Development 111, 601-609 (1991)
Printed in Great Britain © The Company of Biologists Limited 1991

Spatial regulation of the gap gene *giant* during *Drosophila* development

RACHEL KRAUT and MICHAEL LEVINE*

Department of Biological Sciences, Fairchild Center, Columbia University, New York, NY 10027, USA

*Present address: Department of Biology, Bonner Hall, University of California at San Diego, La Jolla, CA 92093-0346

Summary

We describe the regulated expression of the segmentation gene *giant* (*gt*) during early embryogenesis. The *gt* protein is expressed in two broad gradients in precellular embryos, one in anterior regions and the other in posterior regions. Double immunolocalization studies show that the *gt* patterns overlap with protein gradients specified by the gap genes *hunchback* (*hb*) and *knirps* (*kni*). Analysis of all known gap mutants, as well as mutations that disrupt each of the maternal organizing centers, indicate that maternal factors are responsible for initiating *gt* expression, while gap genes participate in the subsequent refinement of the pattern. The maternal morphogen *bicoid* (*bcd*) initiates the anterior *gt* pattern, while *nanos* (*nos*) plays a role in the posterior pattern. Gene dosage studies indicate that different thresholds of the *bcd* gradient might trigger *hb* and *gt* expression, resulting in overlapping but noncoincident patterns of expression. We also present evidence that different concentrations of *hb* protein are instructive in defining the limits of *kni* and *gt* expression within the presumptive abdomen. These results suggest that *gt* is a *bona fide* gap gene, which acts with *hb*, *Krüppel* and *kni* to initiate striped patterns of gene expression in the early embryo.

Key words: maternal morphogens, gap genes, *giant*, segmentation, *Drosophila*.

Introduction

Maternal morphogen gradients have long been postulated as the primary source of positional information in insect embryos. This idea was originally put forth by Seidel (see review by French, 1988) who introduced the notion that maternal 'organizing centers' exist at both poles of the egg and generate pattern in the embryo. More recently, Wolpert (1969) proposed the 'French flag model', whereby pattern could be generated by differential responses of cells to graded concentrations of morphogens. Early support for the presence of morphogens emanating from either pole of the insect egg involved experimental manipulations such as ligature and cytoplasmic transplantation (Sander, 1976). Recent genetic and molecular studies have shown that maternally deposited morphogens are indeed present in the *Drosophila* egg (Frohnhöfer and Nüsslein-Volhard, 1986; Lehmann and Nüsslein-Volhard, 1986; Nüsslein-Volhard *et al.* 1987; Sander and Lehmann, 1988). In particular, the maternal morphogen *bicoid* (*bcd*) has been shown to determine anterior–posterior pattern in a concentration-dependent way by initiating a cascade of patterning events in the early embryo (Driever and Nüsslein-Volhard, 1988*a*,*b*).

Genetic screens carried out by Nüsslein-Volhard and Wieschaus (1980) identified most of the zygotic genes in the regulatory cascade that act downstream of the maternal morphogens. The segmentation gene hierarchy transduces the crude gradients of maternal morphogens into highly refined positional identities. The first step in this process involves the activation of the first zygotically expressed genes, called the gap genes, in broad overlapping domains (Gaul and Jäckle, 1987; Gaul and Jäckle, 1989; Stanojević *et al.* 1989; Struhl, 1989*b*).

The best documented case of an interaction between a maternal factor and a gap gene is the regulation of *hunchback* (*hb*) by the *bcd* morphogen (Driever *et al.* 1989; Struhl *et al.* 1989). The initiation of *hb* has been shown to involve a transcriptional 'on/off' switch, whereby levels of *bcd* protein above a minimal threshold concentration activate *hb* while lower levels fail to do so. The *bcd* protein is distributed in a broad gradient along the anterior–posterior axis of the early embryo, with peak levels present at the anterior pole (Driever and Nüsslein-Volhard, 1988*a*,*b*). High levels of *bcd* in anterior regions activate *hb*, resulting in a relatively sharp border of *hb* expression. The lower levels of *bcd* protein in more posterior regions of the embryo fail to trigger the *hb* promoter. The interaction between *bcd* and *hb* is consistent with the 'French flag' model of cell fate specification suggested by Wolpert

(1969). However, according to that model, the *bcd* gradient should have more instructional capacity, and define additional domains of gap gene expression.

Heterologous promoters containing 5′ *hb* sequences attached to the reporter gene *lacZ* are activated by *bcd* in a concentration-dependent manner. Such gene fusions are expressed in progressively more posterior regions of the embryo as the dose of *bcd* protein is increased by varying the *bcd* gene copy number from 1 to 6 (Struhl *et al.* 1989; Driever *et al.* 1989). Activation of *hb*–lacZ fusions was shown to depend on at least two copies of *bcd* binding sites. Multimerization of a small 5′ *hb* DNA fragment containing two *bcd* sites resulted in the activation of *hb*–lacZ fusions even by low levels of the *bcd* protein. These results prompted the proposal that overlapping, but non-identical, domains of gap gene expression could involve the differential response of gap gene promoters containing different numbers of *bcd* binding sites (Driever *et al.* 1989; Struhl *et al.* 1989). Thus, a promoter such as *hb* is broadly expressed in response to the *bcd* gradient since it contains many *bcd* binding sites, while the expression of a second gap gene might be confined to more anterior regions by possessing fewer *bcd* sites. Although this proposal for establishing distinct domains of gap gene expression is quite appealing, previous studies have failed to identify a second gap gene that is differentially regulated by the *bcd* morphogen. Indeed, Driever *et al.* (1989) refer to such a hypothetical gap gene as 'gene X'. Here we present evidence that the segmentation gene *giant* (*gt*) probably corresponds to gene X.

gt was originally identified in the genetic screens of Wieschaus and Nüsslein-Volhard (1984*a*) as a gap gene on the basis of its effects on segmentation of the larval cuticle, which includes deletion of denticle belts A5–A7 and some head defects. However, these defects are less extensive than those seen for the gap genes *hb*, *Kr* and *kni*. For example, *Kr* mutants show deletions of the entire thorax and anterior abdomen (Wieschaus *et al.* 1984*b*). Thus, *gt* was originally thought not to play an instrumental role in initiating segmentation (Wieschaus *et al.* 1984*a*; Gergen and Wieschaus, 1986; Petschek *et al.* 1987). Here and in the accompanying paper, we present evidence that *gt* belongs to the gap class of genes and is essential for the initiation of segmentation, since it appears to be a primary target of morphogen gradients and interacts with the other identified gap genes.

We describe additional details of the *gt* expression pattern during development (see Mohler *et al.* 1989), and show that it is a nuclear protein that is distributed in a relatively simple pattern at the blastoderm stage, characteristic of gap genes (i.e. Lehmann and Nüsslein-Volhard, 1987; Gaul *et al.* 1987; Nauber *et al.* 1988). The *gt* pattern consists of two broad domains, which are under the separate control of different maternal morphogenetic centers. We have analyzed the distribution of the *gt* protein in all known gap mutants and in mutants that disrupt each representative maternal organizing group, namely *bcd*, *nanos* (*nos*) and *torso* (*tor*) (reviewed in Nüsslein-Volhard *et al.* 1987). None of the gap mutants cause a failure to initiate *gt* expression, suggesting that activation is directly regulated by maternal factors. We present evidence that the anterior portion of the *gt* expression pattern is controlled by *bcd*, while the posterior *gt* domain seems to be regulated by the posterior pattern organizer, *nos*, which controls levels of the maternal *hb* product in posterior regions (Struhl, 1989*a*; Irish *et al.* 1989; Hülskamp *et al.* 1989). We present evidence that the overlapping, but non-coincident patterns of *gt* and *hb* expression involve their differential response to the *bcd* concentration gradient.

Materials and methods

Cloning a full-length gt *cDNA*

A cDNA library in the vector pNB40 (Brown and Kafatos, 1988) was screened with a genomic DNA fragment from the *gt* coding region (Mohler *et al.* 1989). Several clones, all about 1.7 kb in length, were isolated, and those with identical restriction patterns were assumed to represent the major *gt* transcription product. Previous studies have shown that the predominant *gt* mRNA has a length of 1.9 kb (Mohler *et al.* 1989). One of the isolated cDNAs was used to verify that it corresponds to *gt* by making a digoxygenin-labelled probe and hybridizing to whole-mount preparations of embryos (Tautz and Pfeifle, 1989). The pattern was the same as that observed for *gt* by Mohler *et al.* (1989), based on hybridization to tissue sections. This cDNA was then subcloned as a *Hin*dIII–*Eco*RI fragment into the Bluescript SK+ vector (Stratagene), and partially sequenced from the 5′ and 3′ ends. It was found to contain 40 bp of leader sequence and the consensus initiation sequence CACCATG of Kozak (1984), followed by an extended open reading frame. The trailer is 318 bp long and contains a potential polyadenylation signal 300 bp downstream from the stop codon.

Localization of gt *transcripts*

gt transcripts were localized in wild-type embryos according to the protocol of Tautz and Pfeifle (1989). The full-length cDNA was gel-isolated and approximately 200 ng of this fragment was labelled by random priming and digoxygenin-labelled UTP incorporation. About 1/50th of this reaction was used per hybridization reaction, which was done in a total volume of 50 μl in an Eppendorf tube.

Preparation of gt *protein and antisera*

A full-length *gt* protein was produced in *E. coli* using the T7 expression system developed by Studier and Moffatt (1986). Oligonucleotide-directed mutagenesis was performed using the Muta-Gene Phagemid *in vitro* Mutagenesis kit (Bio-Rad). An *Nde*I restriction site was created at the initiating ATG of the cDNA in the Bio-Rad vector pTZ-19 by using the oligonucleotide GCATTAGCATATGGTTCGGTG. The pAR–*gt* expression plasmid was constructed by subcloning a 1.7 kb *Nde*I–*Eco*RI fragment containing the entire *gt* coding region into the unique *Nde*I and *Eco*RI sites of the vector. The protein was extracted from induced cultures of the bacterial strain BL21(DE3), as described previously (Stanojevic *et al.* 1989), and electroeluted from an SDS-polyacrylamide gel and lyophilized. Alternatively, protein was prepared according to the Rubin Lab Methods Book (1986), protocol of D. Rio, p.19.2, plan B and lyophilized. Protein extracts prepared according to both methods were

electrophoresed on SDS–polyacrylamide gels; two major bands at ~50 and ~60×10^3 M_r were observed upon induction of the bacterial cultures. When these two bands were eluted separately and re-run on acrylamide gels, the 60×10^3 M_r band appeared to degrade so that it generated the 50×10^3 M_r species. Therefore, the 60×10^3 M_r species was assumed to be the full-length protein, and the 50×10^3 M_r species a breakdown product. Lyophilized protein prepared both by electroelution from acrylamide gels and by the Rio method was injected into rats and guinea pigs (Pocono Farms, Canadensis, PA).

Embryo fixation and antibody staining

Embryo collections, fixations and staining procedures were done as described in Frasch *et al.* 1987. For anti-*gt* sera, both 50×10^3 M_r and 60×10^3 M_r species of *gt* protein were eluted separately for injection into rats. These sera gave identical staining patterns in embryos. Also crude *gt* bacterial extracts from the Rio protocol were injected into guinea pigs and rats. Rat anti-*gt* sera were diluted 1:250 in a buffer consisting of 1×PBS, 0.5 M NaCl, 0.1 % Tween 80, and 1 % BSA. All other sera used in this study were diluted in the same buffer, and were used at the following dilutions: guinea pig anti-*gt*, 1:500; rabbit anti-*Kr*, 1:100; rabbit anti-*eve* 1:500; mouse anti-*hb*, 1:500; guinea pig anti-*kni* 1:200. *Kr* and *eve* antisera were the same as those used by Stanojević *et al.* (1989), and Frasch and Levine (1987), respectively. The *hb* antiserum was made against a full-length *hb* protein produced in *E. coli* (Stanojević *et al.* 1989). *kni* antiserum was obtained from Dr Ken Howard. Indirect immunofluorescence was done with TRITC-conjugated or FITC-conjugated secondary antibodies to rat, rabbit, guinea pig and mouse, purchased from Jackson Immunoresearch (Bethesda, Md.). Photomicroscopy was done as described by Stanojević *et al.* (1989).

Fly stocks

The following fly stocks were used for embryo collections. *bcd*$^-$: bcd^{E1}, a strong, ems-induced small deletion mutation (Frohnhöfer and Nüsslein-Volhard, 1986; Berleth *et al.* 1988); *bcd*$^-$ embryos were collected from a mixed population of heterozygotes and homozygotes; *bcd* multiple copies: the *BB9+BB16* stock has two wild-type copies of *bcd* and two extra copies balanced over the second chromosome, making the total number of copies four or six in a balanced population of females; *exu*$^-$ and *stau*$^-$: exu^{PJ42}, a strong allele, and $stau^{HL54}$, a hypomorph (mutations are described in Schüpbach and Wieschaus, 1986; Tearle and Nüsslein-Volhard, 1986). All of the above stocks were obtained from Dr Gary Struhl, and *bcd* duplications are described in Struhl *et al.* (1989). Other maternal mutants used in this study were, *nanos*$^-$: $nanos^{L7}$, a hypomorph (G. Struhl, personal communication) provided by Dr Ruth Lehmann (Nüsslein-Volhard *et al.* 1987); *torso*$^-$: $torso^{PM51}$, a strong allele (Tearle and Nüsslein-Volhard, 1987; Schüpbach and Wieschaus, 1986); *torso* dominant gain-of-function mutants: $splc^{RL3}$ was provided by Dr Trudi Schüpbach (Schüpbach and Wieschaus, 1989), and tor^{D4021} was provided by Dr Martin Klingler (Klingler *et al.* 1988). The gap mutants used in this study were *hb*$^-$: hb^{14F21} (Lehmann and Nüsslein-Volhard, 1987); *Kr*$^-$: Kr^9 (Preiss *et al.* 1985; Redemann *et al.* 1988); *kni*$^-$: kni^{IID48} (Tearle and Nüsslein-Volhard, 1987), all of which are strong alleles; *tll*$^-$: tll^1, a hypomorph (Strecker *et al.* 1986, 1988); and *gt*$^-$: gt^{YA82}, a strong allele (Gergen and Wieschaus, 1986; Wieschaus *et al.* 1984*a*). All of the gap mutant stocks were obtained from Dr Jym Mohler.

Results

RNA expression pattern

gt transcripts were localized in whole-mount preparations of wild-type embryos using a full-length *gt* cDNA (see Materials and methods). The results that were obtained (Fig. 1A–D) are consistent with the previously published pattern based on hybridization to tissue sections (Mohler *et al.* 1989). Transcripts are first detected during nuclear cycle 12 as two broad bands encircling the embryo. The anterior band extends from about 80 % to 60 % egg-length (Fig. 1A), while the posterior band extends from about 30 % to 0 % egg-length (data not shown). The distribution of transcripts changes rapidly during the next two cleavage cycles, and the posterior band recedes from the pole, becoming narrower and now covers approximately 35 % to 25 % egg-length. The anterior domain splits into two bands at about mid-nuclear cycle 14, each of which includes approximately one and a half parasegments in width (about 71 % to 63 % and 85 % to 77 % egg-length, respectively). Simultaneously with the splitting of the anterior domains, expression is lost in the ventral-most portion of the anterior stripe (Fig. 1B, C, arrow), and by late cell cycle 14 a third anterior stripe appears in a dorsal patch very close to the anterior pole of the embryo, at 97 % to 91 % egg-length (Fig. 1D, arrow). At the end of cycle 14, a ventral patch reappears at the base of the middle head stripe (Fig. 1D). As the anterior pattern unfolds, the posterior domain fades slightly. By the time gastrulation is under way, the posterior domain is almost completely gone and the head staining becomes very intense. Anterior expression persists throughout germ band elongation and shortening (data not shown).

Protein expression pattern

The same cDNA used for examining the *gt* transcript pattern was used to make full-length protein in bacterial cells, and polyclonal antisera were generated against this product (see Materials and methods). The pattern seen in embryos stained with these sera (Fig. 1E–H) corresponds closely to the RNA expression pattern. The *gt* protein is first detected during nuclear cycle 13, within 10 min of the first appearance of the RNA. An important feature of the *gt* protein distribution is that it is nuclear, which introduces the possibility that *gt* is a transcription factor (see accompanying report by Kraut and Levine (1991); Small *et al.* unpublished data).

Co-localization of gap proteins

Direct comparisons of the gap gene expression patterns with *gt* can help elucidate ways in which *gt* may be functioning to control segmentation. To achieve this, we have carried out double immunolocalization of *gt* in pairwise combinations with each of the other cloned gap genes for which there are antibodies.

We have found that *gt* overlaps extensively with the *hb* and *kni* expression patterns, but is complementary to the *Krüppel* (*Kr*) pattern. The *gt* pattern is consistent with previously identified patterns of gap genes, in that

neighboring gap domains overlap where their expression intensities decline (see Stanojević *et al.* 1989; Gaul and Jäckle, 1989). Double staining for *hb* and *gt* (Fig. 2) reveals that the anterior *hb* pattern entirely encompasses the anterior *gt* domain, while the posterior *hb* stripe overlaps with *gt*'s posterior domain by a few cells (Fig. 2B).

The *knirps* (*kni*) posterior domain similarly overlaps the *gt* posterior pattern by a few cells on the anterior edge of the *gt* band. *gt*'s posterior domain is therefore straddled by *kni* at its anterior and *hb* at its posterior margins. At the anterior of the embryo, the *kni* and *gt* domains are completely exclusive, and remain so throughout the evolution of both patterns during cell cycle 14 (Fig. 2D–F). The central *Kr* domain and the two *gt* domains are entirely non-overlapping. The significance of this finding and the regulatory interactions between the two genes are discussed in the accompanying paper by Kraut and Levine (1991).

gt *expression in gap mutants*

The *gt* staining pattern in all of the known gap mutants demonstrates genetic interactions between *gt* and other gap genes, which are suggested by their relative expression patterns. *Kr* (Wieschaus *et al.* 1984*b*) has a strong effect on *gt* expression, in that both *gt* domains are expanded toward the center of Kr^- embryos (Fig. 3D,E). This result suggests that *Kr* is a strong repressor of *gt* expression since only very low levels of the *Kr* protein are detected at the margins of the *gt* domains. Several lines of evidence suggest that the *gt–Kr* interaction is direct (see Discussion and Kraut and Levine, 1991). The reduced expression of the expanded posterior *gt* domain in Kr^- is probably due to the premature loss of *kni* expression seen in these mutants (Pankratz *et al.* 1989; see below).

The *gt* protein distribution is only slightly altered in *hb* mutants (Fig. 3A–C). The posterior domain is expanded slightly toward the posterior, into the domain which *hb* normally occupies. The anterior domain is slightly shifted toward the anterior of the embryo, possibly due to repression by ectopic *Kr*, which has been shown to expand anteriorly in hb^- embryos (Jäckle *et al.* 1986). This suggests that *hb* has a slight repressive effect on *gt*, which is (notably) only operating in the posterior of the embryo. Not surprisingly, *hb* is not a repressor of the anterior *gt* pattern since proteins encoded by the two gap genes overlap extensively in these regions.

The major effect of kni^- (Nauber *et al.* 1988) is a premature reduction in the level of *gt* protein in the posterior domain (Fig. 4A). In the head domain, the middle stripe persists as an unbroken band covering the entire ventral side, in contrast to wild-type, where it is ventrally and ventral–laterally repressed (see Fig. 1G,H). This result establishes *kni* as a dual regulator of *gt*: in head regions it represses, and in the tail it maintains high levels of *gt*. This maintenance role for *kni* on *gt* in the posterior domain is reminiscent of the positive effect that *Kr* exerts on *kni* (Pankratz *et al.* 1989).

Finally, we tested the effect of *tailless*$^-$ (*tll*) on *gt* expression (Fig. 4D–F, Strecker *et al.* 1986; Pignoni *et al.* 1990). *tll* exerts a negative effect on *gt* expression, in that the posterior domain expands posteriorly, to 12% egg length. This result indicates that *tll* either directly or indirectly represses *gt* expression at the posterior pole, and probably participates in the normal refinement of the *gt* pattern during early development (see Fig. 1A, B). In tll^-, as in all the gap mutants, the relative positioning of the *gt* and *eve* patterns remains constant, (as shown by double stains in Figs 3,4,5,6 and 7), even though both patterns are disrupted. This implies a coordinated movement, so that, for example, in tll^- the posterior *gt* band still encompasses stripe six of *eve*, and the anterior margin of *eve* stripe 1 still coincides with *gt* head stripe 2.

In summary, the results presented here suggest that regulatory interactions with the gap genes modulate *gt* expression, but are not required for its initiation. Below we describe the role of maternal factors in the initiation of *gt* expression.

gt *expression in maternal mutants*

gt is among the earliest zygotic genes to be activated during embryogenesis. Given its very early expression, it seems likely that maternal morphogens could directly determine *gt*'s expression pattern in a way that may be analogous to their action on *hb* and *kni* (Gaul and Jäckle, 1989). In order to assess the effects of maternal products on *gt* expression, one representative mutant from each of the three maternal organizing groups was selected and stained with *gt* antibody.

bcd has been established as the anterior maternal morphogen (Frohnhöfer and Nüsslein-Volhard, 1986; Driever and Nüsslein-Volhard, 1988*a*,*b*) responsible for determining head and thoracic structures. One mode of action is its direct activation of *hb* (Driever and Nüsslein-Volhard, 1989; Struhl *et al.* 1989). But because hb^- exhibits only a subset of the defects seen in the *bcd* mutant, *hb* clearly cannot be the only product that responds to *bcd* (Frohnhöfer and Nüsslein-Volhard, 1986; Driever *et al.* 1989). Therefore, we suspected that *gt*, which is expressed in the head but in a smaller region than *hb*, may also be a target for the *bcd* morphogen. Embryos from *bcd* homozygotes fail to initiate the anterior *gt* domain. Fig. 5 shows changes in the *gt* pattern observed in *bcd* mutants. Although the anterior domain of expression is absent, the posterior *gt* domain is correctly initiated. Co-localization studies with anti-*eve* and anti-*gt* antibodies indicate that there is a coordinated shift of the remaining part of the *gt* pattern with the remaining *eve* stripes, as is seen for all other mutants tested.

We believe that in addition to being a target of *bcd*, *gt* may also respond to the posterior morphogen *nos* (Nüsslein-Volhard *et al.* 1987; Lehmann, 1988). In *nos* mutant embryos, the *gt* posterior domain is usually absent, but is occasionally seen as a very weak and transient stripe (Fig. 6). nos^+ gene activity is known to prevent maternal *hb* expression in the posterior of the embryo, and thereby allow the initiation of the

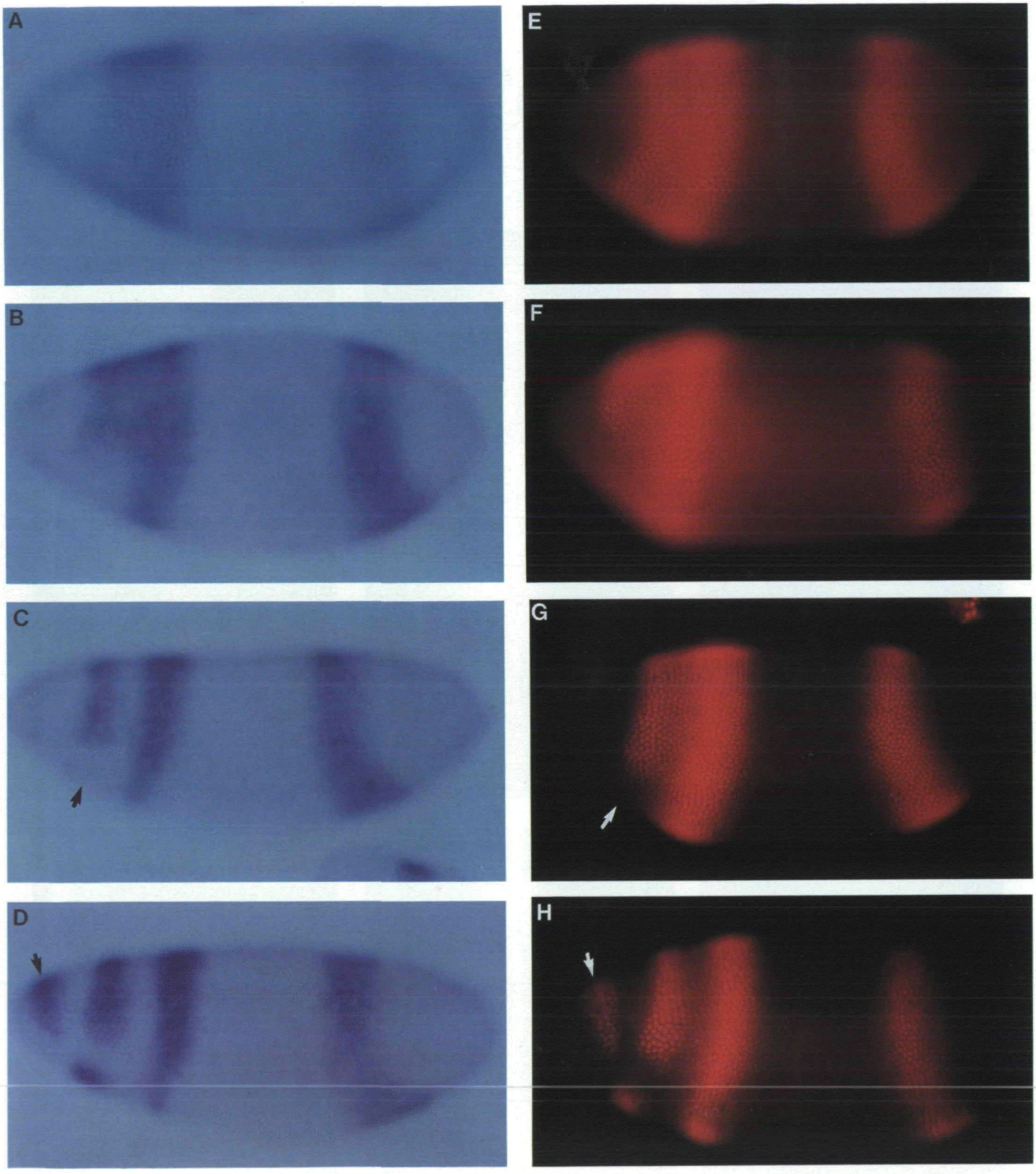


Fig. 1. Wild-type *gt* expression patterns. (A–D) Whole mount *in situs* with digoxygenin-U-labelled probe from *gt* cDNA. (E–H) Embryos stained with anti-*gt* antibodies. (A) Embryo at approximately nuclear cycle 13, showing the early pattern in two domains. Posterior domain extends from 17% to 33% egg length (e.l.) (where 0% is at posterior pole), anterior domain from approx. 61% to 83% e.l. (B) Embryo at early cycle 14. Expression in the anterior domain fades in ventral regions. (C) Mid-cycle 14 embryo. The anterior domain splits into two stripes, each encompassing about one and a half parasegments in width. Note that the anterior-most stripe fades in ventral regions (arrow). (D) During late cycle 14, a third head stripe very near the anterior pole (arrow) arises as a dorsal patch at approx. 91% to 97% e.l. Middle head stripe reappears in ventral regions. (E–H) Embryos stained with antibody to full-length *gt* protein, produced in *E. coli*. Each embryo is at a similar stage to the corresponding RNA pattern shown to its left. Indirect immunofluorescence was done with a secondary antibody conjugated with rhodamine (TRITC).

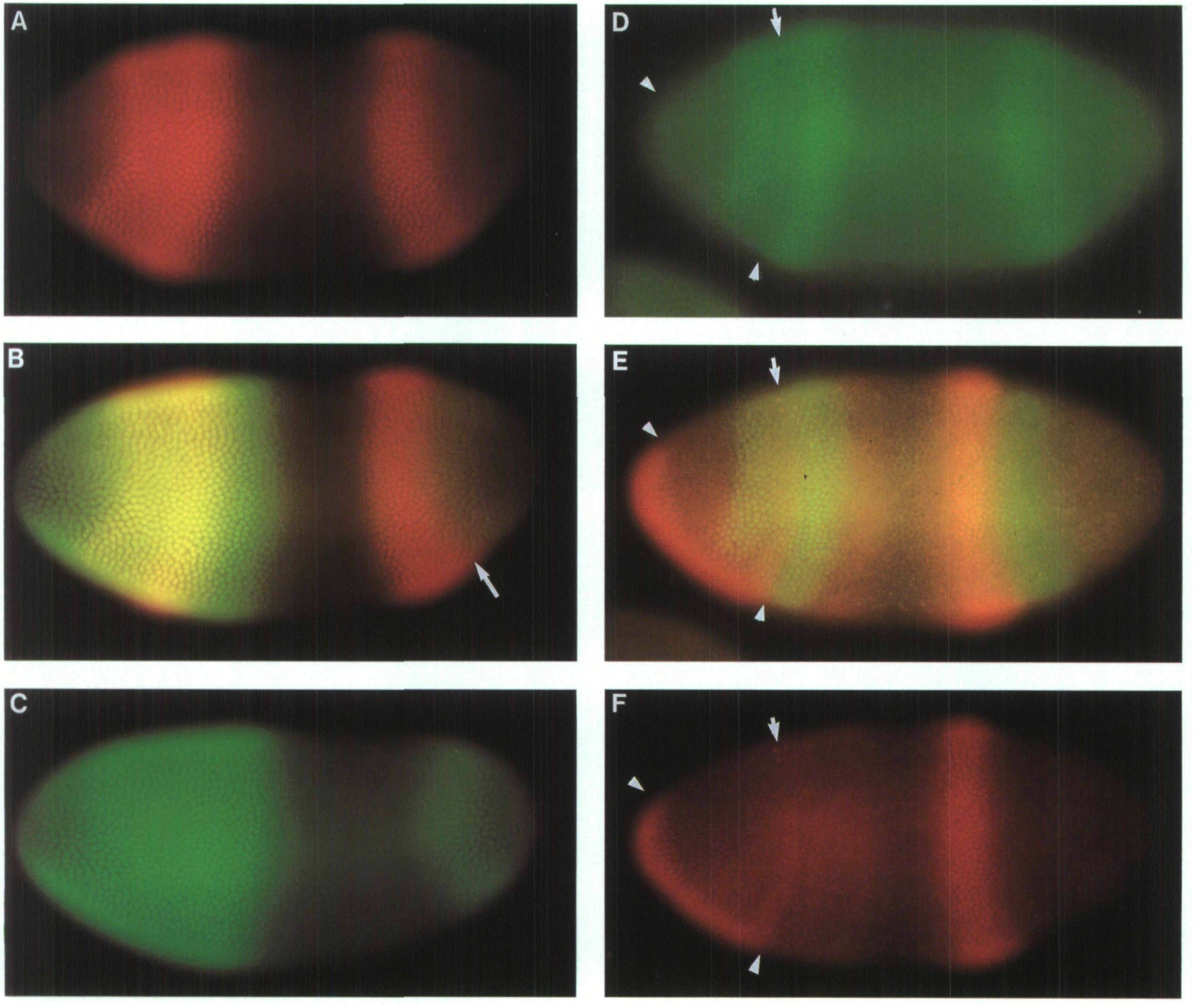


Fig. 2. Double immunolocalization of *giant* with *hunchback* and *knirps*. (A–C) Co-localization of *gt* and *hb* proteins in a cycle 14 embryo. (A) Single exposure showing *gt* pattern alone, (B) Double exposure showing *hb* (green) and *gt* (red); overlap appears yellow. *hb* entirely covers *gt* domain in head region. Substantial overlap (4–5 nuclei) occurs at the posterior edge of the abdominal *gt* band (arrow). (C) *hb* expression in the same embryo shown in A and B. (D–F) Co-localization of *gt* and *kni* proteins in a stage 14 embyro. (D) *gt* alone, with arrowhead pointing to ventral fading of anterior head stripe; arrow indicates the splitting of the anterior domain into two head stripes. (E) Same embryo double-exposed for *gt* (green) and *kni* (red). Arrow in same position as shown in D. Anterior *kni* expression appears exactly in the gap between the two *gt* head stripes; arrowhead at limit of ventral *kni* domain, which appears exactly where *gt* stripe fades ventrally. (F) Arrowheads in D–F indicate the limits of anterior *kni* domain.

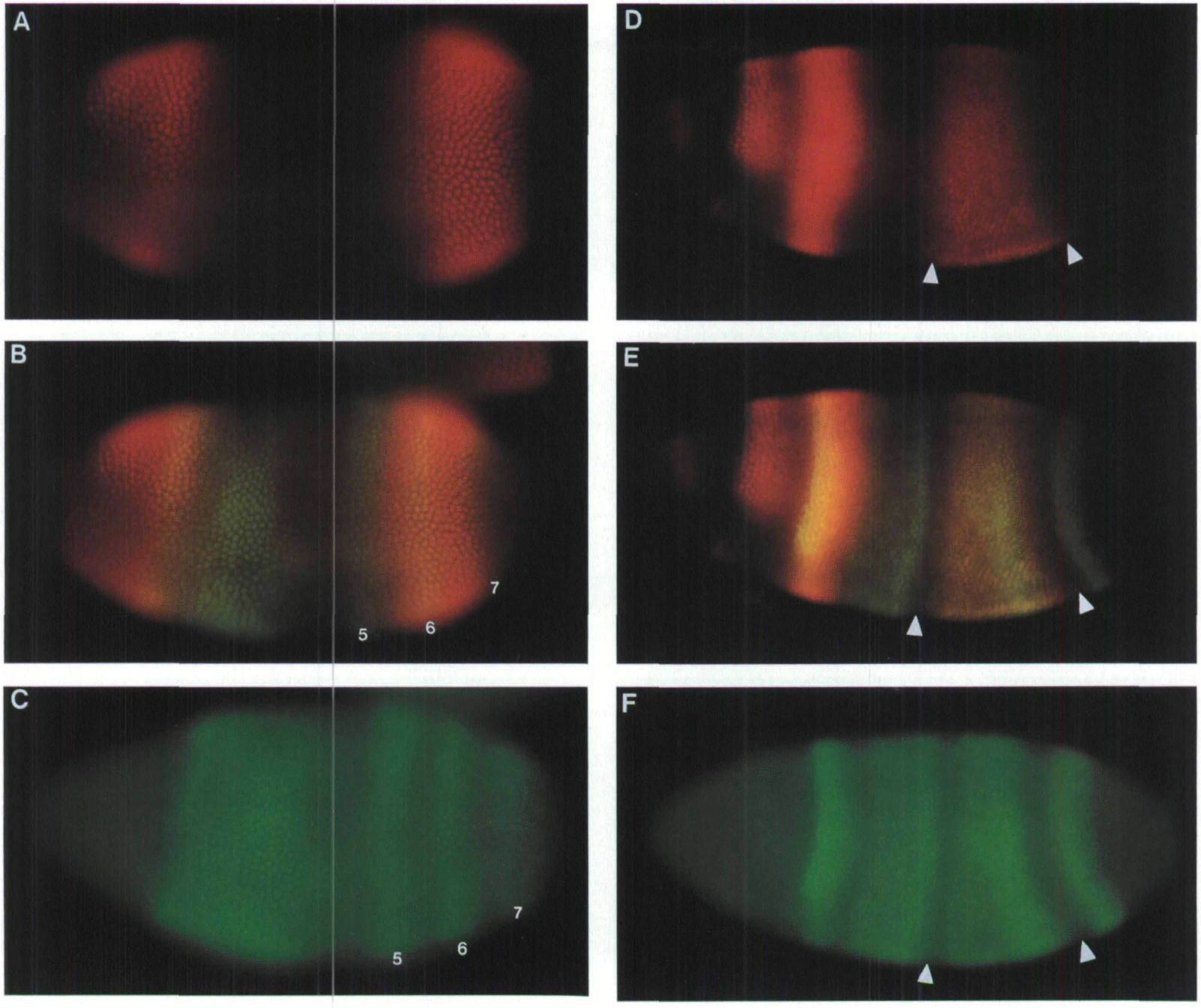


Fig. 3. *giant* expression in gap mutants *hb* and *Kr*. (A–C) *gt* and *eve* patterns in a *hb* mutant embryo at cleavage cycle 14. (A) *gt* expression. The anterior pattern is relatively normal while the posterior band is expanded posteriorly (compare with Fig. 1G). (B) Co-localization of *eve* (green) and *gt* (red). Overlap of patterns appears yellow. Numbers indicate *eve* stripes. Note posterior *gt* pattern covers stripe 6, and abuts stripe 5, as in wild-type. (C) *eve* pattern alone, showing disruption in stripes 1–4. (D–F) *gt* pattern in *Kr* mutant embryo at cleavage cycle 14. (D) *gt* expression. The anterior and posterior domains expand toward center of embryo. Arrowheads point out limits of *gt* posterior domain. (E) *gt* and *eve* co-localization, arrowheads as in D. Overlap of the patterns appears yellow. (F) *eve* pattern alone, showing disruption in stripes 2–6, and arrowheads delimit *gt* domain as in D and E.

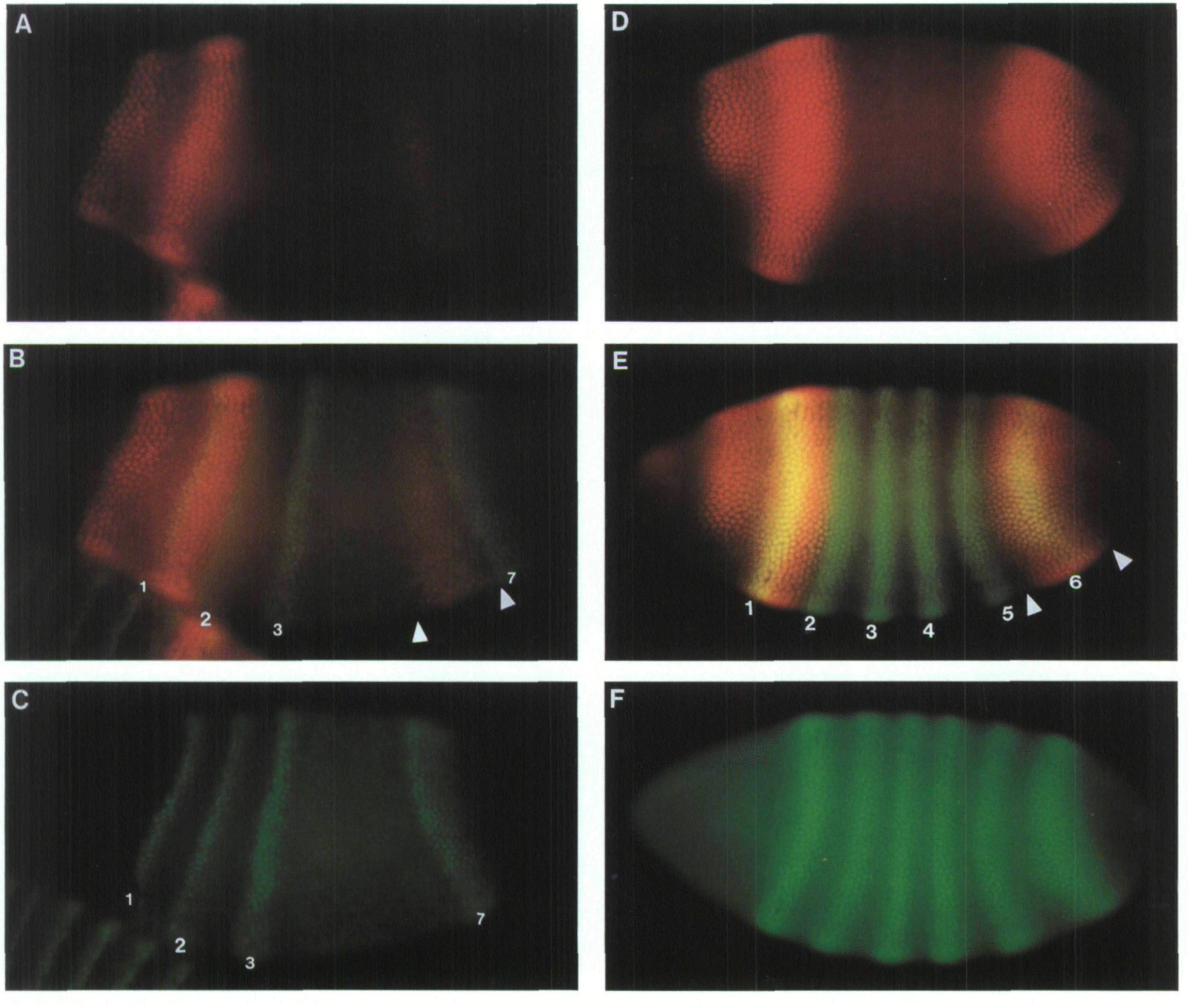
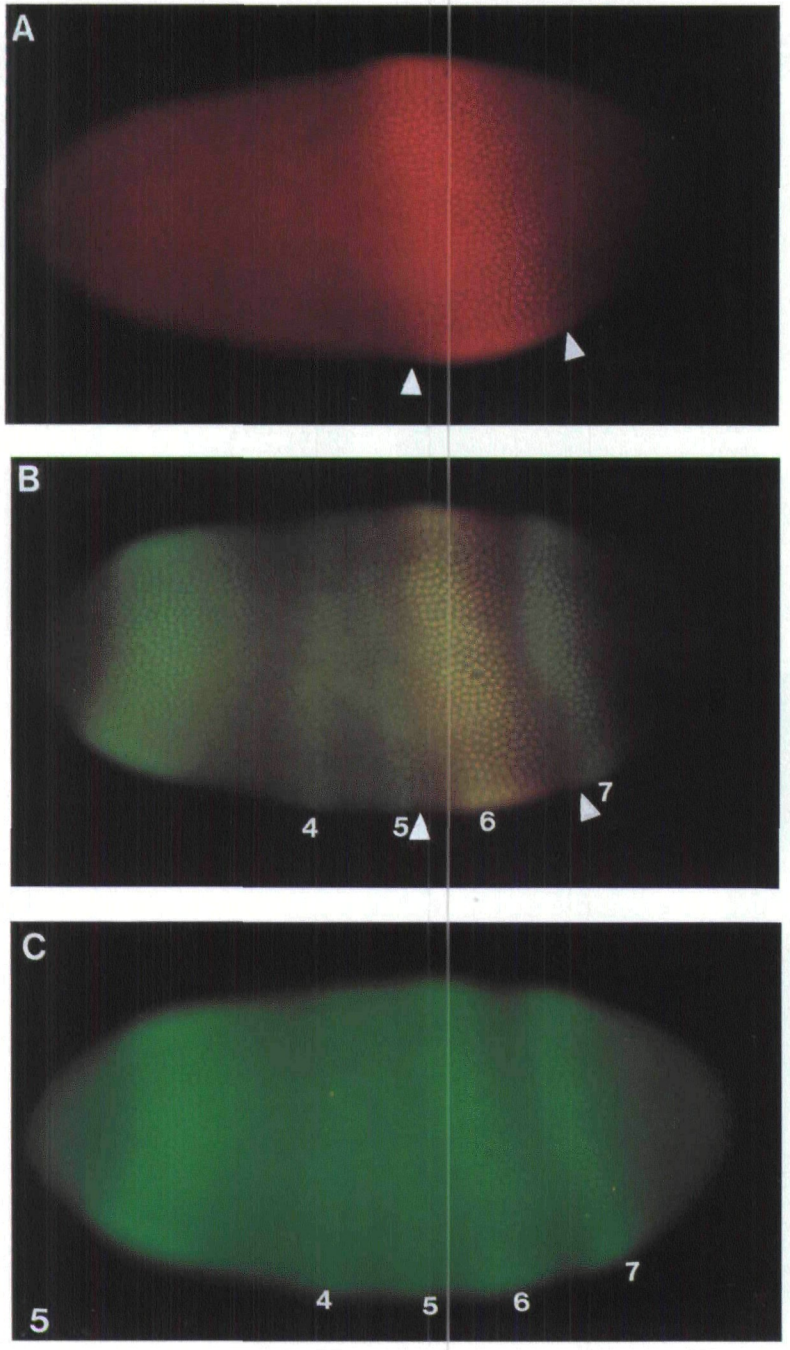


Fig. 4. *gt* expression in gap mutants *knirps* and *tailless*. (A–C) *gt* and *eve* expression in *kni* mutant embryo (cleavage cycle 14). (A) *gt* staining alone, showing premature disappearance of posterior band, and anterior-most head stripe extending all the way ventrally. Normally at this stage, ventral expression of this stripe fades (see Fig. 1H). (B) Co-localization of *gt* and *eve*. Numbers indicate *eve* stripes. Arrowheads bracket the very faint posterior *gt* band. Note that the *gt* pattern coincides with *eve* pattern in normal positions, despite disruptions in *eve* stripes 4–6. (C) Same embryo showing the *eve* staining alone. (D–F) *gt* and *eve* patterns in *tll* mutant embryo. (D) *gt* staining alone, showing slight posterior expansion of posterior domain. (E) Co-localization of *gt* and *eve*. Numbers indicate *eve* stripes, which are also expanded coordinately toward the posterior pole. Arrowheads indicate posterior limits of *gt* domain; note normal overlap with *eve* stripes 1 and 6, despite shifting of both patterns. (F) *eve* pattern alone.



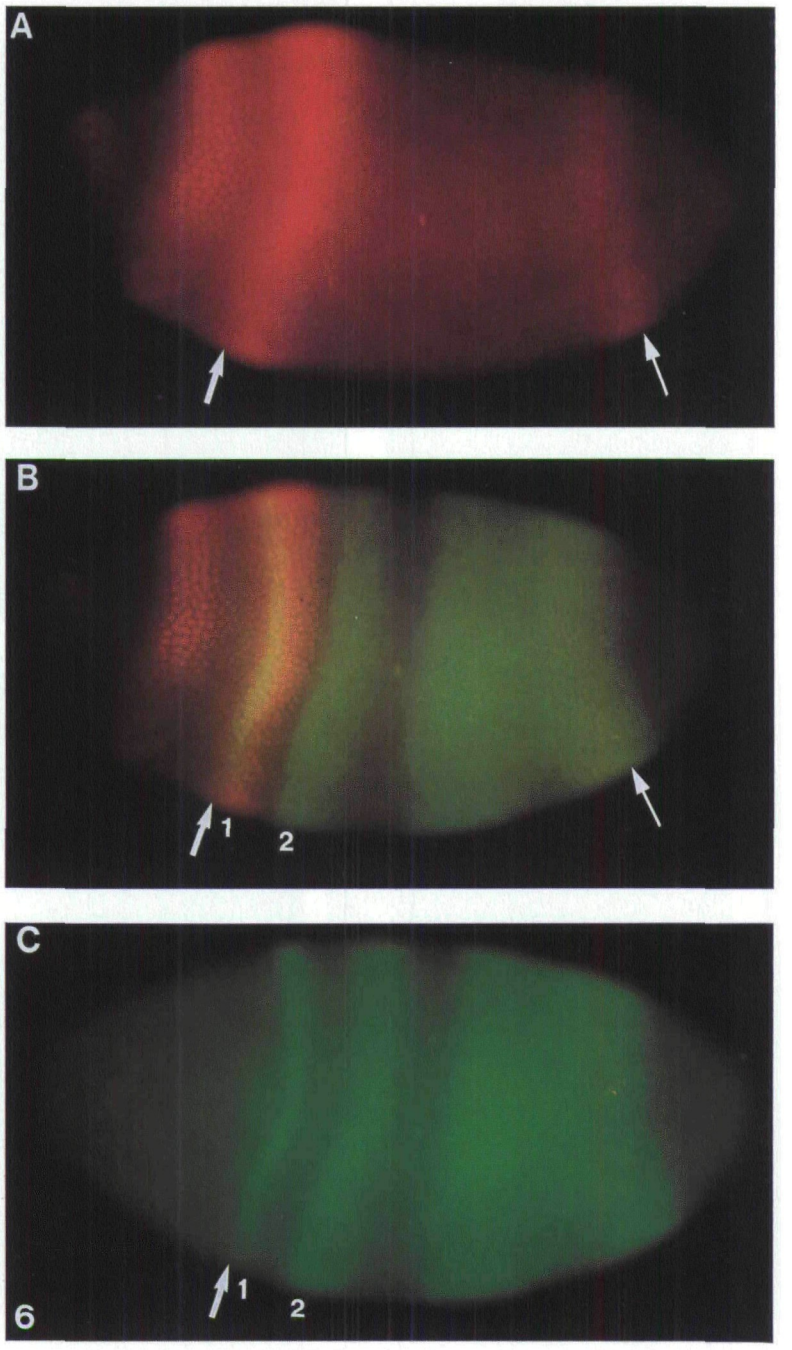
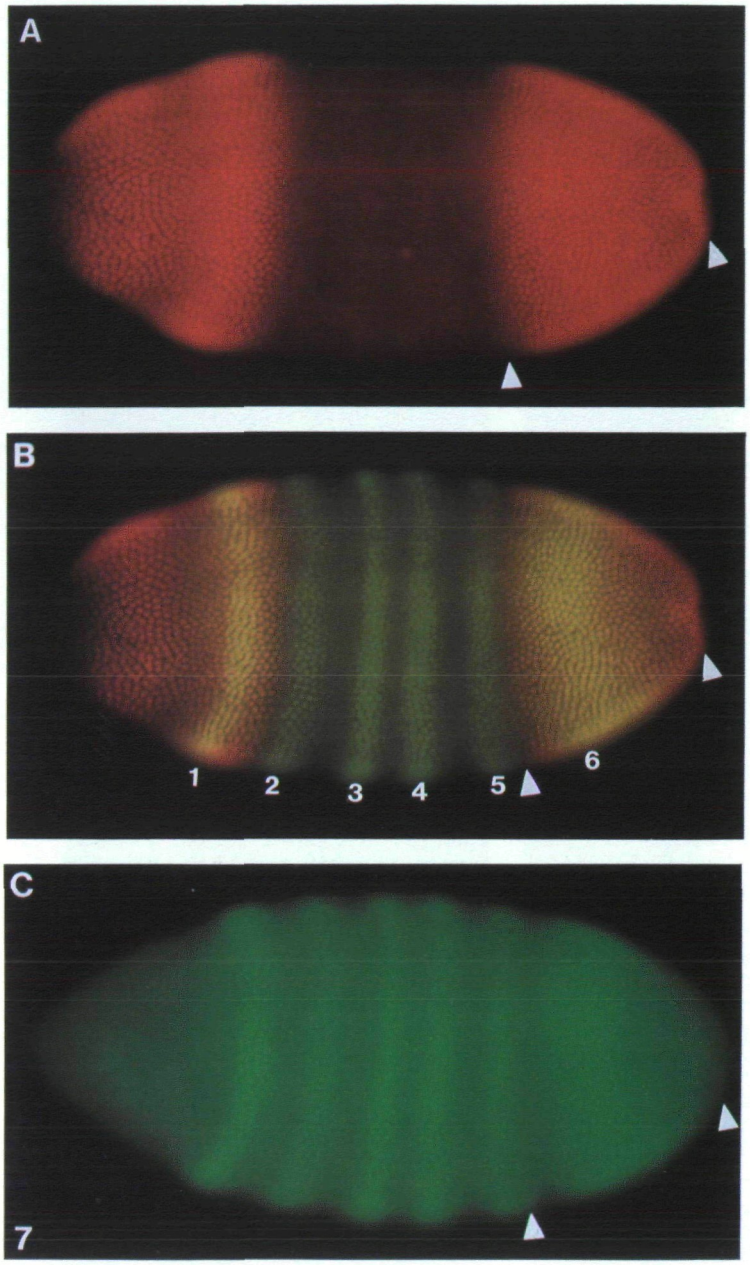


Fig. 5. *gt* expression pattern in a *bcd* mutant.
(A) Alteration of *gt* staining pattern in cleavage cycle 14 bcd^- embryo. The anterior *gt* domain is not initiated, and there is an anterior expansion of the posterior band. Arrowheads indicate limits of posterior domain. (B) Co-localization of *gt* and *eve*. Arrowheads indicate same positions as in A. Numbers indicate *eve* stripes, which are also shifted toward the anterior, and stripes 1–3 are missing. Note that overlap of posterior *gt* band with *eve* stripe 6 is maintained despite shift in both patterns. (C) *eve* pattern alone, numbers again indicating stripe number.

Fig. 6. *gt* and *eve* patterns in a *nanos* mutant.
(A) Alteration in *gt* staining pattern in cleavage cycle 14 nos^- embryo includes a severe reduction of the posterior domain, although very faint staining can be detected in some cases (indicated by small arrow). Anterior domain is unaffected. Anterior thick arrow points to region where *eve* stripe 1 occurs.
(B) Double exposure showing both *gt* and *eve* patterns. Numbers indicate *eve* stripes. Stripes 3–7 are disrupted, but overlap of *gt* pattern with stripe 1 is normal. (C) *eve* pattern alone.



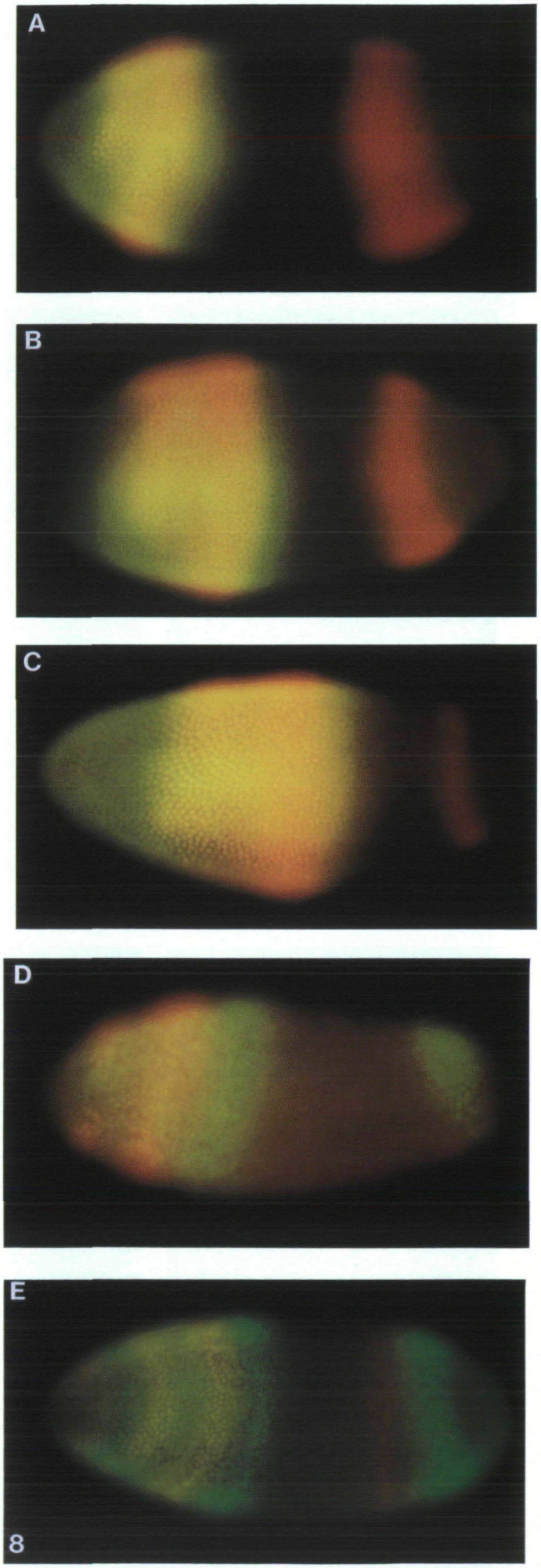


Fig. 7. *gt* and *eve* patterns in a *torso* mutant. (A) Alteration in *gt* staining pattern in tor^- embryo at cleavage cycle 14. Arrowheads point to limits of posterior domain; note posterior expansion, extending all the way to posterior pole. (B) Double exposure showing both *gt* and *eve* in the same embryo as A. Numbers indicate *eve* stripes. Arrowheads point to limits of *gt* posterior domain, as in A. Note correspondence between *gt* domains and *eve* stripes 1 and 6, despite posterior shift of both patterns. (C) *eve* pattern alone. Arrowheads indicate limits of posterior *gt* domain, which covers the greatly expanded stripe 6.

Fig. 8. *gt* and *hb* patterns in the presence of varying copies of *bcd*. A through F show the effect on *gt* (red stain) and *hb* (green stain) of increasing doses of the *bcd* gene. Overlap of the patterns appears yellow. (A) Anterior shift of both patterns is seen in a bcd^+/bcd^- heterozygous embryo. (B) Both patterns are shifted posteriorly in the presence of four copies of *bcd*. (C) Even more severe posterior-ward shift of both patterns is seen in the presence of six copies of *bcd*. Note that the *gt* pattern now extends beyond the middle of the embryo, but its relative positioning to *hb*'s pattern is retained. (D) $stau^-$ embryo, showing anterior shift of both patterns, similar to the *bcd* heterozygote seen in (A). (E) exu^- embryo, showing severe reduction of *gt* staining, and more restricted limits of the *hb* pattern.

abdomen-determining gene *kni* (Hülskamp *et al.* 1989; Irish *et al.* 1989*a*; Struhl, 1989*a*). Again, the apparent positive effect that *nos* exerts on *gt* is likely to be indirect. It has been proposed that *nos* represses maternal *hb* expression post-transcriptionally by either degrading *hb* transcripts and/or blocking their translation. The effect of *nos* on *gt* may be mediated solely through maternal *hb*. In the absence of *nos*, maternal *hb* protein is expressed throughout the egg, and may repress both *gt* and *kni* expression.

The *gt* pattern is also affected in embryos lacking the maternal product *tor*, which is responsible for specifying terminal structures (acron and telson) (Klingler *et al.* 1988; Schüpbach and Wieschaus, 1989). In these mutants, the abdominal *gt* stripe extends all the way to the posterior pole. The anterior pole is also affected in that the anterior-most head patch of *gt* disappears, while the remainder of the anterior pattern is expanded and shifted (Fig. 7). However, expression does not extend all the way to the tip, indicating independent repression by the *bcd* system (see Discussion).

tor$^-$ exceeds *tll*$^-$ in its effect on *gt* expression, implying that *tll* is not the only repressor of *gt* at the poles. Examination of *tor*$^-$ embryos by double staining with both *eve* and *gt* reveals a coordinated movement of the *gt* pattern and the *eve* stripes, similar to the effect seen in *tll*$^-$ mutants. This lends further support to the notion that particular gap gene combinations establish the individual pair-rule stripes (Gaul and Jäckle, 1989; Stanojević *et al.* 1989).

Coordinate regulation of gt *and* hb *by the* bcd *concentration gradient*

According to the 'French Flag' model for localized patterns of gap gene expression, the overlapping, but non-coincident *hb* and *gt* patterns in anterior regions of the early embryo could involve the differential response of these two genes to distinct thresholds of the *bcd* morphogen. Perhaps the *hb* domain extends beyond the limits of the *gt* pattern because it is activated by even low levels of *bcd* protein. *gt* might be restricted to more anterior regions due to a less sensitive response to *bcd*. As a first step towards testing this idea, we examined the relative patterns of *hb* and *gt* expression in embryos containing varying doses of the *bcd* gene. The two patterns show a coordinated shift with increasing doses of *bcd* (Fig. 8), from one copy of the *bcd* gene (*bcd*$^+$/*bcd*$^-$ [Fig. 8A]) where *gt* and *hb* are shifted slightly toward the anterior, to six copies (Fig. 8C), where both *hb* and *gt* show a marked expansion into posterior regions.

We also examined *gt* expression in the maternal mutants *staufen* (*stau*) and *exuperantia* (*exu*) (Schüpbach and Wieschaus, 1986) in order to examine changes in the limits of *gt* and *hb* expression as a consequence of alterations in the slope of the *bcd* gradient (Fig. 8D,E). *exu*$^-$ alters the distribution of *bcd* mRNA, so that low levels of *bcd* protein are present relatively evenly throughout the embryo (Frohnhöfer and Nüsslein-Volhard, 1987; Driever and Nüsslein-Volhard, 1988*b*; Berleth *et al.* 1988). *stau*$^-$ also alters the distribution of *bcd* products, but does not completely eradicate the concentration gradient of *bcd* protein. Instead, it results in a very shallow gradient of *bcd*, with reduced amounts of protein in anterior regions of the embryo (Driever and Nüsslein-Volhard, 1988*b*). *gt*'s posterior domain is abolished in *stau*$^-$, and the anterior domain is shifted anteriorly (Fig. 8D), similar to the shift seen in embryos heterozygous for *bcd*$^-$. *hb* is also shifted anteriorly in concert with *gt*. Both genes are also affected in *exu* mutant embryos, but in a different way from that seen in *stau*$^-$. *exu*$^-$ strongly reduces *gt* expression in both anterior and posterior regions, but the positioning of the pattern is not drastically altered (Fig. 8E). The embryo shown here represents the strongest expression of *gt* seen in *exu*$^-$ mutants. Most mutant embryos completely lack *gt* expression. In contrast, *hb* is expressed at high levels, but is restricted to somewhat more anterior regions than in wild-type.

gt *expression in* nos,Kr *double mutants*

Maternal morphogens are thought to directly activate certain gap genes, such as *hb* (Driever *et al.* 1989; Struhl *et al.* 1989). In addition, it has been proposed that localized gap expression patterns involve regulatory interactions among the gap genes (Jäckle *et al.* 1986). In order to determine the extent to which these two mechanisms operate to regulate *gt* expression, we examined embryos that were doubly mutant for *nos*$^-$ and *Kr*$^-$. The aim here was to determine whether the loss of the *gt* expression pattern observed in the maternal mutant *nos* was due to ectopic expression of maternal *hb* products, or an indirect effect due to the *Kr* repressor. Previous studies have shown that the central *Kr* domain expands posteriorly in *nos*$^-$ mutants (Gaul and Jäckle, 1987*a*). Such an altered *Kr* pattern could repress *gt*, since we have shown that the *gt* domains expand in *Kr*$^-$ embryos (Fig. 3D,E). If *Kr* repression is responsible for the loss of *gt* expression in *nos*$^-$ embryos, then *gt* should 'reappear' in *nos*$^-$,*Kr*$^-$ double mutants. In fact, we found that *Kr*$^-$ does not rescue *gt* expression in a *nos*$^-$ background, since all embryos from a *nos*$^-$/*nos*$^-$;*Kr*$^-$/CyO mutant stock are missing the posterior domain, similar to that seen in *nos*$^-$ (data not shown). This result suggests that *Kr* does not define the limits of *gt*'s posterior domain, but instead the pattern is more likely regulated by maternal *hb* repressor. Once established, the maintenance of the *gt* pattern involves regulatory interactions with *Kr*.

Discussion

The genetic studies presented here suggest that maternal factors are responsible for the initiation of *gt* expression in anterior and posterior regions of the early embryo. The maternal morphogen *bcd* (Nüsslein-Volhard *et al.* 1987) is important for the initiation of the anterior pattern, while posterior expression is controlled in part by *hb* (Lehmann and Nüsslein-Volhard, 1987). The limits of the anterior *gt* domain are set by the levels of the *bcd* protein, and the non-coincident

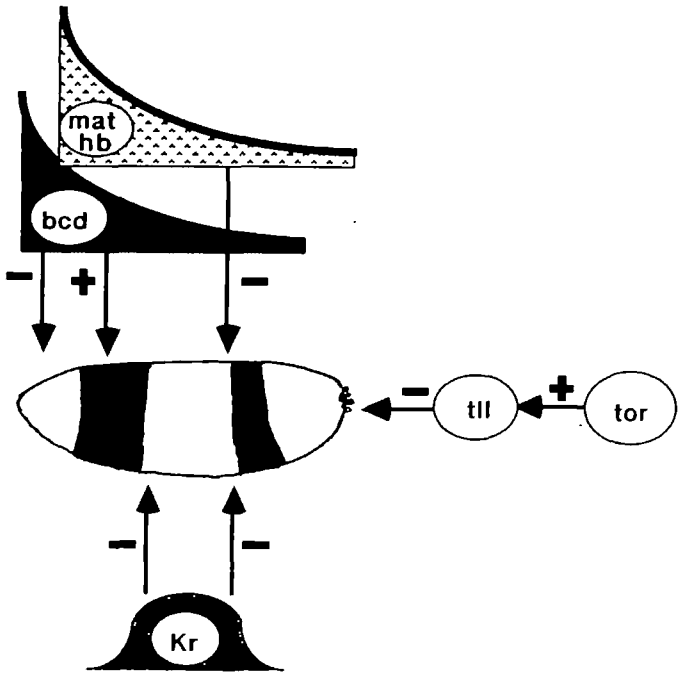


Fig. 9. Summary of the genetic control of *gt* expression. The diagram of the embryo shows the two domains of *gt* expression. The '+' and '–' symbols indicate positive and negative regulatory interactions. For example, activation and repression by *bcd* defines the anterior *gt* domain, while *tor* represses expression in the posterior pole *via tll*. The broad, bell-shaped *Kr* pattern represses *gt* expression in central regions. mat hb: maternal *hb* products.

patterns of *hb* and *gt* expression involve their differential response to distinct thresholds of the *bcd* morphogen. Repression by maternal *hb* products determines the overlapping patterns of *kni* and *gt* expression in posterior regions, which are maintained by cross-regulatory interactions among gap genes (Jäckle *et al.* 1986). The genetic control of the *gt* expression pattern is summarized in Fig. 9.

Initiation of anterior gt *expression*

The *bcd* morphogen appears to regulate the initiation of the anterior *gt* pattern through a 'French flag' mechanism (Wolpert, 1969). The *gt* promoter is either active or repressed depending on the level of the *bcd* protein present in a given nucleus in the syncytial blastoderm. Peak levels of *bcd*, present in the anterior-most regions, coincide with sites where *gt* is not activated. Intermediate levels of the morphogen activate *gt* expression, while low levels, present in more posterior regions, are insufficient for activation. *bcd* probably exerts a direct effect on *gt* expression since null mutations in each of the known gap genes fail to abolish its initiation, and *gt*'s early expression makes intermediate regulatory steps unlikely. It may be that the *bcd* protein binds directly to *gt*'s promoter to activate its expression. The promoter might contain multiple *bcd* binding sites, as was shown for the *hb* promoter (Driever and Nüsslein-Volhard, 1989) and the morphogen might fail to activate *gt* when either all of these sites are occupied or too few are occupied. According to this 'titration' model, activation is achieved only when some of the *bcd* sites are bound with morphogen. Thus, in anterior and posterior regions of the early embryo there are either too high or too low levels of *bcd* protein to activate *gt* expression. A similar model has been proposed for the regulation of the gap gene *Kr* by *bcd* (Gaul and Jäckle, 1989; Hülskamp *et al.* 1990).

An alternative explanation for localized *gt* expression is that repression in the anterior-most regions involves an unknown intermediate gene(s) which is activated by peak levels of *bcd* (Driever *et al.* 1989). This model is consistent with results of transient cotransfection assays, which have shown that *bcd* can function as a sequence-specific transcriptional activator. Such studies have failed to demonstrate a repressor activity for the *bcd* protein (Driever and Nüsslein-Volhard, 1989; Struhl *et al.* 1989). The initiation of any such putative intermediate target gene is also sensitive to the concentration of the *bcd* protein since increasing copies of the *bcd* gene result in a progressively broader zone at the anterior pole where *gt* is repressed (see Fig. 8).

Initiation of posterior gt *expression*

Plausible mechanisms have been put forth for *bcd*'s activation of the gap patterns in the anterior of the embryo, whereas it is still a mystery as to how posterior gap domains are initiated. So far, no abdominal activators of *kni* or *gt* are known; however, we propose that the overlapping patterns of *gt* and *kni* expression in posterior regions involve their differential repression by maternally expressed *hb* protein (Fig. 9). The *hb* transcript is uniformly distributed throughout the cytoplasm of unfertilized eggs and early embryos (Tautz *et al.* 1987). This transcript is not stably translated in all regions of the embryo, such that transcripts in anterior regions are more stably translated than those in posterior regions. This results in a protein concentration gradient by cleavage cycle 11–12 with peak levels in anterior regions, and lower levels in more posterior regions (Tautz, 1988).

Unlike the situation for *bcd*, which is a direct transcriptional activator, the most likely mechanism for *hb*'s action is that it exerts a repressive effect on *kni* and *gt*, thereby restricting their expression to posterior regions lacking maternal *hb* protein (Hülskamp *et al.* 1989; Irish *et al.* 1989*a*; Struhl, 1989*a*). The fact that the patterns of *kni* and *gt* are out of register could be explained if the two genes are repressed by different threshold levels of *hb*, with *gt* being more sensitive to the *hb* repressor. This would restrict *gt* expression to a region posterior to the *kni* pattern. We examined the *gt* and *kni* patterns in embryos where *hb* is overexpressed under the control of the hsp70 promoter (Struhl, 1989*a*). Embryos exposed to a high dose of ectopic *hb* at early stages completely failed to initiate both *kni*'s and *gt*'s posterior domains (Kraul and Levine, 1991).

The concentration gradient of maternal *hb* protein is generated by *nos*, which somehow interferes with *hb* expression in posterior regions (Tautz, 1988). It has been proposed that *nos* constitutes the 'sink' for the *hb*

gradient, either by blocking its translation or stability (Hülskamp *et al.* 1989; Irish *et al.* 1989*a*; Struhl, 1989*a*). *nos* activity is thought to emanate from posterior regions of the egg (Sander and Lehmann, 1988; Nüsslein-Volhard and Roth, 1989). If this is correct, a critical level of *nos* expression near the posterior pole would define the site where *gt* can be activated due to the complete absence of *hb* repressor. In slightly more anterior regions, there are somewhat lower levels of *nos*, permitting the expression of just enough *hb* to repress *gt*. In nos^- embryos, the *hb* protein is distributed along the entire length of the embryo (Tautz, 1988), thereby blocking the initiation of *kni* and *gt* in posterior regions.

Recent studies have shown that the only purpose of *nanos* is to suppress maternal *hb* in posterior regions of early embryos (Hulskamp *et al.* 1989; Irish *et al.* 1989*a*; Struhl, 1989*a*). Double mutants that lack both maternal *hb* and *nanos* show a normal segmentation pattern, and even survive to adulthood. Although not specifically tested, it is likely that *kni* and *gt* are correctly initiated in such mutants, suggesting that maternal *hb* is not an essential morphogen. However, it is conceivable that in wild-type embryos the zygotic *hb* gradient functions redundantly with the maternal *hb* gradient to trigger overlapping patterns of *kni* and *gt* expression. The maternal and zygotic *hb* gradients show similar slopes along the anterior–posterior axis. Zygotic *hb* expression is correctly activated by the *bcd* morphogen in embryos lacking both *nos* and maternal *hb* products, giving a gradient of expression that is quite similar to the transient maternal gradient seen in wild-type. Perhaps this zygotic gradient is sufficient to generate overlapping *kni* and *gt* expression patterns in embryos lacking both maternal *hb* and *nos* products.

Since we have seen that *hb* is capable of repressing *gt* in the posterior of the embryo, the problem arises as to how *gt* escapes repression by the high levels of *hb* that exist in anterior regions. A competitive mechanism between *bcd* and *hb* might allow *gt* to be expressed in anterior regions, whereby the *bcd* activator occupies *gt*'s promoter more avidly, excluding *hb*. It is also possible that the *gt* promoter contains at least two discrete and autonomous elements, one responsible for expression in anterior regions and another that directs posterior expression. As discussed above, it is likely that *bcd* would activate a putative anterior promoter element directly *via bcd* binding sites. This element would be insensitive to repression by *hb* if it lacks *hb* binding sites. In contrast, a posterior promoter element might contain numerous *hb* binding sites, thereby making it very sensitive to repression by even low levels of the *hb* protein. An important implication of this model is that repression by *hb* does not occur over long distances within the *gt* promoter. In anterior regions there are high levels of *hb* protein, which should fill all hypothetical *hb* binding sites within the posterior promoter element. According to the model, the *hb* proteins bound to the posterior element are unable to repress over a long enough distance to interfere with the activation of the anterior element by *bcd*.

Differential regulation of gt *and* hb *by* bcd

Overlapping patterns of *hb* and *gt* expression in anterior regions, and *kni* and *gt* in posterior regions, could arise from the differential regulation of these genes by a common set of maternal factors. As discussed above, the out-of-register patterns of *kni* and *gt* in posterior regions could involve their differential repression by distinct thresholds of the *hb* gradient. Similarly, the noncoincident *hb* and *gt* patterns in anterior regions could be due to their differential response to the *bcd* gradient. It has been proposed that there might be a gene whose domain of expression is a subset of the anterior *hb* pattern, designated 'gene X' (Driever *et al.* 1989). This was originally based on the observation that the phenotype of hb^- is not as severe as that seen in bcd^- (Frohnhöfer and Nüsslein-Volhard, 1986). Recent support for this notion stems from the finding that progressively more posterior patterns of expression are obtained from fusion genes driven by synthetic promoters containing increasing numbers of *bcd* binding sites (Driever *et al.* 1989; Struhl *et al.* 1989). This result established the principle that the limits of a *bcd* target gene are determined by the configuration, strength and number of *bcd* binding sites present in its promoter. Target genes containing relatively few and/or low affinity binding sites would be activated only by peak levels of the *bcd* protein, and therefore be restricted to the anterior-most regions of the embryo. In contrast, a target gene such as *hb* that contains multiple, high affinity binding sites should be activated by even low levels of *bcd*, and therefore be expressed from the anterior pole into relatively posterior regions of the embryo.

We propose that the early pattern of *gt* expression fulfils the requirement for a gene 'X'. The restriction of *gt* to a subset of the *hb* anterior pattern could be due to the presence of fewer high affinity *bcd* binding sites in the *gt* promoter as compared with the *hb* promoter (Driever and Nüsslein-Volhard, 1989). During the final stages of preparing this manuscript other ostensible targets of the *bcd* morphogen have been identified, including *empty spiracles* (Dalton *et al.* 1989) and *orthodenticle*, which are homeobox genes, and *buttonhead* (Cohen and Jürgens, 1990; Finkelstein and Perrimon, 1990). It is conceivable that all of these genes are direct targets of *bcd*, although it is possible that some are only indirectly regulated by *bcd*. Here we have shown that changes in the number of *bcd* copies results in a correlative alteration in the limits of *gt* expression at very early stages of embryogenesis (prior to cellularization; see Fig. 8). This early effect of *bcd* dose on *gt* expression suggests that *bcd* is likely to act directly on *gt*.

Mutations in the maternal genes *exu* and *stau* (Schüpbach and Wieschaus, 1986) were used to examine the effects of altering the shape and amplitude of the *bcd* gradient (Berleth *et al.* 1988; Driever and Nüsslein-Volhard, 1988*b*) on the patterns of *hb* and *gt* expression. *exu* causes such a severe reduction in the overall levels of *bcd* protein that *gt* expression is nearly abolished. In contrast, the reduced levels of *bcd* are still

sufficient to permit activation of *hb*, albeit in a narrower domain than normal. These results provide further evidence that *hb* can be activated by a lower threshold of *bcd* morphogen than that required to initiate *gt*, which is probably the basis for *gt*'s restriction to a subset of the *hb* pattern in wild-type embryos. The coordinated shift of the *gt* and *hb* patterns seen in *stau* mutants is also consistent with a direct response of both *hb* and *gt* to *bcd* levels, since the effect of *stau* is to drop the overall amplitude of the *bcd* concentration gradient (Berleth *et al.* 1988; Driever and Nüsslein-Volhard, 1988*b*).

Cross-regulation between gt *and other gap genes*

It has been proposed that the maintenance of discrete gap gene expression patterns involves cross-regulatory interactions among the gap genes (Jäckle *et al.* 1986). Weak repressive interactions have been shown to occur between *hb* and *Kr*, in that mutations in either gene causes a slight expansion in the limits of the other. There is evidence that the maintenance of the posterior *kni* pattern depends on positive regulation by *Kr* (Pankratz *et al.* 1989). Here we have provided further support for the notion that cross-regulatory interactions are important for refining the limits of gap gene expression.

The maintenance of the two initial domains of *gt* expression near the anterior and posterior poles involves repression by Kr (Fig. 3D). And, in a manner analogous to the role *Kr* plays in the maintenance of the *kni* pattern, we have found that *gt* is sustained by *kni* (Fig. 4A). Although it has been claimed that *Kr* is a 'direct' activator of *kni* expression (Pankratz *et al.* 1989), it is equally likely that it functions as an indirect anti-repressor which 'buffers' *kni* from *hb*. In Kr^- embryos, the *hb* domain expands posteriorly, where it would be expected to exert a strong repressive effect on *kni* expression. Consistent with this model is the finding that the *Kr* protein functions as a strong transcriptional repressor in transient cotransfection assays; thus far these studies have failed to reveal an activating activity for *Kr* (Licht *et al.* 1990). Other genetic interactions involving *Kr* can also be interpreted as being brought about by either repression or anti-repression (i.e. Harding and Levine, 1988; Irish *et al.* 1989*b*). Similarly, the positive effect that *kni* exerts on the posterior *gt* pattern could involve an anti-repression mechanism, whereby *kni* restricts the *Kr* repressor to central regions of the embryo.

We thank Christine Rushlow for the *Kr* antibody, Ken Howard for the *knirps* antibody, Gary Struhl for fly stocks, and David Kosman for technical assistance. This work was funded by grant GM34431 from the NIH.

References

Berleth, T., Burri, M., Thoma, G., Bopp, D., Richstein, S., Frigerio, G., Noll, M. and Nüsslein-Volhard, C. (1988). The role of localization of *bicoid* RNA in organizing the anterior pattern of the *Drosophila* embryo. *EMBO J.* **7**, 1749–1756.

Brown, N. H. and Kafatos, F. C. (1988). Functional cDNA libraries from *Drosophila* embryos. *J. molec. Biol.* **203**, 425–437

Cohen, S. M. and Jürgens, G. (1990). Mediation of *Drosophila* head development by gap-like segmentation genes. *Nature* **346**, 482–485.

Dalton, D., Chadwick, R. and McGinnis, W. (1989). Expression and embryonic function of *empty spiracles*: a *Drosophila* homeobox gene with two patterning functions on the anterior–posterior axis of the embryo. *Genes and Dev.* **3**, 1940–1956.

Driever, W. and Nüsslein-Volhard, C. (1988*a*). A gradient of *bicoid* protein in *Drosophila* embryos. *Cell* **54**, 83–93.

Driever, W. and Nüsslein-Volhard, C. (1988*b*). The *bicoid* protein determines position in the *Drosophila* embryo in a concentration-dependent manner. *Cell* **54**, 95–104.

Driever, W. and Nüsslein-Volhard, C. (1989). The *bicoid* protein is a positive regulator of *hunchback* transcription in the early *Drosophila* embryo. *Nature* **337**, 138–143.

Driever, W., Thoma, G. and Nüsslein-Volhard, C. (1989). Determination of spatial domains of zygotic gene expression in the *Drosophila* embryo by the affinity of binding sites for the *bicoid* morphogen. *Nature* **340**, 363–367.

Finkelstein, R. and Perrimon, N. (1990). The *orthodenticle* gene is regulated by *bicoid* and *torso* and specifies *Drosophila* head development. *Nature* **346**, 485–488.

Frasch, M., Hoey, T., Rushlow, C., Doyle, H. and Levine, M. (1987). Characterization and localization of the *even-skipped* protein of *Drosophila*. *EMBO J.* **6**, 749–759.

Frasch, M. and Levine, M. (1987). Complementary patterns of *even-skipped* and *fushi-tarazu* expression involve their differential regulation by a common set of segmentation genes in *Drosophila*. *Genes and Dev.* **1**, 981–995.

French, V. (1988). Gradients and insect segmentation. *Development* **104 Supplement**, 3–16.

Frohnhöfer, H. G. and Nüsslein-Volhard, C. (1986). Organization of anterior pattern in the *Drosophila* embryo by the maternal gene *bicoid*. *Nature* **324**, 120–125.

Frohnhöfer, H. G. and Nüsslein-Volhard, C. (1987). Maternal genes required for the anterior localization of *bicoid* activity in the embryo of *Drosophila*. *Genes and Dev.* **1**, 880–890.

Gaul, U. and Jäckle, H. (1987). Pole region-dependent repression of the *Drosophila* gap gene *Krüppel* by maternal gene products. *Cell* **51**, 549–555.

Gaul, U. and Jäckle, H. (1989). Analysis of maternal effect mutant combinations elucidates regulation and function of the overlap of *hunchback* and *Krüppel* gene expression in the *Drosophila* blastoderm embryo. *Development* **107**, 651–662.

Gaul, U., Seifert, E., Schuh, R. and Jäckle, H. (1987). Analysis of *Krüppel* protein distribution during early *Drosophila* development reveals posttranscriptional regulation. *Cell* **50**, 639–647.

Gergen, J. P. and Wieschaus, E. F. (1986). Localized requirements for gene activity in segmentation of *Drosophila* embryos: analysis of *armadillo*, *fused*, *giant*, and *unpaired* mutations in mosaic embryos. *Roux' Arch. devl. Biol.* **195**, 49–62.

Harding, K. and Levine, M. (1988). Gap genes define the limits of Antennapedia and Bithorax gene expression during early development in *Drosophila*. *EMBO J.* **7**, 205–214.

Hülskamp, M., Pfeifle, C. and Tautz, D. (1990). A morphogenetic gradient of *hunchback* protein organizes the expression of the gap genes *Krüppel* and *knirps* in the early *Drosophila* embryo. *Nature* **346**, 577–580.

Hülskamp, M., Schroder, C., Pfeifle, C., Jäckle, H. and Tautz, D. (1989). Posterior segmentation of the *Drosophila* embryo in the absence of a maternal posterior organizer gene. *Nature* **338**, 629–632.

Irish, V. F., Lehmann, R. and Akam, M. (1989*a*). The *Drosophila* posterior-group gene *nanos* functions by repressing *hunchback* activity. *Nature* **338**, 646–648.

Irish, V. F., Martinez-Arias, A. and Akam, M. (1989*b*). Spatial regulation of the *Antennapedia* and *Ultrabithorax* homeotic genes during *Drosophila* early development. *EMBO J.* **8**, 1527–1537.

Jäckle, H., Tautz, D., Schuh, R., Seifert, E. and Lehmann, R.

(1986). Cross-regulatory interactions among the gap genes of *Drosophila*. *Nature* **324**, 668–670.

KLINGLER, M., ERDELYI, M., SZABAD, J. AND NÜSSLEIN-VOLHARD, C. (1988). Function of *torso* in determining the terminal anlagen of the *Drosophila* embryo. *Nature* **335**, 275–277.

KOZAK, M. (1984). Compilation and analysis of sequences upstream from the translational start site in eukaryotic mRNAs. *Nucl. Acids Res.* **12**, 857–872.

KRAUT, R. AND LEVINE, M. (1991). Mutually repressive interactions between the gap genes *giant* and *Krüppel* define middle body regions of the *Drosophila* embryo. *Development* **111**, 611–621.

LEHMANN, R. (1988). Phenotypic comparison between maternal and zygotic genes controlling the segmental pattern of the *Drosophila* embryo. *Development* **104 Supplement**, 17–27.

LEHMANN, R. AND NÜSSLEIN-VOLHARD, C. (1986). Abdominal segmentation, pole cell formation, and embryonic polarity require the localized activity of *oskar*, a maternal gene in *Drosophila*. *Cell* **47**, 141–152.

LEHMANN, R. AND NÜSSLEIN-VOLHARD, C. (1987). *hunchback*, a gene required for segmentation of an anterior and posterior region of the *Drosophila* embryo. *Devl Biol.* **119**, 402–417.

LICHT, J. D., GROSSEL, M. J., FIGGE, J. AND HANSEN, U. M. (1990). *Drosophila Krüppel* protein is a transcriptional repressor. *Nature* **346**, 76–79.

MAHONEY, P. A. AND LENGYEL, J. A. (1987). The zygotic segmentation mutant *tailless* alters the blastoderm fate map of the *Drosophila* embryo. *Devl Biol.* **122**, 464–470.

MOHLER, J., ELDON, E. D. AND PIRROTTA, V. (1989). A novel spatial transcription pattern associated with the segmentation gene, *giant*, of *Drosophila*. *EMBO J.* **8**, 1539–1548.

NAUBER, U., PANKRATZ, M., KIENLIN, A., SEIFERT, E., KLEMM, U. AND JÄCKLE, H. (1988). Abdominal segmentation of the *Drosophila* embryo requires a hormone receptor-like protein encoded by the gap gene *knirps*. *Nature* **336**, 489–492.

NÜSSLEIN-VOLHARD, C., FROHNHÖFER, H. G. AND LEHMANN, R. (1987). Determination of anteroposterior polarity in *Drosophila*. *Science* **238**, 1675–1681.

NÜSSLEIN-VOLHARD, C. AND ROTH, S. (1989). Axis determination in insect embryos. In *Cellular Basis of Morphogenesis*, Ciba foundation symposium **144**, 37–55.

NÜSSLEIN-VOLHARD, C. AND WIESCHAUS, E. (1980). Mutations affecting segment number and polarity in *Drosophila*. *Nature* **287**, 795–801.

PANKRATZ, M. J., HOCH, M., SEIFERT, E. AND JÄCKLE, H. (1989). *Krüppel* requirement for *knirps* enhancement reflects overlapping gap gene activities in the *Drosophila* embryo. *Nature* **341**, 337–339.

PETSCHEK, J. P. AND MAHOWALD, A. P. (1990). Different requirements for *l(1)giant* in two embryonic domains of *Drosophila melanogaster*. *Developmental Genetics* **11**, 88–96.

PETSCHEK, J. P., PERRIMON, N. AND MAHOWALD, A. P. (1987). Region-specific defects in *l(1)giant* embryos of *Drosophila melanogaster*. *Devl Biol.* **119**, 175–189.

PIGNONI, F., BALDARELLI, R. M., STEINGRIMSSON, E., DIAZ, R. J., PATAPOUTIAN, A., MERRIAM, J. R. AND LENGYEL, J. A. (1990). The *Drosophila* gene *tailless* is expressed at the embryonic termini and is a member of the steroid receptor superfamily. *Cell* **62**, 151–163.

PREISS, A., ROSENBERG, U. B., KIENLIN, A., SEIFERT, E. AND JÄCKLE, H. (1985). Molecular genetics of *Krüppel*, a gene required for segmentation of the *Drosophila* embryo. *Nature* **313**, 27–32.

REDEMANN, N., GAUL, U. AND JÄCKLE, H. (1988). Disruption of a putative Cys-zinc interaction eliminates the biological activity of the *Krüppel* finger protein. *Nature* **332**, 90–92.

SANDER, K. (1976). Specification of the basic body pattern in insect embryogenesis. *Adv. Insect Physiol.* **12**, 125–138.

SANDER, K. AND LEHMANN, R. (1988). *Drosophila* nurse cells produce a posterior signal required for embryonic segmentation and polarity. *Nature* **335**, 68–70.

SCHRODER, C., TAUTZ, D., SEIFERT, E. AND JÄCKLE, H. (1988). Differential regulation of the two transcripts from the *Drosophila* gap segmentation gene *hunchback*. *EMBO J.* **7**, 2881–2887.

SCHÜPBACH, T. AND WIESCHAUS, E. (1989). Female sterile mutations on the second chromosome of *Drosophila melanogaster*. I. Maternal effect mutations. *Genetics* **121**, 101–117.

SCHÜPBACH, T. AND WIESCHAUS, E. (1986). Maternal-effect mutations altering the anterior–posterior pattern of the *Drosophila* embryo. *Roux's Arch devl Biol.* **195**, 302–317.

SPRENGER, F., STEVENS, L. M. AND NÜSSLEIN-VOLHARD, C. (1989). The *Drosophila* gene *torso* encodes a putative receptor tyrosine kinase. *Nature* **338**, 478–483.

STANOJEVIĆ, D., HOEY, T. AND LEVINE, M. (1989). Sequence-specific DNA-binding activities of the gap proteins encoded by *hunchback* and Krüppel in *Drosophila*. *Nature* **341**, 331–335.

STRECKER, T. R., KONGSUWAN, K., LENGYEL, J. AND MERRIAM, J. R. (1986). The zygotic mutant *tailless* affects the anterior and posterior ectodermal regions of the *Drosophila* embryo. *Devl Biol.* **113**, 64–76.

STRECKER, T. R., MERRIAM, J. R. AND LENGYEL, J. A. (1988). Graded requirement for the zygotic terminal gene, *tailless*, in the brain and tail region of the *Drosophila* embryo. *Development* **102**, 721–734.

STRUHL, G. (1989*a*). Differing strategies for organizing the anterior and posterior body pattern in *Drosophila* embryos. *Nature* **338**, 741–744.

STRUHL, G. (1989*b*). Morphogen gradients and the control of body pattern in insect embryos. In *Cellular Basis of Morphogenesis*, Ciba foundation symposium **144**, 65–86.

STRUHL, G., STRUHL, K. AND MACDONALD, P. (1989). The gradient morphogen *bicoid* is a concentration-dependent transcriptional activator. *Cell* **57**, 1259–1273.

STUDIER, F. W. AND MOFFATT, B. A. (1986). Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. *J. molec. Biol.* **189**, 113–130.

TAUTZ, D. (1988). Regulation of the *Drosophila* segmentation gene *hunchback* by two maternal morphogenetic centres. *Nature* **332**, 281–284.

TAUTZ, D., LEHMANN, R., SCHNURCH, H., SCHUH, R., SEIFERT, E., KIENLIN, K. AND JÄCKLE, H. (1987). Finger protein of novel structure encoded by *hunchback*, a second member of the gap class of *Drosophila* segmentation genes. *Nature* **327**, 383–389.

TAUTZ, D. AND PFEIFLE, C. (1989). A nonradioactive *in situ* hybridization method for the localization of specific RNAs in *Drosophila* embryos reveals a translational control of segmentation gene *hunchback*. *Chromosoma* **98**, 81–85.

TEARLE, R. AND NÜSSLEIN-VOLHARD, C. (1987). Tubingen mutants and stocklist. *Dros. inf. Serv.* **66**, 209–269.

WIESCHAUS, E., NÜSSLEIN-VOLHARD, C. AND JÜRGENS, G. (1984*a*). Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster*, III. Zygotic loci on the X-chromosome and fourth chromosome. *Wilhelm Roux' Arch. devl. Biol.* **193**, 296–307.

WIESCHAUS, E., NÜSSLEIN-VOLHARD, C. AND KLUDING, H. (1984*b*). *Krüppel*, a gene whose activity is required early in the zygotic genome for normal embryonic segmentation. *Devl Biol.* **104**, 172–186.

WOLPERT, L. (1969). Positional information and the spatial pattern of cellular differentiation. *J. theor. Biol.* **25**, 1–47.

(*Accepted 26 October 1990*)