlarged and further apart than normal. No loss of stripes is observed in *exu* embryos, but the anterior stripe is shifted far toward the anterior pole, reaching 80% EL (compared to 65% in the wild-type) (Fig. 3F, 4). Anterior expansion and posterior crowding appear as almost discontinuous and distinct alterations of the fate map: the reduced distance and size of the four posterior stripes is in sharp contrast to the more than doubled size and distance between the anterior two stripes. It is likely that the posterior segmentation defects occasionally observed in *exu* embryos are caused by the crowded parasegmental anlagen, whereas the substantial enlargement of the anterior anlagen results in the oversized gnathal and thoracic segments. In their study of *ftz* expression in

Figure 4. Patterns of *ftz* expression in mutant and wild-type embryos. (Left) Each line represents one embryo, and for every genotype four embryos are represented. The maternal genotypes are indicated at the right. If not otherwise stated, eggs were collected at 18°C. (Right) Average of the data presented. The anterior margins of the bands corresponding to parasegments, 2, 6, and 14 are connected by heavy lines to indicate the relative shift of head, thorax, and abdomen. For further explanation see text.

mutants affecting the posterior center, Carroll et al. (1986) have also observed that compression of the Anlagen results in pattern defects, whereas expansion is tolerated with very little effects on the final pattern.

Although following the same tendency, the patterns of *ftz* expression in *swa* embryos are more variable than those in *exu*. In weak alleles and generally at elevated temperatures the stripes appear expanded at the anterior and crowded at the posterior (Fig. 4). The transition between crowding and expansion is more continuous than the rather abrupt change in *exu* embryos. At low temperature, many embryos do not reveal a *ftz* expression pattern due to lack of normal cellularization, whereas the stripe pattern in the developing embryos is often quite irregular. The anterior three stripes persist but their orientation with respect to the dorso-ventral or right-left axis is often oblique. The posterior stripes are weaker and less sharply defined, and frequently they disappear altogether (Fig. 3G).

Reduction of anterior *bcd*⁺ activity in *exu* and *swa* embryos

To test whether the head defects observed in *exu* and *swa* embryos can be related to the *bcd*⁺ activity at the anterior pole, anterior cytoplasm from *exu* and *swa* was transplanted into *bcd* embryos. Whereas cytoplasm taken from the anterior tip of unfertilized wild-type embryos induces at least thoracic but frequently also head structures, the cytoplasm from *exu* and *swa* embryos is much less efficient (Fig. 5). None of the injected embryos was able to differentiate head structures. The reduction in *bcd*⁺ activity appears to be stronger in *exu* than in *swa*, in agreement with the more pronounced effect of *exu* on anterior head development; indeed, in *exu* embryos a *bcd*-rescuing activity is barely detectable.

Removal of anterior cytoplasm

Leakage of anterior cytoplasm from wild-type eggs creates embryos with reduced head structures, similar to weak *bcd* phenotypes. When cytoplasm is removed from embryos with an already reduced amount of *bcd*⁺ activity, phenocopies of *bcd* with all phenotypic features including the abdominal segmentation defects result (Frohnhofer and Nüsslein-Volhard 1986). These experiments suggest that the *bcd* phenotype is caused entirely by the reduction or lack of anteriorly localized cytoplasmic activity. When cytoplasm is removed from the anterior tip of *exu* or *swa* embryos, a different result is obtained. Rather than increasing in phenotypic strength, the phenotypes remain more or less unmodified (Fig. 6). Thus, a reduction of *bcd*⁺ activity in eggs of weak *bcd* alleles confers an increased sensitivity to loss of anterior cytoplasm, whereas in *exu* and *swa* embryos a reduced activity at the anterior pole is associated with an increased resistance against this treatment. This finding suggests that *bcd*⁺ activity in *exu* and *swa* embryos is not restricted to the anterior tip but that it is distributed toward more posterior positions in the embryo.

Figure 5. *bcd*⁺ activity at the anterior of *exu* and *swa* embryos. *bcd* embryos were injected with cytoplasm taken from the anterior of wild type *exu*^{PI}/*exu*^{QR} or *swa*¹⁴/*swa*¹⁵ eggs as indicated. The resulting phenotypes were classified according to the degree of anterior development: (class 0) normal (appearing in Fig. 6 only); (class 1) development of thorax and a substantial portion of the head including antennal sense organs; (class 2) thorax and head structures but no antennae developed; (class 3) thorax but no head structures; (class 4) unmodified *bicoid* phenotype as observed in uninjected control embryos.

Double mutant phenotypes

Though *exu* and *swa* embryos are not responsive to loss of anterior cytoplasm, their phenotypes are strongly enhanced when *bcd*⁺ is reduced by genetic means. In embryos from *bcd*^{-/+}, *exu/exu* females, both head structures and prothorax are lacking; in combinations of *exu* with homozygous weak *bcd* alleles, further thoracic segments are deleted in a strictly polar order, leaving a somewhat enlarged but otherwise normally segmented abdomen (Fig. 7B). Thus, *exu* confers a localized deletion mode to all levels of the *bcd* hypomorphic series. However, in combinations with strong alleles (*exu/exu*; *bcd/bcd*), *exu* no longer influences the phenotype (Fig. 7A), supporting the notion that all phenotypic features of *exu* are mediated by its effect on *bcd*⁺.

swa exhibits similar but less pronounced effects in combinations with *bcd* alleles. The abdominal defects of *swa* seem to be ameliorated by reduced levels of *bcd*⁺ activity, in accordance with the view that wrongly localized *bcd*⁺ activity is responsible for them. In contrast, the early cellularization defects are not suppressed by this means. In strong double mutant combinations (*swa/swa*; *bcd/bcd*, 18°C) a novel phenotype, bicaudal embryos, is frequently observed (30–60% of all developed embryos). The facilitation of bipolar development by *swa* mutant conditions suggests that *swa*, in contrast to *exu*, does not act solely on *bcd* but is also involved in other functional circuits, which may not be immediately related to pattern formation, however.

Double mutants of *exu* and *swa* show an enhanced frequency of strong pattern defects; most embryos show the strong *swa* phenotype with rather distorted development in both the anterior and posterior parts of the em-

Figure 6. Modification of anterior development by removal of cytoplasm. Embryos from wild type, *Df(3R)9A99/+*, *exu^{PI}/exu^{QR}*, and *swa¹⁴/swa¹⁵*, respectively, were pricked at the anterior tip in early cleavage and the leaking cytoplasm removed. The resulting phenotypes were classified as in Fig. 5. The narrow black bars indicate the phenotypic frequencies of unpicked control embryos.

bryo. However, no entirely new phenotype is created, nor do the effects of *exu* and *swa* add up to a strong *bcd* phenotype.

In addition to the anterior *bcd* activity, the activity localized in the posterior pole plasm (posterior center) confers polarity to the early embryo (Lehmann and Nüsslein-Volhard 1986). The elimination of the posterior polarizing center in *exu* embryos results in mirror-image duplications of anterior structures: *vasa exu* double mutant females produce embryos with duplicated gnathal regions whereas thorax, abdomen, and telson structures are lacking (Schüpbach and Wieschaus

1986). Similar duplications can be found in the combination *exu;oskar (osk)*: Mouthhooks or only prothoraces may be formed at both ends of the embryo. There are always only a few cuticular structures expressed in these animals. Frequently they do not differentiate any cuticular markers, possibly because little gradation of positional values is left (Fig. 7).

The combinations suggest that it is mainly the posterior pole plasm that accounts for polarity in *exu* single mutants, whereas almost no polarity is conferred by the *bcd* distribution on its own. Because the pole plasm is able to suppress the anterior *bcd⁺* activity (Frohnhofer

Figure 7. Double mutant phenotype. (A) Combination of *exu* with strong *bcd* alleles: *exu^{PI}/exu^{PI};bcd^{E1}/bcd^{E2}*. This embryo is indistinguishable from a strong *bcd* embryo. (B) Combination of *exu* with weak *bcd* alleles: *exu^{PI}/exu^{PI};bcd^{E5}/bcd^{E4}*. (C) Combination of *exu* with *osk*: *exu^{PI}/exu^{PI};osk¹⁶⁶/osk¹⁶⁶*. The embryo displays prothoracic denticle bands at both ends.

et al. 1986; Lehmann and Nüsslein-Volhard 1986), it can convert the symmetric state of *exu;osk* embryos toward the polar *exu* phenotype.

In *swa*, more of the anterior polarity seems to be maintained, as *swa;osk* combinations do not assume a symmetric state. Rather, defects are superimposed on each other in the cuticle phenotype (whereas in the anlagen plan a posterior shift of the anterior structures would be predicted).

Discussion

Mutations in *exu* and *swa* affect both the anterior and posterior domain of the longitudinal pattern. Therefore, possible functions of these genes would be to affect the establishment of both polar centers or influence their interactions; alternatively, they might act on *bcd* alone, which, in turn, mediates the abdominal defects. Our data support the latter interpretation. Specifically, they indicate that *exu* and *swa* are required for the localization of the *bcd* product at the anterior pole. In *exu* and *swa* mutant embryos, the posterior activity is not affected (data not shown), whereas the *bcd* activity at the anterior pole is severely reduced. However, comparison with weak *bcd* alleles shows that there is no simple reduction in anterior activity. Removal of cytoplasm from the anterior tip has almost no effect on the phenotype of *exu* and *swa*, whereas hypomorphic alleles of *bcd* react to this treatment with a strong enhancement of the phenotype. Because double mutants suggest that *exu* and *swa* are sensitive to reduction of *bcd*⁺, resistance to loss of anterior cytoplasm suggests that in *exu* and *swa* the *bcd*⁺ activity has lost its normal, predominantly anterior localization and is present in more posterior positions.

Changes in the fate map demonstrate how the absence or mislocalization of *bcd*⁺ affects the general pattern. It has been shown before that *bcd*⁺ has some suppressive effect on abdominal development; in particular, if transplanted toward posterior positions it may cause the entire abdomen to disappear. In *bcd* mutant embryos, the abdominal anlagen are spread anteriorly, obviously due to the lack of anterior restrictive influence. Thus, a more homogeneous distribution of *bcd*⁺ can account for the posterior pattern defects in *exu* and *swa*. Whereas anteriorly the *bcd*⁺ activity is no longer sufficient to induce the anterior-most structures of the head, its presence in more posterior positions pushes the posterior anlagen backwards. The spreading of intermediate levels of *bcd*⁺ activity causes enlarged regions to become thorax and gnathocephalon.

It is difficult to measure *bcd*⁺ activity in medial and posterior egg positions by transplantation tests, because these regions also contain abdomen-specific factors that complicate the analysis by antagonistic interactions with *bcd*⁺ activity. Positive evidence for ectopic positioning of *bcd*⁺ activity in *exu* embryos rests mainly on the double mutants, with *vasa* and *osk* giving rise to phenotypes with a dicephalic symmetry. When the pole plasm in *exu* embryos is removed, almost no polarity is maintained, suggesting a fairly homogeneous distribution of *bcd*⁺ activity. In *swa*, the *bcd*⁺ distribution may

be less even. However, we do not know whether this difference is allele or locus specific.

All mutant effects of *exu* may be mediated by an even distribution of *bcd*⁺ activity, whereas *swa* appears to have additional functions. The reduced capacity to produce pole cells observed in *swa* embryos (data not shown) may result from a reduced rate of early cleavage (Okada 1986); further, the frequent failure of proper cellularization points to a weakness in more general cellular physiology. Whereas in double mutants with *bcd* the effects of *exu* are all eliminated or suppressed, the cellularization defects of *swa* cannot be suppressed by the elimination of the *bcd* product. *swa* also deviates from *exu* with respect to pattern formation. The two mutants might differ in the degree of *bcd* homogeneity as well as in the total quantity of *bcd*-dependent product present in the egg. Transplantation experiments using *exu* and *swa* as source for *bcd*⁺ activity and interpreting the double mutants with *osk* suggest that in *swa*, residual polarity is preserved and more *bcd*⁺ activity is present at least at the anterior pole, although these differences might be allele rather than locus specific. However, the weak effect on the abdomen compared with that on the head is a gene-specific peculiarity of *exu* that can be attributed to a lowered average amount of *bcd*⁺ activity.

bcd mRNA is transcribed during oogenesis by the nurse cells and accumulates at the anterior end of the oocyte (Frigerio et al. 1986). As the oocyte grows, the RNA remains essentially in position and thus ends up at the anterior pole of the egg. No elaborate transport systems in the oocyte have to be assumed; the RNA could simply be trapped upon entry into the egg. At the anterior pole of young eggs, inhomogeneities can be seen in the yolk/cytoplasm distribution, but no cytoskeletal specializations have been described. Judging from our work, it seems that *exu* is more directly associated with the *bcd* product, fixing it and possibly giving it some protection, whereas *swa* may be part of the general cellular framework facilitating localization. This could explain inversion of abdominal segments in double mutants of *swa* and *bcd* or *swa* and *hunchback* (not shown). However, such instabilities occurring with incomplete penetrance in both combinations may allow other interpretations.

It has been suggested that *bcd* RNA acts as a source to produce a *bcd* protein gradient, which specifies spatially restricted transcription of zygotic segmentation genes of the gap class and thereby organizes the anterior pattern (Frohnhofer and Nüsslein-Volhard 1986). Assuming a shallow distribution of *bcd* RNA in *exu* embryos, the expansion of the gnathal and thoracic region in the fate map is the most direct evidence for a concentration-dependent specification of the pattern along the antero-posterior axis.

Materials and methods

Mutant strains

The *bicoid* alleles and *DF(3R)LIN*, *bcd*⁻ have been described (Frohnhofer and Nüsslein-Volhard 1986). The *exu* alleles were

obtained from T. Schüpbach (Schüpbach and Wieschaus 1986). Since no chromosomal deficiency is available for *exu*, the cytological localization was determined by constructing synthetic deficiencies using *T(Y;R)* translocations (Lindsley and Sandler et al. 1972). Females of the genotype *C(1)RM; T(Y;2R)L139P/T(Y;2R) L107^D/exu^{PI}*, although poorly viable, produced some embryos with a strong *exu* phenotype placing *exu* into the cytological interval 56E;57B. The *swallow* alleles *swa¹⁴* and *swa¹⁵* are *fs(1)1497* and *fs(1)1502* (Gans et al. 1975). The *swa* deficiency, *Df(1)JF5*, was kindly supplied by E. Stephenson and is described in Stephenson and Mahowald (1987). The wild-type stock was Oregon R. All mutant chromosomes contained suitable visible markers. Flies were grown under standard conditions (Nüsslein-Volhard et al. 1984), and eggs were collected and processed for the determination of the cuticular phenotypes as described (Wieschaus and Nüsslein-Volhard 1986).

Temperature shift experiments

The temperature-sensitive period of the allele *bcd^{E3}* was determined. Eggs were collected from *bcd^{E3}* homozygous females on yeast agar plates for 3 hr at 29°C or 7 hr at 18°C and shifted to the other temperature at the end of the egg collection period. The eggs were covered with Voltalet 3S oil and visibly staged by selecting gastrulating embryos (stage 6; Campos-Ortega and Hartenstein 1985) at regular intervals after the shift (20 min at 29°C, 1 hr at 18°C). The batches of embryos were kept to develop at the final temperature. The embryos were fixed and mounted. The phenotype was scored as strong if the embryos lacked most head structures and had less than two thoracic segments. The cold-sensitive period of *exu* and *swa* lies before egg deposition. Females of the genotype *exu^{PI}/exu^{PI}* or *swa¹⁴/Df(1)JF5* were shifted from 18°C to 29°C and vice versa. Eggs were collected at regular intervals after the shift and the phenotypes determined. For *exu*, the phenotype was graded as strong if the dorsal arms and the ventral plate of the cephalopharyngeal skeleton (Jürgens et al. 1986) was absent. The *swa* embryos were scored for incidence of developmental failure and segmentation defects in the posterior abdomen. They were scored as strong mutants when they had less than seven abdominal segments. Although the temperature-critical period using either criteria covers about the same developmental time, data concerning abdominal segmentation yielded the clearest results and are presented here. The frequency relates to the total number of embryos developing a scorable cuticular pattern.

Antibody staining

Embryos were dechorionated, fixed, and devitellinized with the heptane-methanol standard procedure (Mitchinson and Sedat 1983). The fixation was performed in 4% fresh paraformaldehyde in 0.1 M PIPES (pH 6.9), 2 mM MgSO₄, 1 mM EGTA, and the devitellinization was carried out at room temperature rather than applying dry ice–hot water treatment. The first antibody, a polyclonal rabbit *α-ftz*, was kindly obtained from S.B. Carroll and M.P. Scott (Carroll and Scott 1985). Antibody binding and detection using horseradish peroxidase staining was according to the protocol of MacDonald and Struhl (1986). Embryos were dehydrated with ethanol, mounted in 50% dried Canada balsam dissolved in methyl salicylate, and hardened by overnight incubation at 60°C. The positions of the strips were determined from camera lucida drawings of optical sections using Nomarski interference contrast. The mean lateral position of the stripes along the longitudinal egg axis was calculated using standard geometrical procedures. The four embryos of each genotype, the patterns of which are shown in Fig. 4a, were

selected according to their age (middle stage 5) and suitable orientation and no corrections were applied. For several genotypes, a larger sample of embryos was used with little, if any, effect on the mean values shown in Figure 4B, with the exception of *swa* which shows considerable variability.

Cytoplasmic transplantation

Transplantation of cytoplasm from the anterior tip of donor embryos into the anterior tip of stage 2 *bcd* recipient embryos was carried out as described (Frohnhofer and Nüsslein-Volhard 1986). The recipient embryos were from *bcd^{E1}/bcd^{E2}* females. Uninjected control embryos show exclusively the strong *bcd* phenotype (class 4). The donor eggs were from *swa¹⁴/swa¹⁵*, *exu^{PI}/exu^{QR}*, or from Oregon-R females kept at 18°C. All donor eggs were unfertilized and used within 3 hr (18°C) after egg deposition. Removal of cytoplasm from the anterior tip of embryos from females of various genotypes was performed as described (Frohnhofer et al. 1986; Frohnhofer and Nüsslein-Volhard 1986). The frequency of embryonic phenotypes was calculated for embryos surviving the experimental procedure and developing a scorable cuticular pattern, usually 50–80% of all treated embryos. Each point represents an average of at least 35 embryos.

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