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Segmental polarity and identity in the abdomen of *Drosophila* is controlled by the relative position of gap gene expression

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Summary

The establishment of the segmental pattern in the *Drosophila* embryo is directed by three sets of maternal genes: the anterior, the terminal and the posterior group of genes. Embryos derived from females mutant for one of the posterior group genes lack abdominal segmentation. This phenotype can be rescued by transplantation of posterior pole plasm into the abdominal region of mutant embryos. We transplanted posterior pole plasm into the middle of embryos mutant either for the posterior, the anterior and posterior, or all three maternal systems and monitored the segmentation pattern as well as the expression of the zygotic gap gene *Krüppel* in control and injected embryos. We conclude that polarity and identity of the abdominal segments do not depend on the relative concentration of posterior activity but rather on the position of gap gene expression. By changing the pattern of gap gene expression, the orientation of the abdomen can be reversed. These experiments suggest that maternal gene products act in a strictly hierarchical manner. The function of the maternal gene products becomes dispensable once the position of the zygotically expressed gap genes is determined. Subsequently the gap genes will control the pattern of the pair-rule and segment polarity genes.

Key words: *Drosophila* embryo, segmental pattern, zygotic genes, maternal genes.

Introduction

The anterior–posterior pattern in the *Drosophila* egg is initially evident both in specialized structures of the egg cell covers and within the cytoplasm. Characteristic specializations of the chorion include the dorsal appendages and micropyle at the anterior end and the aeropyle at the posterior end of the egg. Within the egg cytoplasm at the posterior pole are the polar granules which are determinants of the germ line fate (Mahowald, 1962). As development proceeds, additional pattern is established as segments of the embryo become arranged into functional units such as the head, thorax and abdomen. Each segment subsequently acquires a polarized pattern as rows of denticles appear at the anterior margin of each segment followed by a region of naked cuticle. The anterior and posterior ends of the embryo do not develop segmental characteristics, but rather specialized structures referred to as the acron and telson at each end respectively.

The establishment and maintenance of the polar pattern in the embryo is directed by a genetic pathway whose principles can be summarized as follows (for review see Akam, 1987; Ingham, 1988; Nüsslein-Volhard *et al.* 1987): the basic information for the embryonic pattern is provided by maternal genes. Mutations in these genes affect the development of specific regions of the embryo. By and large, these mutations interfere only with the embryonic pattern without disturbing the pattern of the extra-embryonic egg covers. On the basis of their phenotype the maternal genes can be grouped into three sets: the anterior group that affects the development of the head and thorax; the posterior group that affects the development of the abdomen; and the terminal group that affects the most anterior (acron) and most posterior (telson) structures. Maternal gene products provided by the mother and stored in the egg cell are required for the spatial regulation of transcription of the first genes expressed by the embryo, the gap genes.

The gap genes are expressed in nonrepetitive domains and the products of neighboring gap genes are thought to overlap at the borders (U. Gaul *et al.* in preparation). Several different types of experiments indicate that gap gene expression is controlled both by the products of the maternal genes described above and by the products of the gap genes themselves. First, the phenotype of mutations in gap genes resembles the phenotype of mutations in the maternal genes (for review see Lehmann, 1988). Second, mutations in one

gap gene affect expression of other gap genes (Jäckle *et al.* 1986). Accordingly, the maternal anterior system controls the zygotic expression of the gap gene *hunchback* (*hb*) in the anterior half of the embryo (Tautz, 1988), the maternal posterior system affects expression of *knirps* (*kni*) in the prospective abdominal region (Nauber *et al.* 1988), and the maternal terminal system regulates the activity of the gap gene *tailless* (*tll*) at the termini (Klingler *et al.* 1988). Later in development, the polar arrangement of individual segments is established by the patterned expression of pair-rule and segment polarity genes (for review see Ingham, 1988; Ingham and Gergen, 1988).

What are the mechanisms that establish the polarity described above? Do the maternal gene products directly control the expression of gap genes, pair-rule genes and segment polarity genes, or are the maternal gene products at the top of a hierarchy in which the only genes they control directly are the gap genes? Presently, most of our information comes from studies of the gene, *bicoid (bcd)*, involved in development of the anterior pattern. *Bicoid* encodes an RNA that is highly concentrated at the anterior end of the egg (Berleth *et al.* 1988). The product of this RNA is a homeo-domain containing protein that is distributed in an anterior–posterior gradient (Driever and Nüsslein-Volhard, 1988). The *bcd* protein activates transcription by binding to the promoter of the gap gene *hb* (Driever and Nüsslein-Volhard, 1989). Activation of hb is dependent on the concentration of the *bicoid* protein and consequently the *hb* gene is activated only in the anterior half of wild-type embryos (Struhl *et al.* 1989; Driever *et al.* 1989). These experiments show that the anterior system controls gap gene expression directly by transcriptional activation. However, these experiments do not resolve whether the anterior system operates as a hierarchical system or whether the maternal genes also directly control the expression of pair-rule and segment polarity genes.

A key gene within the posterior group of genes is *nanos* (*nos*) (Nüsslein-Volhard *et al.* 1987; Nüsslein-Volhard and Lehmann in preparation). Genetic experiments argue that transcriptional activation of the gap gene *kni* by *nos* involves *hb* (Hülskamp *et al.* 1989; Irish *et al.* 1989; Struhl, 1989). In addition to the zygotic *hb* expression which is under the transcriptional control of *bcd*, *hb* is also expressed maternally. The distribution of the maternal *hb* product is controlled by the posterior system. *nos* does not affect the transcription of the maternal product but seems to interfere with the stability and/or translation of the maternal *hb* product such that *hb* is absent from the posterior region of the embryo. Loss of *nos* function results in uniform, rather than anterior, distribution of maternal *hb* RNA and protein (Tautz, 1988). The abnormally high *hb* concentration within the posterior half of the embryo is correlated with the suppression of transcription of *kni* (Nauber *et al.* 1988). Finally embryos that are derived from a germ line deficient for the *nos* and *hb* products can develop a normal segmental pattern suggesting a normal pattern of *kni* expression. This leaves us with the question of how, in the absence of posterior maternal information, a normal abdominal segmentation pattern develops. One hypothesis is that the distribution of gap gene expression plays a critical role in the establishment of the segmental pattern independently of maternal information. According to this hypothesis, maternal genes would act in a strict hierarchical manner and maternal information would become dispensable, once the gap gene expression pattern is established. This hypothesis was tested by transplanting posterior pole plasm into embryos of different genetic backgrounds. We show that the polarity and identity of the posterior segments can be determined by the autonomous action of gap genes.

Polarity and identity of posterior pattern does not depend on the relative concentration of posterior activity

Embryos derived from females homozygous for any one of the posterior group genes lack all abdominal segmentation, while the head, thorax and telson develop normally (Boswell and Mahowald, 1985; Schüpbach and Wieschaus, 1986, 1989; Lehmann and Nüsslein-Volhard, 1986, 1987). The abdominal phenotype of posterior group mutants can be completely rescued by injection of wild-type cytoplasm into mutant embryos (Lehmann and Nüsslein-Volhard, 1986, 1987, and in preparation). Only posterior pole plasm is active and best rescue is achieved when posterior pole plasm is transplanted into the prospective abdominal region. The site of localization, the posterior pole, is separated from the prospective abdominal region by the telson, whose development is under the control of the terminal genes. After transplantation of posterior pole plasm into the middle of the embryo, a normal anteroposterior pattern of abdominal segmentation is restored (Fig. 1B,C). Since at the site of injection the normal orientation of abdominal segments is retained, we conclude that posterior activity can not control the abdominal pattern in a concentration-dependent manner. It is more likely that a certain threshold of posterior activity is required for segmentation to occur, but that the orientation of segments within the segmented region is established independently. In the case of *bcd*, on the other hand, a direct quantitative relationship between pattern and concentration has been shown (Frohnhöfer and Nüsslein-Volhard, 1986): injection of high amounts of anterior cytoplasm into the anterior pole of embryos derived from *bcd* females leads to the development of head structures, while lower concentrations give rise to thoracic structures.

Polarity of posterior pattern depends on anterior and terminal information

Although normal pattern requires all three maternal systems, some pattern can be generated in the presence of only one or two of the systems. The precise type of

pattern that develops, however, depends upon which maternal systems are active. This point is addressed by injection of posterior pole plasm into the middle of *bcd, nos* embryos, which lack both anterior and posterior information, or by injection into *bcd, nos,torsolike (tsl)* mutant embryos, which lack all three systems of maternal information (Fig. 1). *tsl* is a maternal gene and a member of the terminal group (Frohnhöfer, 1987).

If left uninjected, embryos derived from *bcd, nos* females will develop two telsons in mirror image, due to the presence of the terminal system (Fig. 1D, Nüsslein-Volhard *et al.* 1987). Embryos derived from triple mutant *bcd, nos, tsl* females develop no anterior–posterior pattern (Fig. 1G). After injection of posterior pole plasm into the middle of the double or triple mutant embryos, abdominal segments are formed in mirror image. The polarity of the mirror image depends on the maternal genotype. In *bcd, nos* embryos the duplicated sets of segments are formed in a posterior–anterior–posterior (P–A–P) orientation such that the posterior segments (A8) are juxtaposed to the two telsons (Fig. 1E). After injection into *bcd, nos, tsl* embryos, however, the embryos develop the reverse mirror image with abdominal segments oriented toward the middle and the more anterior abdominal segments formed at both ends (A–P–A, Fig. 1H). These findings indicate that, although posterior activity is crucial for the establishment of segmentation within the abdominal region, the anterior–posterior polarity of the segments depends upon additional positional information. Since parts of the normal abdominal pattern can be established in the absence of anterior and terminal maternal systems, it is possible that this additional positional information is encoded by gap genes.

Polarity of the abdomen can be predicted by the pattern of gap gene expression within the embryo

Three gap genes, *hb*, *Kr*, and *kni* have been characterized on the molecular level and the distribution of their RNA and protein patterns have been described. During the early syncytial blastoderm stages, *hb* is found within the anterior 50 % of the embryo (for RNA: Tautz *et al.* 1987, for protein: Tautz, 1988) *Kr* is found between 40 % and 55 % egg length (for RNA: Knipple *et al.* 1985, for protein: Gaul *et al.* 1987), and *kni* is expressed between 30 % and 45 % egg length (for RNA: Nauber, 1988) (0 % = posterior pole). Although the distribution of the *tll* product has not been determined, genetic evidence suggests that *tll* is active at the anterior and posterior ends of the embryo (Strecker *et al.* 1986; Klingler *et al.* 1988). Thus, the following anterior–posterior order of gap gene expression would be predicted: *tailless*, *hunchback*, *Krüppel*, *knirps* and *tailless* (Fig. 3B). (All gap genes analyzed so far have additional domains of expression which have been omitted from this discussion.)

To test the idea that neighboring gap genes may determine the orientation of the abdominal segments in the embryo, we analyzed the pattern of gap gene expression in different mutant backgrounds. These experiments allowed us to interpret changes in the segmentation as a consequence of changes in gap gene juxtaposition. As in the previous experiments, posterior pole plasm was injected into the middle of mutant embryos. We studied changes in *Kr* expression in embryos lacking either the posterior, the anterior and posterior, or all three systems of maternal information. We also examined the pattern of the *Kr* protein after injection of posterior pole plasm into embryos of these different maternal backgrounds. When embryos had reached the appropriate developmental stage, the distribution of *Kr* protein was detected with anti-*Krüppel* antibodies (Fig. 2A,B). The results of this study, and of studies by others (Gaul and Jäckle, 1987), indicate that all maternal systems act negatively on the expression of *Kr*. In uninjected *nos* mutant embryos *Kr* protein extends further posteriorly than in wildtype (Fig. 2C). In the double mutant *bcd, nos* embryos, the *Kr* domain is expanded anteriorly as well as posteriorly (Fig. 2E). Finally in the triple mutant, *Kr* is expressed homogeneously throughout the embryo (Fig. 2G). Injection of posterior pole plasm into mutant embryos leads to the suppression of *Kr* at the site of injection. After injection into a *nos* mutant embryo, the *Kr* domain narrows, and resembles the domain of *Kr* expression found in wildtype embryos (Fig. 2D). After injection into the *bcd nos* double mutant, *Kr* is completely suppressed from the middle region in some embryos, while in other embryos weak staining remains (Fig. 2F). After injection of posterior activity into the triple mutants, *Kr* expression is confined to both ends of the embryo (Fig. 2H).

In our injection experiments, we monitored the distribution of *Kr* protein but not the expression pattern of the other gap genes. In order to reconstruct the approximate pattern of different gap genes in injected embryos, we extrapolated the pattern of the other gap genes from previous experiments (Gaul and Jäckle, 1987; Tautz, 1988; Nauber *et al.* 1988). Since injection of posterior pole plasm into the middle of a *nos* mutant embryo (Fig. 3A) can restore the wildtype pattern, it seems likely that the expression pattern of all gap genes in such an embryo resembles that of the wildtype. We therefore infer that the injection of posterior pole plasm will activate *kni* expression at the site of injection. A normal orientation of the segmental pattern would thus require that the *kni* domain be bordered anteriorly by *Kr* and posteriorly by *tll* (Fig. 3B). After injection into the *bcd nos* double mutant, *Kr* is suppressed and *kni* is presumably expressed throughout the central region. Thus the *kni* domain must be flanked by the *tll* domains at either end (Fig. 3D). The juxtaposition of *kni* and *tll* would lead to a P–A–P pattern duplication (cf. Fig. 1E). Finally, after injection into a triple mutant embryo, *Kr* is suppressed in the central region and *kni* is presumably expressed in this region. In contrast to the previous experiment, in these mutant embryos *kni* must be flanked by *Kr* whose expression remains at the ends (Fig. 3F). This order of *kni* and *Kr* expression would lead to an A–P–A pattern (cf. Fig. 1H) in accordance

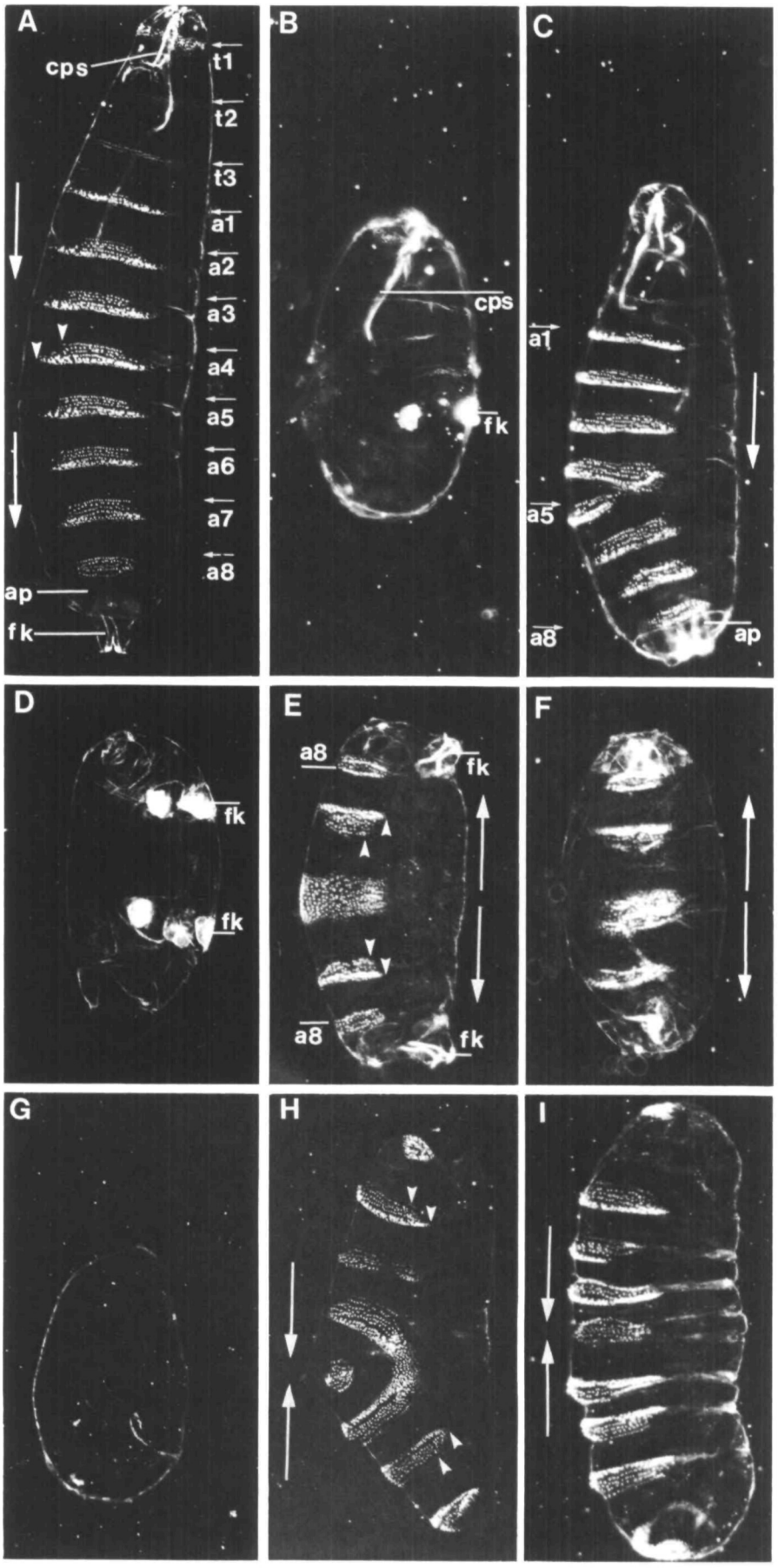
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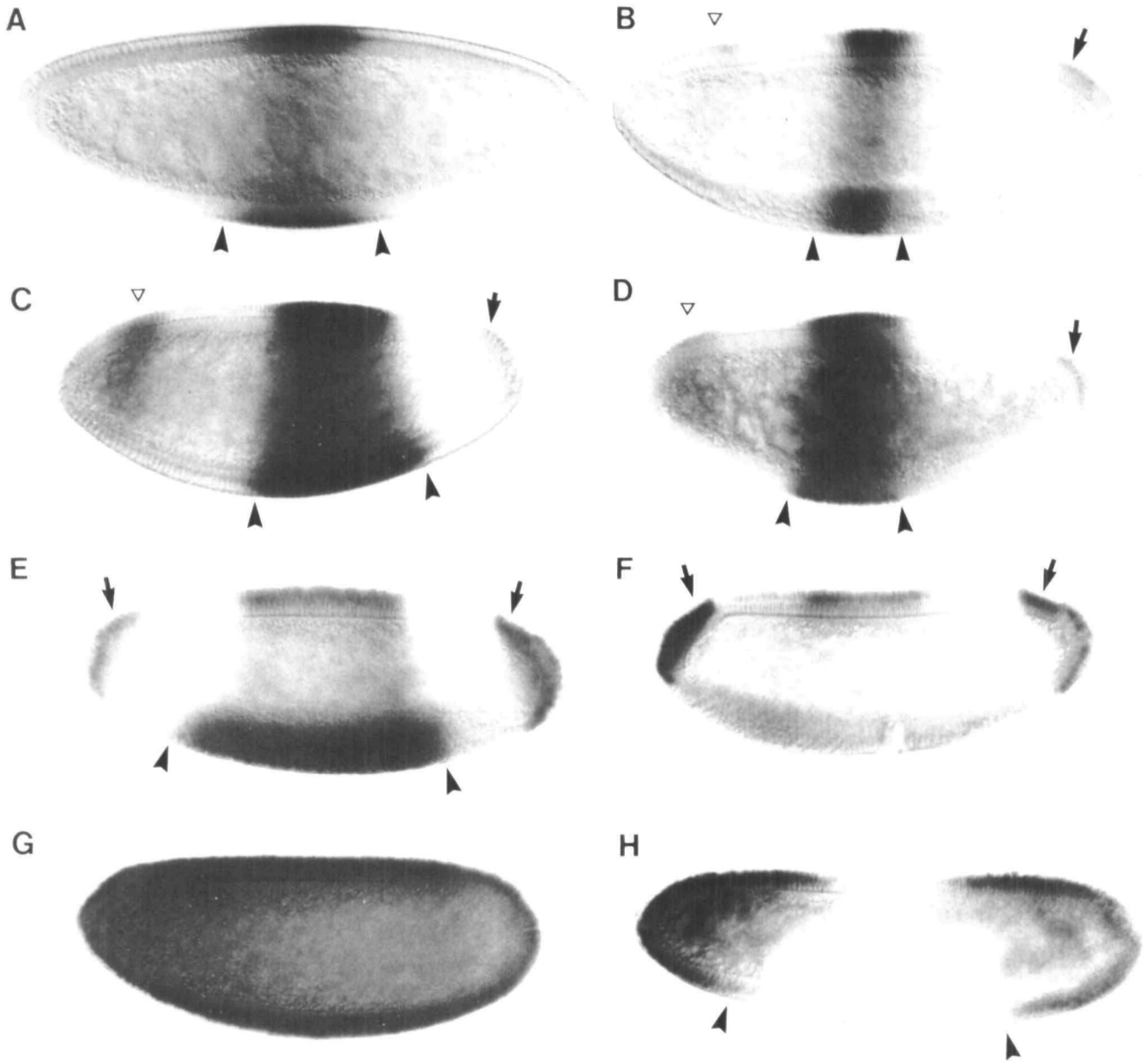
Fig. 1. Cuticle preparations of control and injected embryos. (A) Wildtype embryo. Polarity within each abdominal segment is manifested by the shape of the denticle band located at the anterior margin of each segment. Within each band the more anterior rows of denticles are narrower than the more posterior ones (small arrows, for description of wildtype pattern refer to Lohs-Schardin *et al.* 1979). (B) Control embryo derived from a female homozygous mutant for nos^{L7}. No abdominal segmentation is formed but head, thorax and telson are normal. (C) Rescued *nos* embryo. After injection this embryo formed an almost complete set of abdominal segments in normal anteroposterior orientation.
(D) Control embryo, derived from a female mutant for bcd^{E1} and nos^{L7}. This embryo received only maternal information provided by the terminal system. Two telsons in mirror image are formed. (E) *bcd,nos* embryo after injection. Two abdomens in mirror image are formed. The orientation of the denticle bands and the characteristics of the eighth abdominal segment indicate the orientation and character of the segments (see arrows). (F) Injected embryo derived from female homozygous for *bcd, nos* and heterozygous for tll^{L10}. This embryo resembles that in E. It is phenotypically wildtype for *tll* and thus did develop a normal telson. An uninjected embryo of this genotype shows the same phenotype as the embryo in D.
(G) Embryo derived from female homozygous mutant for bcd^{E1}, nos^{L7}, tsl^{146}. This embryo developed a cuticle but no segmental pattern. Some embryos of this maternal genotype form a field of denticles normally found in the abdominal region. The denticles point medially. Dorso-ventral polarity seems unaffected in these embryos since they form a ventral furrow which spans the entire anterior–posterior axis.
(H) Injected embryo of the same maternal genotype as embryo in G. Orientation of segments is reverse from that of embryo in E and F. The most anterior abdominal segments are formed toward the ends. In some embryos of the same genotype, the two terminal segments show the characteristics of an A1 abdominal segment. (I) Embryo of the same maternal genotype as embryo in F but homozygous for *tll*. The pattern of this embryo is very similar to the embryo in H. Four and half anterior abdominal segments are formed in mirror image with the most anterior structures towards the ends. Uninjected embryos of this genotype can not be distinguished from embryos lacking all maternal information on the basis of their cuticle phenotype. They differ, however, since *tll* embryos (in contrast to *tsl* embryos) form a labrum and a posterior midgut (Strecker *et al.* 1986). Orientation of embryos: anterior up in A,B,C. The orientation of embryos shown in D–I is arbitrary since the anterior–posterior orientation of the egg cannot be reconstructed after the chorion and vitelline membrane have been removed. Ventral is to the left, except for embryos in A and F where a frontal view on ventral side is shown. Arrows mark the orientation of segments and point in anterior–posterior direction. Arrowheads indicate the polarity of a single band of denticles. ap, anal plate; a1–8, abdominal segments 1–8; cps, cephalo-pharyngeal skeleton; fk, Filzkörper; t1–3, thoracic segments 1–3.
Methods: embryos were injected as previously described (Lehmann and Nüsslein-Volhard, 1986). Injection was carried out with posterior pole plasm from wildtype donors into the middle of mutant recipients. To test whether the presence of anterior or terminal activity in the donor embryos influenced the injection result, we injected *bcd, nos, tsl* and *bcd, nos* embryo with posterior pole plasm from embryos derived from homozygous *bcd, tsl* females. Although injection of high dosage of cytoplasm into the triple mutant embryo can lead to the induction of Filzkörper material (H.G. Frohnhöfer unpubl.), a structure characteristic of the telson, under the conditions used in the experiments described, we could not detect any difference between injections with wildtype or mutant cytoplasm. After injection, embryos were left to develop at 18°C for 48 h and their cuticles were prepared according to van der Meer, 1977.
The cuticle of about 50 embryos was examined for each genotype. In one of the *bcd, nos, tll* experiments we examined 53 cuticles; 42 embryos had developed telson structures and thus were not mutant for *tll*. Of these, 36 developed abdominal segments, 24 embryos showed clear symmetric or asymmetric mirror image duplications as described in the text and shown in Fig. 1. In a few exceptional cases, where only very few abdominal segments (3–4 total) were formed, the opposite orientation of the denticle bands was observed. This suggests that *Kr* expression was not completely suppressed after injection. 11 embryos developed no telson and were thus mutant for *tll*, 10 of these developed abdominal segments and all but one of these showed symmetric or asymmetric duplications of the abdomen. The orientation was always (A–P–A) as described in the text. In summary, of the examined cuticles 20 % were mutant for tll which is slightly less than the expected 25 % and may be due to the fact that not-rescued *bcd, nos, tll* embryos are very fragile and some may have been lost during cuticle preparation.

with the more anterior expression of *Kr* in the normal pattern.

The expression of *kni* together with either *tll* or *Kr* seems sufficient to promote the formation of some part of the abdomen. The cooperation of either pair of gap genes determines both the polarity and the identity of the abdominal segments. If *tll* is juxtaposed to *kni,* a posterior–anterior mirror-image duplication of the posterior abdomen (A8–A4/5–A8, c.f. Fig. 1E) is formed. Juxtaposition of *kni* with *Kr* on the other hand results in an anterior– posterior mirror-image duplication of the anterior abdomen (A1/2–A4/5–A1/2, c.f. Fig. 1H). Thus the polarity as well as the identity of each abdominal segment is controlled coordinately by interactions among gap genes and their products. This suggests that the relative position of the domains of gap gene expression establishes the pattern of homeotic gene expression, which determines the identity of each segment (White and Lehmann, 1986; Irish *et al.* 1989).

Polarity of the abdomen can be determined without maternal information

To test whether changes in the relative position of the domains of gap gene products do indeed affect polarity independently of maternal information, we injected embryos of identical maternal genotype but which differed in their zygotic genotype. Females homozygous



for *bcd* and *nos* and heterozygous for the zygotic lethal gene *tll* were crossed with heterozygous *tll* males. While all of the progeny lacked anterior and posterior maternal information, a quarter of these embryos were homozygous mutant for *tll* and lacked telson structures. Injection into embryos from this cross resulted in two phenotypes. The majority of embryos developed duplications of the posterior abdomen in P–A–P orientation. As described for *bcd nos* embryos (Fig. 1F) this indicates that *kni* is flanked by *tll* and *Kr* expression is suppressed. One quarter of the embryos, the *tll* embryos, developed anterior duplications of abdominal segments in the A–P–A orientation. As described for injected *bcd, nos, tsl* embryos (Fig. 1I) we infer that in these embryos *kni* is flanked by *Kr*. This result indicates that abdominal polarity is established according to the spatial arrangement of gap gene expression. In the wildtype, however, the different maternal genes ensure the correct positioning of the gap genes and thus ensure normal polarity.

Discussion and Conclusions

A hierarchical system of pattern formation

The activity of the posterior group genes is required for the normal expression pattern of zygotic segmentation genes (Gaul and Jäckle, 1987; Carroll *et al.* 1986; Lehmann, 1988). Cytoplasmic transplantation experiments suggested that posterior activity is distributed in a gradient with its source at the posterior end (Lehmann and Nüsslein-Volhard, 1986). It might have been conceivable that at a given position along the anterior–posterior axis the concentration of posterior activity

Fig. 2. Expression pattern of *Kr* protein in control and injected embryos. (A) Wildtype embryo in late syncytial blastoderm stage (nuclear cycle 14, Foe and Alberts, 1983, stage 4 according to Campos-Ortega and Hartenstein, 1985). *Kr* is expressed in a domain between 55 and 40 % egg length. (0 % egg length=posterior pole). (B) Wildtype embryo at the beginning of gastrulation, stage 6. Central *Kr* domain has narrowed, new *Kr* expression appears in the anterior (open triangles) and at the posterior (arrow). (C) Embryo derived from homozygous *nos* female at nuclear cycle 14, stage 5. The central domain of *Kr* is extended posteriorly (57–27 % egg length). Anterior (open triangles) and posterior domain (arrow) are normal. (D) Injected *nos* embryo of similar age as embryo in C. The central domain of *Kr* is reduced in comparison to uninjected embryo (60–41 %). (E) Control embryo derived from *bcd, nos* female at onset of gastrulation (stage 6). The central *Kr* domain is extended toward anterior and posterior to 68 and 31 % egg length, respectively. At both ends a posterior midgut forms which is marked by the posterior *Kr* domain (arrow). (F) Injected embryo of same maternal genotype and developmental stage as embryo in E. This embryo shows no expression of *Kr* in the central domain ventrally and only slight expression dorsally. In this context it is worth mentioning that injections were carried out by introducing the injection needle into the dorsal side in order to deposit the cytoplasm ventrally. (G) Embryo derived from triple mutant females (*bcd, nos, tsl*) at cellular blastoderm (late nuclear cycle 14, stage 5). Central domain of *Kr* is expressed homogeneously throughout the embryo. (H) Embryo of same maternal genotype and stage as embryo in G. *Kr* is only expressed at the ends. We interpret this expression as remnants of the extended central domain. Similar to the embryo in F, suppression of *Kr* is stronger ventrally than dorsally after injection of posterior activity. Similar results to those shown in E–H were obtained with embryos derived from females homozygous for *bcd,nos* and heterozygous for *tll* crossed to *tll* heterozygous males. Methods: embryos of maternal and zygotic genotypes as described above were injected as described in Fig. 1. When most embryos had reached the late cellular blastoderm–early gastrula stage, embryos were fixed in paraformaldehyde for antibody staining. We chose to stop development at this stage since the posterior domain of *Kr* expression can be used as an internal control for the preparation. Embryos were manually freed from the vitelline membrane and incubated with anti-*Kr* antibody. A biotinylated secondary anti-rabbit antibody was detected histochemically as described by McDonald and Struhl (1986). After dehydration embryos were embedded in Araldite.

would specify the segmental pattern and its orientation. However, this study suggests that the posterior group genes do not control the polarity of the segmental pattern in a concentration-dependent manner. Indeed, we can show that, irrespective of the maternal genotype, the polarity of the abdominal segments depends on the presence or absence of the zygotic gap gene *tll*. Our data do not rule out that the expression of other yet unknown gap genes is important for abdominal segmentation as well. We would predict that in different genetic backgrounds the pattern of expression of such genes would be altered coordinately with *kni* and *Kr*.

Our conclusions may not be limited to the posterior system: double mutants between *exuperantia* which affects the localization of *bicoid* RNA (Berleth *et al.* 1988) and mutants which affect the posterior system result in mirror image duplications of anterior segments (Schüpbach and Wieschaus, 1986). The polarity and identity of the pattern elements formed seem independent of the orientation of the *bicoid* gradient (Struhl *et al.* 1989) and may thus be directed by the relative position of gap gene expression. These findings may suggest that all maternal systems act in a strict hierarchical manner, such that once the domains of gap gene expression have been established, maternal information becomes dispensable.

It is not clear how the pattern of gap gene expression is translated into the repetitive transverse stripes of pair-rule and segment polarity gene expression. It is conceivable from the experiments presented here that a particular combination of two gap genes initiates the expression of a given stripe of a pair-rule gene. In a particular region, however, the relative concentration of a single gap gene product may be critical for the expression of a particular pair-rule gene stripe. Indeed, the medial abdominal segments (A3–A6) are most sensitive to reduction in *kni* activity. According to the fate map, the domain of *kni* expression (between 30 % and 45 % egg length, Nauber, 1988) gives rise to the primordia of segments A3–A5 (Lohs-Schardin *et al.* 1979). Thus a direct correlation can be established between the development of the medial abdominal segments and the domain of *kni* expression.

Pattern formation without maternal information

The activation of *kni* by the posterior group genes is indirect and mediated by the negative effect of posterior activity upon *hb*. In wildtype embryos, the maternal *hb* product is distributed in a shallow anterior–posterior gradient. This gradient depends on posterior activity (Tautz, 1988) and small changes in posterior activity are reflected in changes in the fate map (Lehmann, 1988). In the absence of posterior activity, the maternal *hb* product is evenly distributed in the embryo. This even distribution of maternal *hb* product permits an extension of the *Kr* domain posteriorly while *kni* expression is completely suppressed (Tautz, 1988; Nauber *et al.* 1988). The expression of the gap genes *Kr* and *kni* may thus be very sensitive to changes in the *hb* gradient within the abdominal region. Since the concentration of maternal *hb* product is quite low in comparison to the concentration of the zygotic product, low concentrations of *hb* may have a positive influence on *Kr* expression while high concentrations of *tll* have a negative effect on *Kr* expression (Jäckle *et al.* 1986).

A direct positive influence of *hb* upon *Kr* may also account for the establishment of a normal pattern in the absence of the maternal *hb* product. As we have shown, the proper position of the domain of *Kr* expression is critical for the establishment of normal polarity. Therefore, knowledge of the mechanisms by which the positions of *Kr* and *kni* expression are controlled in these embryos is critical to understand how normal

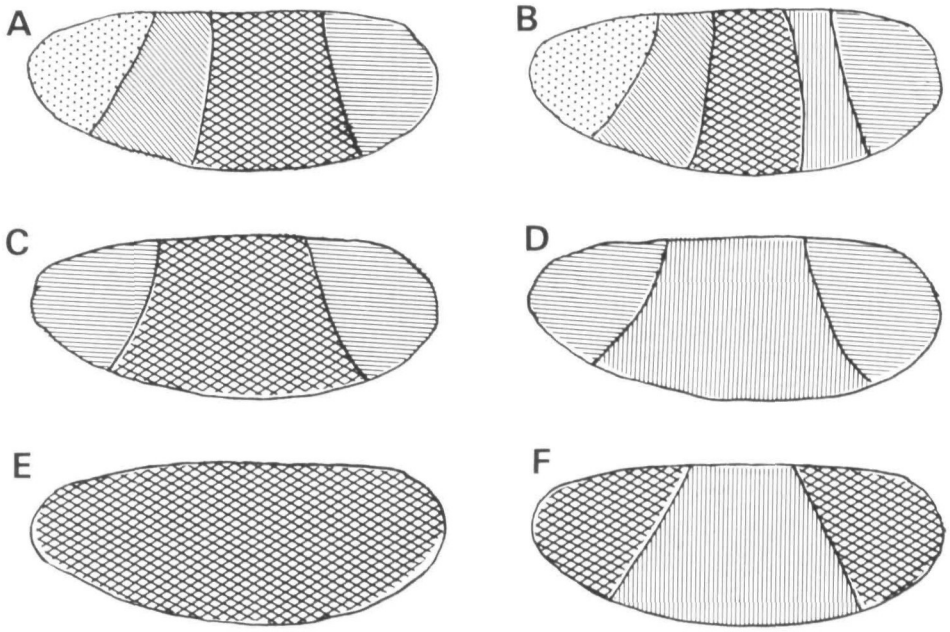


Fig. 3. Schematic presentation of hypothetical expression domains of gap genes in control and injected embryos. This scheme illustrates that the reversal in orientation of the abdominal segments in *bcd, nos* and *bcd, nos, tsl* embryos can be attributed to the pattern of gap gene expression produced after injection (c.f. Figs 3D, 1E and 2F with Figs 3F, 1H and 2H.). (A) Control *nos* mutant embryo. (B) Wild type pattern (expected to be similar to that of rescued embryo). (C) Control *bcd, nos* mutant embryo. (D) Injected *bcd, nos* mutant embryo. (E) Control *bcd, nos, tsl* mutant embryo. (F) Injected bcd, nos, tsl embryo. Stipple: in this anterior domain *hb* and *tll* and a hypothetical terminal gap gene (Martin Klingler and Detlef Weigel per. com.) are thought to be active. Diagonal lines: *hb* domain. Cross hatch: *Kr* domain. Vertical lines: *kni* domain. Horizontal lines: domain where *tll* and the hypothetical terminal gap gene are expressed. Note that the actual patterns of expression have so far only been described for *hb*, *kni* and *Kr*. Overlaps between gap gene products and the graded distribution of individual gap gene products have not been considered in this presentation. The domains of the most terminal areas (marked with stipples and with horizontal lines respectively) are based on genetic and developmental evidence and may not represent regions where a single gap gene is expressed or where its product is active.

polarity is established in the absence of maternal *nos* and *hb* products. At present we can only speculate. Perhaps low levels of zygotic *hb* product are sufficient to activate *Kr* and repress *kni* in the anterior abdominal region and thereby set up an asymmetry in the expression of the two gap gene products. Alternatively, it is conceivable that a low concentration of *bcd* activates *Kr* while a high concentration of *bcd* inhibits *Kr*. Although attractive, this model seems unlikely since, in the absence of *bcd*, *Kr* is expressed (Gaul and Jäckle, 1987 and this study). Analysis of this model and other models will require a more detailed understanding of the mechanisms that control *kni* and *hb* expression in the early embryo.

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