The EMBO Journal vol.8 no.5 pp.1539 - 1548, 1989

A novel spatial transcription pattern associated with the segmentation gene, *giant*, of *Drosophila*

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Communicated by V.Pirrotta

The segmentation gene, *giant*, is located in 3A1 within a cloned chromosome region surrounding the *zeste* locus. Rearrangement breakpoints associated with *giant* mutations were localized on the genomic clone map, and nearby transcription units were identified. One transcription unit is active during early embryogenesis and its transcripts are spatially localized from blastoderm into extended germband stages, consistent with expected expression patterns predicted by the 'gap' phenotype of *giant* mutants. Germ line transformation experiments using a 10-kb DNA fragment containing this transcription unit gave complete rescue of the abdominal *giant* defect but only partial correction of the head defect. The effect of mutations in three other gap loci, *Kr*, *kni* and *hb*, were also analyzed.

Key words: gap gene/head segmentation/*in situ* hybridization/germ line transformation

Introduction

The segmental pattern of the larva of *Drosophila melanogaster* is under the control of several zygotically active genes. These genes can be divided into three classes, depending on the nature of the defects associated with mutations in a given gene (Nüsslein-Volhard and Wieschaus, 1980). Mutations of genes of the segmental polarity class cause defects in homologous positions in every segment, and therefore these genes act on a single segment unit. Mutations of genes of the pair-rule class cause defects spaced at double segment intervals, indicating that these genes act on a double segment unit. Mutations of genes in the gap class result in the loss of contiguous blocks of segments from the body plan, indicating that these genes are required for particular regions of the segmental pattern. Because lethal mutations in the *giant* gene locus cause defects in abdominal segments 5–7 and loss of cuticular head structures (Gergen and Wieschaus, 1986a; Petschek *et al.*, 1987), the *giant* gene has been included in the gap class.

Analysis of many of the genes of the pair-rule and gap classes has indicated that each of these genes is expressed at the blastoderm stage in regions corresponding to parts of the segmental pattern fated to be deleted in mutants for that gene (see review by Akam, 1987), although for most genes the areas affected by the mutations slightly exceed the regions of gene expression. The two genes of the gap class that have been analyzed (*Kr*, Knipple *et al.*, 1985; *hb*, Jäckle *et al.*, 1986) are both expressed in continuous regions of the blastoderm that correspond to portions of the regions deleted by mutations in those loci. Genes of the pair-rule class typically are expressed at the blastoderm stage in seven stripes, spaced at intervals of approximately seven cells, between ~15% and 65% egg length, consistent with the observation that the larval segments arise from primordia three to four cells wide from this region of the blastoderm (Campos-Ortega and Hartenstein, 1985). Finally, each of three segment polarity mutations (*en*, Kornberg *et al.*, 1985; *gsb*, Baumgartner *et al.*, 1987; *wg*, Baker, 1987) is expressed in 14 or more stripes during early gastrulation at intervals corresponding to every segment. In addition, analysis of larvae mosaic for one of various X-linked mutations (*gt*, *runt*, *arm*) revealed that these gene loci functioned primarily autonomously (although some local non-autonomy was observed; Gergen and Wieschaus, 1985), indicating that the gene function is required in those tissues fated to be affected by mutations in that locus. It was, therefore, expected that the *giant* gene would also be expressed only in those tissues that are affected by mutations of the *giant* locus.

The *giant* locus is located in polytene band 3A1,2 and is included in a set of overlapping genomic clones isolated by Mariani *et al.* (1985) surrounding the zeste locus. Using this set of overlapping DNA clones, we identified genomic rearrangements associated with mutations in the *giant* locus and identified the *giant* transcription unit within this region. The temporal and spatial pattern of expression of this transcription unti is consistent with the phenotypic lesions of *giant* lethal mutations but more complex than was anticipated.

Results

Phenotype of giant lethal alleles

As described by Gergen and Wieschaus (1985) and Petschek *et al.* (1987), larvae mutant for deficiencies or strong hypomorphic lethal *giant* alleles are missing denticle bands of abdominal segments 5–7 and occasionally 8 (Figure 1a). However, unlike mutations in the other gap loci, *giant* does not delete all the ventral cuticular tissues in this region. In these mutant animals some naked cuticle is present, occasionally interspersed with patches of denticles between the fourth and eight denticle bands; the length of the larva between the fourth and eight denticle bands is reduced by only ~45%. The dorsal cuticle is similarly shortened in length and occasional secondary filz-körper (up to six) can be found in this region. In weaker alleles (e.g. gt^{XH}), only the sixth abdominal denticle band is missing and in the weakest embryonic lethal allele, gt^{L2}, all the abdominal segments are present.

Mutant *giant* larvae possess a characteristic 'buttonhead' phenotype, caused by the loss of labral and labial structures and by the failure to complete head involution, such that the

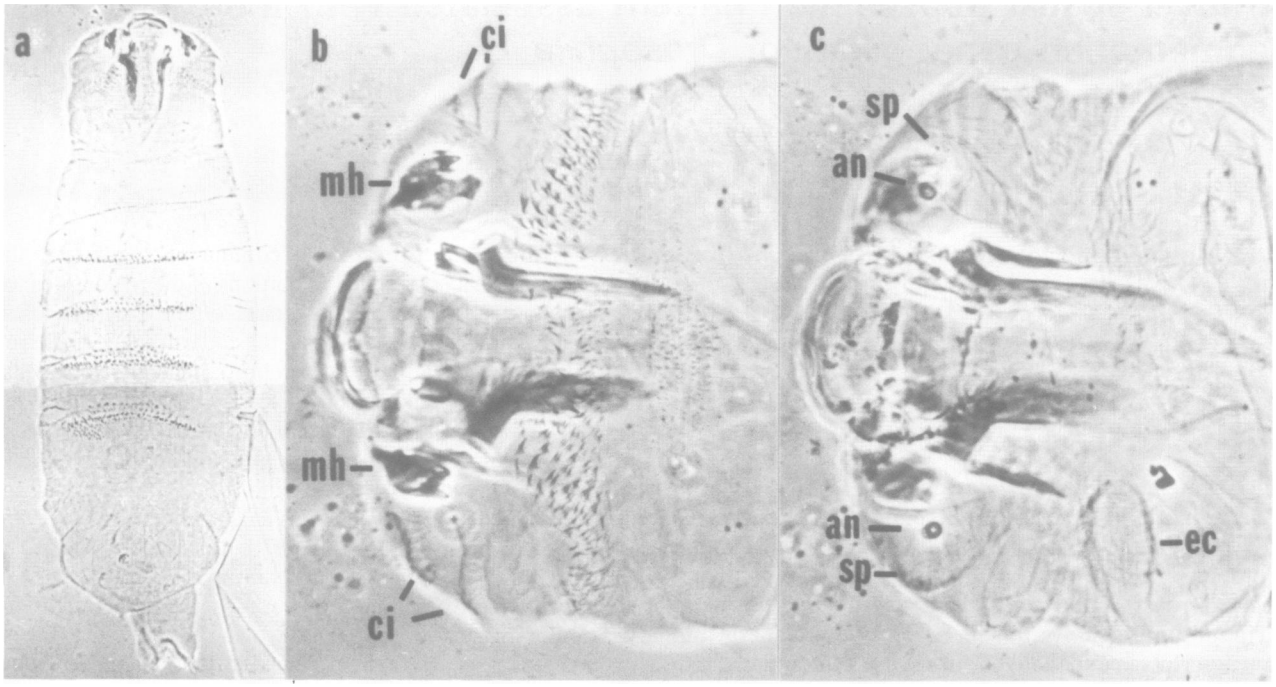

Fig. 1. Cuticular phenotype of a strong *giant* mutation, gt^{YA82}. (**a**) Ventral cuticle, photomontage of a fully developed homozygous mutant embryo. (**b,c**) Ventral and dorsal aspects of the head region. mh, mouthhooks; ci, cirri; an, antennal sense organ; sp, sensory papilla; ec, ectopic cuticle.

ventral portion of the cephalopharyngeal skeleton is extruded out of the anterior end of the larva. Larvae mutant for strong alleles (e.g. gt^{YA82}, Figure 1b and c) lack the labrum, epistomal sclerite, H-piece, hypostomal sclerite and dorsal bridge and retain in the pseudocephalon: cirri, ventral organ, antennal sense organ, maxillary sense organ (including the dorso-lateral and dorso-medial papillae), mouthhooks, the dorsal and ventral arms of the cephalopharyngeal skeleton, the ventral T-ribs and the hypopharyngeal organ. The lateralgräten are present but reduced and disorganized. Small, additional ectopic patches of naked cuticle, or cuticle covered with structures similar to dorsal hairs, can be found both dorsally and ventrally between the prothorax and the maxillary structures. Strong hypomorphic *giant* larvae also possess salivary glands, but these are attached to the exoskeleton at the anterior edge of the prothorax. Placement of the affected structures on the blastoderm fate map of Jürgens *et al.* (1986) reveals two regions of the anterior blastoderm fated to give rise to defective structures in mutant *giant* larvae (Figure 2): the labral segment on the dorsolateral side of the blastoderm embryo at ~90–95% egg length and the labial segment at ~60–65% egg length of the blastoderm. In weaker alleles (e.g. gt^{XH}) labral structures are missing, but part of the labial H-piece is present. In weak, hypomorphic alleles (e.g. gt^{L2}) most of the structures are present to variable extents, but the labrum is misshapen and the mouthhooks are displaced.

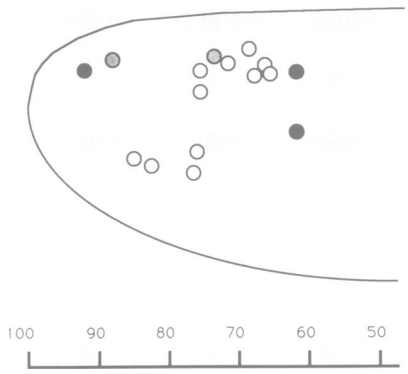

Fig. 2. Blastoderm fate map of the larval head. The closed circles indicate structures missing, open circles, structures present in the heads of gt^{YA82} mutant embryos. The cross-hatched circles represent two foci necessary for the dorsal bridge which is defective in gt^{YA82}. The fate map is taken from Jürgens *et al.* (1986).

Genomic rearrangements associated with giant mutations

Mariani *et al.* (1985) mapped two breakpoints which served as proximal and distal limits for the position of the *giant* locus in the genomic map. *Df(1)* w^{rJ1}, which extends proximally from position +4 on the map shown in Figure 3, complements *giant* mutations, whereas *Df(1) 62g18*, which extends proximally from position +63, does not. The gt^{1} mutation, a spontaneous adult viable allele causing delayed pupariation and enlarged larvae, pupae and adults, is associated with two insertions of DNA at two positions in this region, one near +17 and one near +32. The gt^{L2} mutation, *Tp(1;1) L2*, is a weak lethal allele of *giant* generated by irradiation and associated with the transposition of chromosomal region 8D-10B1 into 3A2. Genomic Southern blots indicated the presence of a breakpoint in the interval +13.5 to +15.8 (Figure 4). Of two alleles generated by P element hybrid dysgenesis, one, $gt^{\#30}$, had no alterations detectable in genomic Southern blots, while the other, $gt^{1/2B/3}$, proved to be a deletion of *giant, zeste* and *1(1)zwl* with a breakpoint near +40. Many other alleles of *giant* are known but their genomic analysis is complicated by the fact that the genomic interval +10 to +20 contains several restriction site polymorphisms and several polymorphic regions which differ in their arrangement in

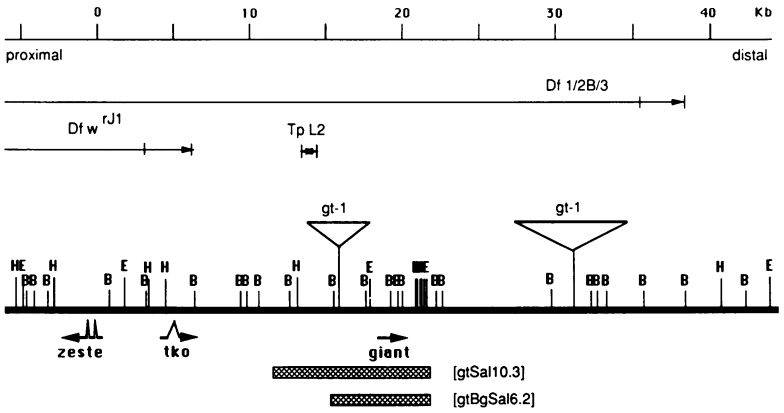

Fig. 3. Map of the *zeste–giant* genomic region. The coordinates and restriction map are taken from Mariani *et al.* (1985). Deletion breakpoints are diagrammed above the restriction map with the solid line indicating the deleted region. The approximate positions of the *L2* transcription breakpoint and of the gt^1 insertion are indicated. The transcription units (*zeste* from Pirrotta *et al.*, 1987; *tko* from Royden *et al.*, 1987) are shown below the restriction map and the cross-hatched bars represent the transposon constructions used for germ line transformation.

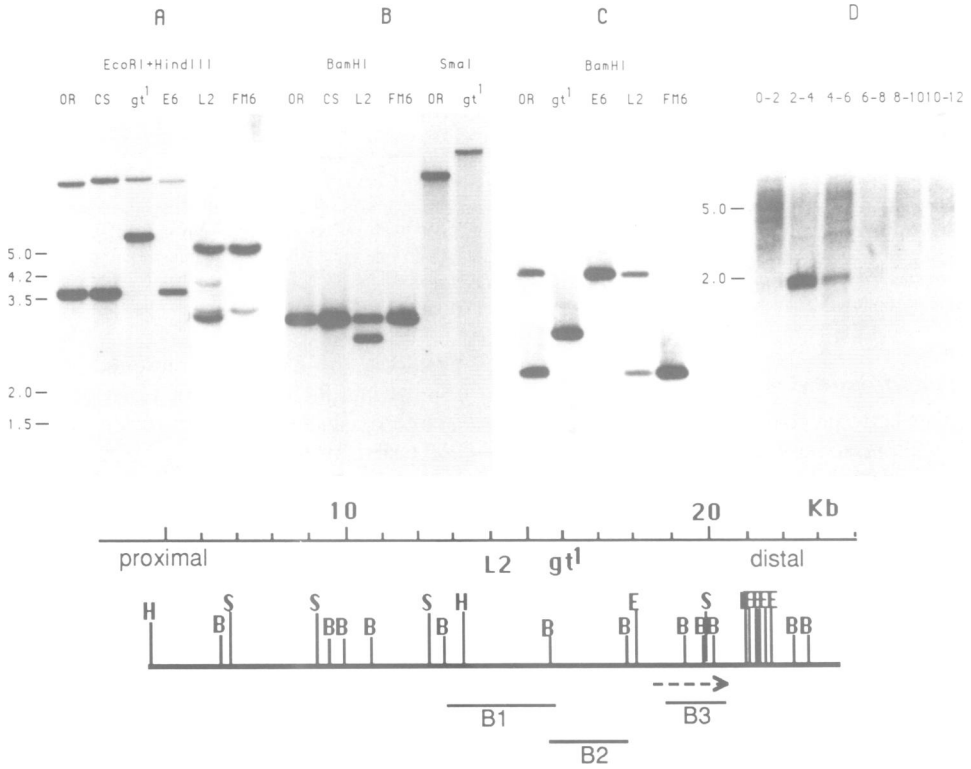

Fig. 4. Genomic Southern blot analysis and Northern blot hybridization. (**A**) Genomic DNAs digested with *Eco*RI + *Hind*III, hybridized with probe B1. (**B**) Genomic DNAs digested with *Bam*HI or with *Sma*I and hybridized with probe B1. (**C**) Genomic DNAs digested with *Bam*HI and hybridized with probe B2. OR and CS are Oregon R and Canton S wild-type strains; gt^1 and *E6* are two homozygous viable *giant* alleles; *L2* is gt^{L2}/FM6; FM6 is a balancer X chromosome. Note that some of the OR flies used here as well as the CS, *L2* and *E6* flies but not FM6 have the insertional polymorphism of 2 kb near position 17. The FM6 chromosome has a new *Hind*III site near position 9. (**D**) Northern blot of RNA extracted from Oregon R embryos at 2-h intervals after deposition and hybridized with probe B3. Size markers are indicated in kb and the origin of the probes is illustrated in the map below.

different wild-type strains. Examples of these polymorphisms are a 2-kb insertion around +17, found in 30% of wild-type Oregon R chromosomes but not in Canton S flies (Mariani *et al.*, 1985); a 0.18-kb tandem repeat unit at position +21 found in variable numbers in different individuals and in different strains; a 0.3-kb tandem repeat unit at position +24 which is also found in variable numbers.

Collectively, the analysis of the mutants places part or all of the *giant* locus between +4 and +40 with strong implications that the region around position +16 is important for *giant* function. Because Mariani *et al.* (1985) found no embryonic transcription units between +4 and +10, we concentrated on the analysis of transcription patterns for DNA between +10 and +40.

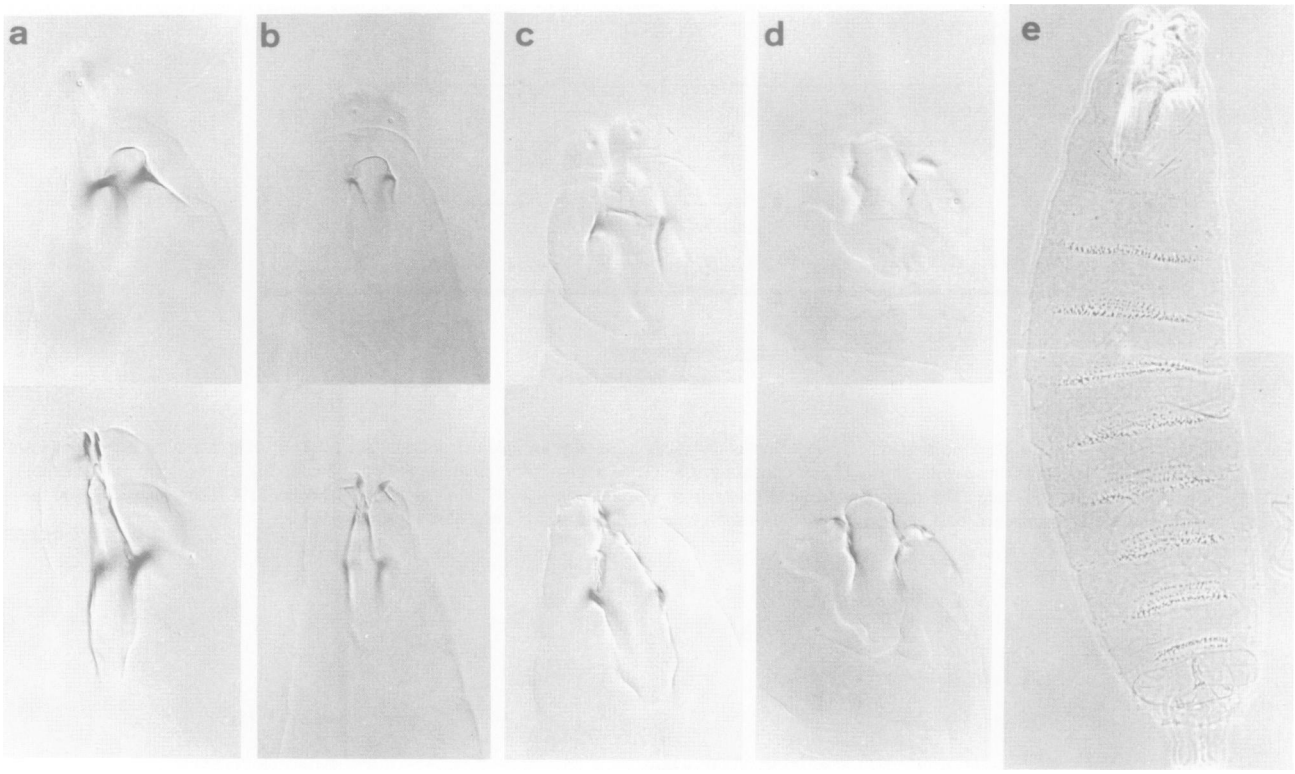

Fig. 5. Phenotypes of partially rescued embryos. In each case, the upper picture shows a more dorsal focal plane, the lower picture a more ventral focal plane. (**a**) Head region of Oregon R wild-type first instar larva (**b**) Line 1−4, with one copy of transposon gtSal10.3 in a gt^{X11} mutant background. This represents the best degree of rescue obtained. (**c**) Line 10−6, with one copy of the same transposon at a different genomic site, representing a very incomplete rescue. (**d**) gt^{X11} mutant embryos. (**e**) Rescue of abdominal phenotype in line 10−6 with a head phenotype similar to that in (c). (a−d) Nomarski optics, (e) phase contrast.

Embryonic transcripts from the region of +17 to +40

Three genomic regions between positions +17 and +40 are homologous to RNA expressed during embryonic development. The region around position +37 is homologous to a 1.6-kb RNA most abundant in 4- to 6-h embryos and to a 2.4-kb RNA found in adults. A family of RNAs of 0.5, 3.5 and 5.0 kb is revealed by probes from the region +21 to +28. These are expressed throughout embryonic, larval and pupal stages. This region consists of tandem repeats of a 0.18-kb *EcoRI* fragment followed by tandem repeats of a 0.3-kb *Pvu*II fragment present in variable numbers in different strains of flies. The region +18 to +21 is homologous to a 1.9-kb RNA that is expressed almost exclusively in 2- to 4-h embryos and is barely detectable in 4- to 6-h embryos (Figure 4D). Hybridization with single-stranded probes indicated that its transcription most likely orginates just to the right of the *Eco*RI site at position +18.1 and proceeds from left to right in the map shown in Figure 3. The temporal specificity of this RNA species, as well as its proximity to DNA rearrangements affecting *giant* expression (gt^{L2} and gt^{1}), suggested that this transcription until might correspond to the *giant* locus. To confirm this hypothesis we attempted to rescue the *giant* mutant phenotype by germ line transformation with corresponding DNA fragments.

Germ line transformation

We first constructed a transposon for germ line transformation using the 6.5-kb *Bgl*II−*Sal* fragment, spanning positions +15.8 to +22.3, inserted in the pUChsneo vector (Steller and Pirrotta, 1985). Lines carrying this transposon at autosomal sites were constructed using the G418 selection and tested for their ability to rescue strong *giant* mutations by crossing males homozygous for the transposon with females gt^{X11}/FM6. No rescue, total or partial, was observed with one or two doses of the transposon: males that received the gt^{X11} allele died as late embryos with the head and abdominal defects of typical gt^{X11} embryos. A second transposon was constructed using the 10.3-kb *Sal*I fragment (position +11.7 to +22.0) inserted in the *Sal*I site of the Carnegie 20 (Rubin and Spradling, 1983). Lines transformed with this construct, tested for ability to rescue the mutant phenotype, still gave no viable gt^{X11} males but examination of the embryos that failed to hatch revealed that the abdominal segmentation defect of gt^{X11} could be completely rescued by one copy of the transposon in most individuals. In contrast, the head defects, including failure to complete head involution and the variable loss of labial and labral parts, were only partly alleviated (Figure 5). The degree of rescue varies considerably from one individual to another as well as among independent transformed lines. In the more poorly rescued cases the dorsal bridge is missing or incomplete, the H piece, epistomal and hypostomal sclerites are absent and the lateralgräten, although present, are shorter and appear as disorganized bundles of fibers (Figure 5C). In the better rescued cases (Figure 5B) head involution is more nearly complete, the lateralgräten appear normal and the H-piece may be present but with short or no anterior arms; the epistomal and hypostomal sclerites are

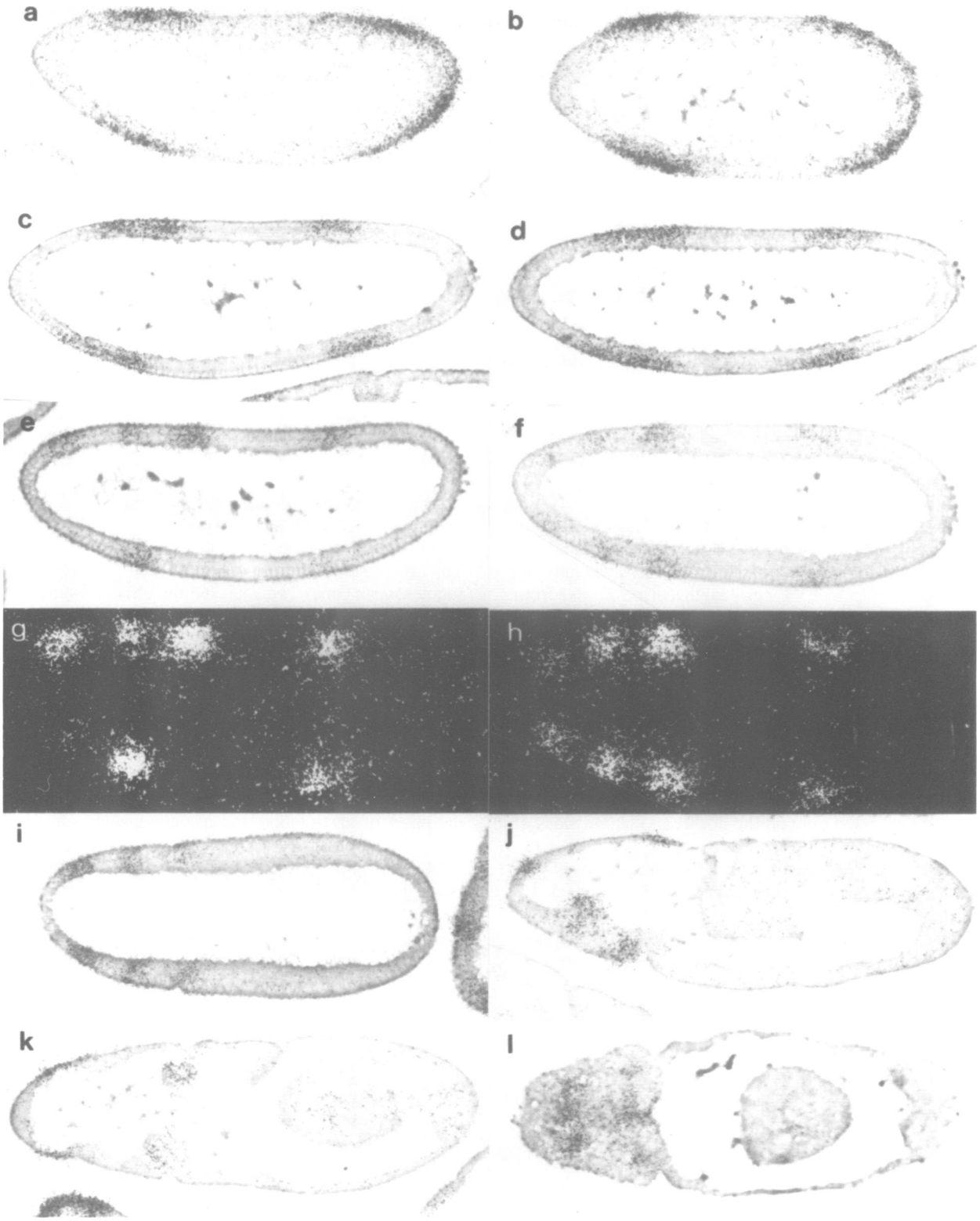

Fig. 6. *In situ* hybridization to *giant* RNA. The RNA probe from the 1.6 *Bam*HI genomic fragment (position 17.8−19.4) was hybridized to embryonic thin sections. (**a,b**) Early cycle 13 embryos. (**c,d**) Early cycle 14 embryos. (**e−h**) Cellular blastoderm. (**g, h**) Dark-field photomicrographs of embryos in (e) and (f). (**i**) Early gastrula. (**j−l**) Extended germ band embryos. (a, c, e, g, j) Sagittal sections; (b, d, f, h, i, k, l) horizontal sections.

either absent or abnormally short. Although, in most cases, the abdominal region is more likely to be rescued, the different body parts show a remarkable independence of one another. In some cases both head and abdomen are poorly rescued and occasionally partial head rescue is found in the absence of abdominal rescue.

Two copies of the transposon do not result in an appreciably greater degree of rescue, suggesting that the

remaining defect is not quantitative but qualitative. The partially rescued embryos resemble those produced by the gt^{L2} hypomorphic allele, suggesting that, like *L2*, the gtSal10.3 transposon may lack some additional head-specific regulatory element. We conclude that the 1.7-kb RNA originating from the region +18 to +21 represents the transcripts of the *giant* gene and that the gene and the regulatory region required for its proper expression cover >10 kb.

Spatial pattern of expression of giant

During blastoderm and germ band extension stages of embryogenesis, *giant* RNA is detectable by *in situ* hybridization in peripheral blastoderm nuclei and their descendants but not in internal yolk nuclei. When this RNA is first detected, during cell cycle 12, it is expressed predominantly in two regions of the syncytial blastoderm: a broad band of expression anteriorly from ~60 to 82% egg length and an even broader posterior region from 0 to ~33% egg length (Figure 6a and b). During cell cycle 13 the region of posterior expression narrows to a band from ~20 to 33% egg length (Figure 6c and d). During cellularization of the blastoderm at mid-cycle 14, a new region of expression appears at the anterior end of the embryo (stripe 1, Figure 6e–h) from ~91 to 97% egg length. The broad anterior band present in the syncytial blastoderm is resolved into two bands: from ~75 to 83% egg length (stripe 2) and from ~62 to 70% egg length (stripe 3). The posterior band present in the syncytial blastoderm narrows further during cellularization to a stripe from ~25 to 33% egg length (stripe 4); a comparison of the hybridization pattern of *giant* and a pair-rule gene, *eve*, on adjacent sections (not shown) indicates that stripe 4 includes the sixth stripe of *eve* expression, corresponding approximately to parasegment 11 (posterior A5 and anterior A6). Unlike stripes 3 and 4, which extend around the circumference of the embryo, stripes 1 and 2 are expressed only on the dorsal and lateral sides of the embryo and vanish completely in the ventral region. Thus, at cellular blastoderm *giant* RNA is expressed in four stripes ~5–6 cells wide; the anterior three stripes are separated by 4–5 non-expressing cells.

During cellular blastoderm, stripe 4 begins to disappear and little detectable RNA is present in the early gastrula (Figure 6i). In contrast, RNA continues to be expressed in regions corresponding to stripes 1–3 during germ band elongation (Figure 6j–l). Cells expressing stripe 1 move anteriorly to the region forming the clypeolabrum. Cells expressing stripe 2 are present in the procephalic lobe and the lateral portions of the anterior midgut invagination. Cells expressing stripe 3 become sequestered into the cephalic furrow. Expression of this RNA continues in these regions in the fully extended germ band embryo and gradually decays before germ band retraction. These spatial patterns of expression are summarized in Figure 7.

Gap genes and giant expression

Studies of the spatial expression patterns of various gap and pair-rule genes in mutants of other segmentation genes have suggested that the gap gene loci regulate the spatial expression pattern of the pair-rule loci (see review by Akam, 1987). For all pairs examined to date, mutations in a given gap gene affect the spatial expression pattern of any pair-rule gene in the vicinity of the gap gene domain, whereas

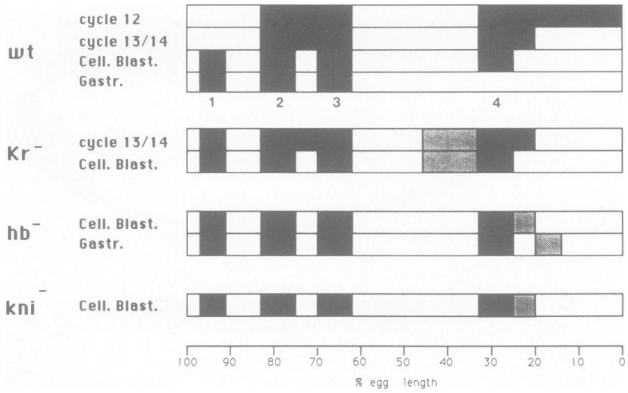

Fig. 7. Schematic diagram of the spatial pattern of *giant* expression. The position of transcripts revealed by hybridization is shown in black at positions along the embryo indicated in percentage egg length. wt, wild-type embryos at cycle 12, cycle 13/14, cellular blastoderm and gastrula. Hybridization stripes are labelled 1–4. Kr⁻, *Krüppel* mutant embryos. The shaded regions indicate new domains of expression. hb⁻, *hunchback* mutant embryos. kni⁻, *knirps* mutant embryos.

no mutations in any pair-rule gene affects the spatial expression pattern of any gap class gene. In addition, mutations in each of the gap genes (*Kr, hb, kni*) appear to affect the pattern of the other gap genes (Jäckle *et al.*, 1986). To investigate the role of *giant* in the hierarchy of segmentation genes, we analyzed the effect of mutations in three gap genes (*Kr, hb* and *kni*) and one pair-rule gene (*eve*) on the spatial expression pattern of *giant*.

No effect on the spatial expression pattern of *giant* was observed in *even-skipped* mutants. In contrast, mutations in the gap genes *Kr, hb* and *kni* altered the expression of *giant* in the posterior part of the embryo. Surprisingly, these mutations did not affect the more complex expression patterns of *giant* in the anterior region. In these mutant embryos, the anterior three stripes were ~5 cells wide at the onset of gastrulation and indistinguishable from those in wild-type embryos.

Mutations in each of the three gap loci modified the posterior transcription pattern of *giant* as summarized in Figure 7. In embryos homozygous for Kr^1 (Figure 8a and c), the first alteration in the transcription pattern can be detected during early cycle 14, when the posterior expression domain broadens to cover a region from ~20 to 45% egg length. During cellularization in mid-cycle 14, the posterior margin of posterior region expression in mutant *Kr* embryos moves to 25% egg length, similar to wild-type (Figure 8b and d), and posterior region expression vanishes at about the onset of gastrulation. Thus, the effect of *Kr* mutations on the expression pattern of *giant* is to extend the anterior limit of posterior region expression from 33 to 45% egg length during cycle 14, with no effect in the anterior region of the embryo, on the posterior limit of expression in the posterior region, or on the timing of expression in the anterior or posterior regions.

In embryos homozygous for hb^{14F}, the first alteration in the transcription pattern can be detected during mid-cycle 14 when the posterior limit of expression remains at ~20% egg length (Figure 8e and g). At the onset of gastrulation, expression in the posterior region from 20 to 33% egg length ceases as in the wild-type, but new expression is initiated in the region from 14 to 20% egg length (Figure 8f and h), where it persists through the fully extended germ band stage

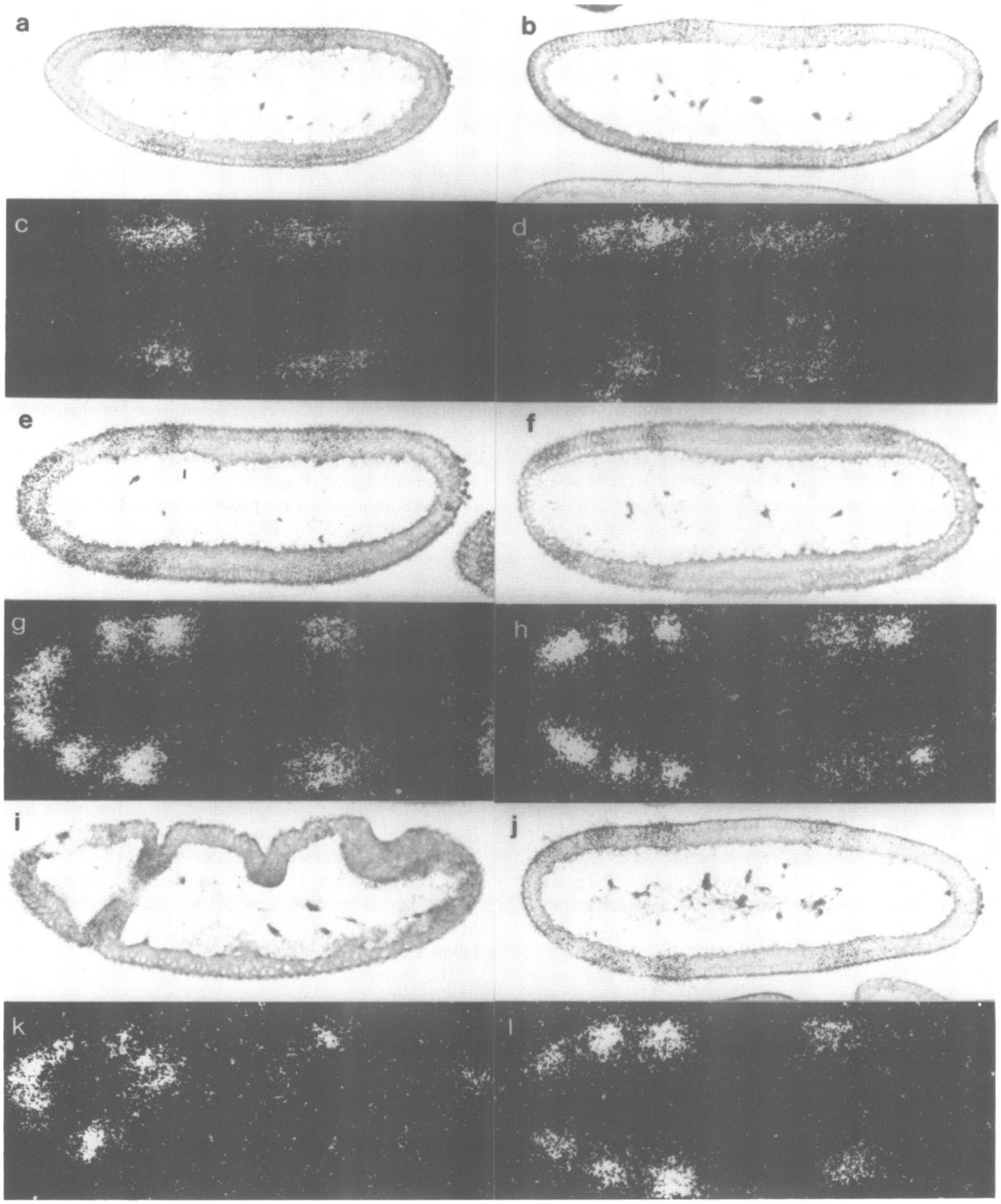

Fig. 8. Transcription patterns of *giant* in gap mutant embryos. (**a–d**) *Krüppel* mutant embryos. (**a, c**) Early cycle 14, sagittal section (**b,d**) Cellular blastoderm, sagittal section. (**e–i, k**) *hunchback* mutant embryos. (**e,g**) Cellular blastoderm, horizontal section. (**f,h**) Gastrula, horizontal section. (**i, k**) Early germ band extension, sagittal section. (**j, l**) *knirps* mutant embryos, cellular blastoderm, horizontal section.

(Figure 8i and k). Thus, the effect of mutations in *hb* on the spatial expression pattern of *giant* is confined to the posterior-most region of the embryo, blocking the normal narrowing of the posterior expression domain during cellularization and allowing expression in a new posterior domain during gastrulation and germ band extension.

Mutation in *kni* also have no effect on the spatial expression of *giant* in the anterior region of the embryo and have only a subtle effect on the pattern of expression in the posterior region. In embryos homozygous for kni^{2D}, the only alteration is detected during cellularization when the posterior expression domain fails to narrow completely to a stripe of 25–33% egg length (Figure 8j–l). In these embryos the size of the posterior domain at the onset of

gastrulation is variable and its posterior limit may be anywhere between ~20 and 25% egg length.

Discussion

Several lines of evidence confirm that we have identified the *giant* locus. Deficiency breakpoints define an interval that must contain all or part of the locus. Rearrangements found in *giant* mutants are located in this interval. Only one transcriptional unit in this interval has the developmental expression peaking at 2–4 h expected for a segmentation gap gene. The spatial distribution of the transcript in the embryo is in rough agreement with the fate map of the regions affected by *giant* mutations. Finally, germ line transformation with a 10.5-kb fragment from this region results in partial rescue of the *giant* embryonic phenotype. We are confident therefore that the *giant* locus maps in the interval +11 to +22 in the map shown in Figure 3 and that its transcript is a 1.9-kb RNA species found predominantly in 2- to 4-h embryos. Breakpoints in the 5′ flanking region of *giant* alter its expression. A very mild and viable allele, gt^1 is associated with an insertion of foreign DNA very close to position +16 >2 kb before the transcription start. This mutation does not have a detectable embryonic phenotype but is apparently weakly deficient in the function of some head derivatives such as the ring gland, resulting in ecdysone insufficiency (Schwartz *et al.*, 1984). However, a natural polymorphism, frequently found in wild-type stocks, due to an insertion of 2 kb approximately at position 16.5 (Mariani *et al.*, 1985) causes no apparent phenotypes. Stronger defects are produced by the gt^{12} breakpoint which is very close to position +14. These defects are limited to the head region, while the abdominal segmentation pattern is entirely normal. These results suggest that the 5′ flanking region of *giant* contains discrete regulatory elements, some of which are closer to the promoter and are required for expression in the abdominal region, while some are more distant and contain head-specific determinants. It remains possible, however, that normal head development simply requires higher concentrations of *giant* product than those sufficient for abdominal development.

These conclusions are supported by the germ line transformation experiments. A 6.5-kb transposon failed to give any detectable rescue of either head or abdominal phenotypes, but a 10.5-kb transposon with the same distal endpoint gave complete rescue of the abdominal defect and partial rescue of the head phenotype. Since altering the dosage of the transposon had little detectable effect on the degree of rescue, we conclude that there is still a qualitative insufficiency in the distribution of gene activity or in the nature of the product. The simplest explanation is that additional upstream sequences are required for expression in all the blastoderm cells that require *giant* product.

Giant stripes and phenotypic defects

The spatial distribution of *giant* RNA includes regions of the blastoderm fated to be defective in mutant *giant* larvae. These defects are in labral and labial head structures (derived from ~93 and 63% egg length in the blastoderm fate map) and in the abdominal segments 5–7. Expression of *giant* RNA at the cellular blastoderm stage occurs in four stripes 5–6 cells wide at ~95, 80, 65 and 30% egg length.

However, some discrepancies between the expression pattern of *giant* and the structures missing in *giant* larvae are worth noting. Most conspicuous is the lack of cuticular defects corresponding to stripe 2. The most obvious explanation is that few or no cuticular structures are derived from the region of stripe 2. Jürgens *et al.* (1986) placed the primordia for the dorsal arms, ventral plate, ventral arms and T-ribs of the cephalopharyngeal skeleton at ~75% egg length and the hypopharyngeal organ and posterior pharyngeal wall at 82 and 85% egg length respectively, essentially flanking stripe 2. This stripe should then correspond to a large portion of the brain primordium, in which Petschek *et al.* (1987) found no effect of *giant* mutations. In contrast, extensive cell death of the neurogenic region is found in the region of segments A5–A7 of *giant* mutants (Honisch and Campos-Ortega, 1982; Petschek *et al.*, 1987). Therefore, if *giant* played a similar role in tissues derived from the region of stripe 2, we would expect mutants to be noticeably affected. An alternative explanation would be that, although *giant* is transcribed in the region around 80% egg length, its expression is not necessary for the formation of structures in that region. However, little superfluous transcription has been observed for the pair-rule genes and other gap loci (see review by Akam, 1987). On the contrary, in the case of *ftz, hairy* and *runt*, incorrect or over-expression results in segmentation defects (Struhl, 1985; Gergen and Wieschaus, 1986b; Ish-Horowicz and Pinchin, 1987).

A similar discrepancy exists with respect to stripe 3. Although this stripe is 5–6 cells wide, only labial structures from this region appear to be defective in mutant *giant* larvae. Both the flanking maxillary structures and prothoracic structures appear to be present and intact. In addition, the salivary glands, which are products of the labial segment (Campos-Ortega and Hartenstein, 1985), are present, but displaced. However, Petschek *et al.* (1987) found that in *giant* embryos just after germband retraction (6–8 h), the labial prothoracic and mesothoracic segments are fused, although they are resolved at later stages (12–15 h). Thus, although the defects associated with stripe 3 initially extend posteriorly to cover three segments, they become resolved in subsequent development and limited to a portion of the labial segment.

The abdominal defects in mutant *giant* larvae correspond well to the stripe 4 domain of expression at syncytial blastoderm although this domain later narrows considerably at cellular blastoderm. The 'gap' in the abdominal segmentation pattern should be contrasted with the gaps caused by other segmentation mutations. The physical length between the fourth and eighth abdominal denticle belts in mutant *giant* larvae is only reduced by 45% despite the fact that the denticle belts for A5, A6 and A7 are missing. In contrast, the denticle belts of A1 and A7 are fused in *knirps* mutants, producing a true gap. In *hunchback* and *Krüppel* mutants, an extensive gap is associated with a mirror-image duplication of the adjoining posterior segments. Thus, *giant* mutants have a region in the abdominal portion of the larva containing extensive defects, but not a true gap.

The spatial transcription pattern of the *giant* gene is also markedly different from that of the other gap loci. Both *Kr* and *hb* are expressed in extensive regions covering multiple segments, both in blastoderm and yolk nuclei. Whereas *giant* is also initially expressed in an extensive region (but only in blastoderm nuclei), expression becomes fixed in stripes, wider than a single segment but narrower than two segments,

which in the head appear with a period corresponding to three germband segments. This periodic repeat is apparently tissue specific, as expression of these stripes can be found in regions fated to develop into ectoderm, mesoderm and neural tissue, but not that fated to develop into endodermal structures.

Possible roles of giant

The mutational effects of other segmentation genes upon the spatial expression pattern of *giant* described here are sufficiently weak to imply that *giant* may be acting in a process essentially independent of the other segmentation genes. The fact that *giant* expression is not affected by mutations in the *eve* locus, which radically alter the expression patterns of pair-rule and segmental polarity genes (Harding *et al.*, 1986; Frasch and Levine, 1987), suggests that the expression of *giant* is independent of pair-rule and segmental polarity genes. The other gap genes have an effect on the spatial expression pattern of *giant*, but only after the initial pattern is established. The effect is limited to the posterior domain of *giant* and, in contrast to the interactions among the other gap loci, it appears to be indirect; mutations in the *Kr* gene, which is normally expressed in a broad stripe at 48–60% embryonic length, cause the extension of *giant* expression from 33 to 45% embryonic length. This late, indirect effect of gap gene mutations on *giant* expression may indicate the existence of unknown intermediate genes, whose products act directly on the *giant* gene.

Three possibilities are apparent for the functional role of the *giant* gene product. First, *giant*, like other gap and pair-rule genes, may be necessary to establish the normal segmentation pattern of the embryo. In this case, the *giant* gene locus may be responsible for the establishment of the 'segments' of the embryonic head and to a lesser extent of the abdomen. The different periodic pattern of *giant* in the head as compared to the two segment repeat pattern of the pair-rule genes in the germ band might indicate a difference between the processes that determine the segments of the germ band and the 'segments' of the procephalon. The relatively weak effect of *giant* mutants on the expression pattern of *ftz* (Carroll and Scott, 1986), where seven stripes are present but the width and spacing of stripes are perturbed, emphasizes the independence of these processes. A second possibility is that *giant* may in effect be a homeotic-type gene, mutations in which transform those regions of the embryo that normally express *giant* into other regions. A similar case would be that of the *spalt* gene whose action in the head region is required to prevent the labium from assuming prothoracic character and in the tail region to prevent abdominal segments A9 and A10 from resembling A8 (Jürgens, 1988). The observation of up to six secondary filzkörper (one pair for each missing abdominal segment) in the abdominal defect of *giant* larvae and of extra, ectopic naked cuticle associated with the anterior defect suggest this possibility. For example, it may be possible to interpret the *giant* phenotype as a partial transformation of A5–A7 into posterior A8–A9, while in the head, the labial segment and the labrum might take on aspects of the prothorax and of the hypopharyngeal or mandibular segments, respectively. An interesting possibility is therefore that *giant* might act directly on anterior homeotic genes such as *Dfd, Scr* and *Antp* and on posterior genes like *AbdB*.

The third possibility is that *giant* is in part directly responsible for head involution. Stripes 1, 2 and 3 roughly correspond to those tissues that need to be brought together in the center of the procephalon: the labrum, the labial structures and the anterior portion of the cephalopharyngeal skeleton respectively. Thus, expression of *giant* might 'mark' portions of the embryo that must be brought together during head involution. The phenotypic defects associated with *giant* might be expected if displaced, non-involuted tissues normally undergo selective cell death. This interpretation does not explain the defects in the abdominal region.

None of these possibilities account fully for the post-embryonic defects associated with the gt^1 allele. This mutation causes delayed pupariation from ecdysone insufficiency, resulting in large larvae, pupae and adults (Schwartz *et al.*, 1984). The explanation proposed by Petschek *et al.* (1987) that delayed pupariation (and hence continued larval growth) is due to defects in the structure of the ring gland is plausible but only weakly supported by the observation that the ring gland is smaller or disrupted in the strong, lethal allele gt^{X11}. An increased mutation rate found associated with heterozygotes of gt^1 and lethal *giant* alleles (Narachi and Boyd, 1985) might be due to the nature of the gt^1 allele, rather than the physiological function of the *giant* locus. The gt^1 allele is associated with DNA insertions near the *giant* locus. If either of these insertions represents mobile genetic elements that can be destabilized by outcrossing, an increased spontaneous mutation rate would be the result.

Materials and methods

Mutant strains

gt mutant strains were obtained from E.Wieschaus [*YA82, XH34, Df(1)1/2B/3)*], G.Lefevre (*L2*) and the Indiana University Collection (*E6*, a homozygous viable allele with no phenotype but producing high mortality when heterozygous with a strong *giant* allele). Gap mutants for *in situ* hybridization were obtained from M.Levine. Cuticle preparations were obtained according to the protocols of Wieschaus and Nüsslein-Volhard (1987).

Southern and Northern analysis

Southern analysis of genomic DNAs from *giant* mutants was performed according to standard protocols (Maniatis *et al.*, 1982). Southern blots of *giant* mutant DNAs were hybridized with ^{32}P-labeled DNA fragments. Northern analysis of RNAs of Oregon-R flies collected at daily intervals or of embryos collected at 2-h intervals were performed according to standard procedures, using nick-translated probes (Thomas, 1980).

In situ hybridization

In situ hybridization to paraffin-embedded embryonic sections was performed according to the protocols of Ingham *et al.* (1985) using ^{35}S-labeled single-strand RNA probes. Probes for *giant* were made using the 1.6-kb *Bam*HI fragment inserted into the pGem1 vector and transcribed from the T7 promoter. The orientation of the fragment is such that this probe is antisense (the 5′ end of the probe is from the chromosome-distal end of the gene). No hybridization was detected using transcripts from the Sp6 promoter (sense probe). Mutant gap embryos were identified by altered *Kr* expression patterns detected in hybridization to adjacent sections. The position of the stripes of hybridization was calibrated against *eve* stripes obtained by hybridization to adjacent sections.

Germ line transformation

Transposon constructions were assembled in the pUChsneo vector (Steller and Pirrotta, 1986) or in Carnegie 20 (Rubin and Spradling, 1983). Embryos from *Df(1)* $w^{67c23(2)}$ or ry^{506} flies were injected with a solution containing 400 μg/ml transposon and 80 μg/ml phsπ helper plasmid (Steller and Pirrotta, 1986). Transformed G1 flies were identified either by selection on food containing 0.8 mg/ml G418 or by the ry^+ eye color. After establishing stocks homozygous for the transposons, *giant* function was tested by crossing females gt^{X11}/FM6 with males carrying the transposon. Embryos that failed to hatch were collected, dechorionated and cuticle preparations were made according to Van der Meer (1977).

Acknowledgements

We thank Kate Harding and Chris Rushlow for assistance in *in situ* hybridization and Michael Levine for providing space and supplies. We also thank Helen Salz, Doug Coulter, Mark Benson and Elaine McGuffin for technical advice. This research was supported by a grant from the NIH to V.P. and a NIH Biomedical Research Support Grant to Barnard College.

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Received on January 3, 1989; revised on February 10, 1989