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Graded requirement for the zygotic terminal gene, *tailless*, in the brain and tail region of the *Drosophila* embryo

TERESA R. STRECKER[1,*], JOHN R. MERRIAM[1] and JUDITH A. LENGYEL[1,2]

[1]*Department of Biology and* [2]*Molecular Biology Institute, University of California, Los Angeles, CA 90024-1606, USA*

* Present address: Division of Biology, California Institute of Technology, Pasadena, CA 91125, USA

Summary

We have used hypomorphic and null *tailless* (*tll*) alleles to carry out a detailed analysis of the effects of the lack of *tll* gene activity on anterior and posterior regions of the embryo. The arrangement of *tll* alleles into a continuous series clarifies the relationship between the anterior and posterior functions of the *tll* gene and indicates that there is a graded sensitivity of anterior and posterior structures to a decrease in *tll* gene activity. With the deletion of both anterior and posterior pattern domains in *tll* null embryos, there is a poleward expansion of the remaining pattern. Using anti-horseradish peroxidase staining, we show that the formation of the embryonic brain requires *tll*. A phenotypic and genetic study of other pattern mutants places the *tll* gene within the hierarchy of maternal and zygotic genes required for the formation of the normal body pattern. Analysis of mutants doubly deficient in *tll* and maternal terminal genes is consistent with the idea that these genes act together in a common pathway to establish the domains at opposite ends of the embryo. We propose that *tll* establishes anterior and posterior subdomains (acron and tail regions, respectively) within the larger pattern regions affected by the maternal terminal genes.

Key words: pattern formation, segmentation, acron, *Drosophila*, *tailless*, terminal gene, brain, gene activity, mutant.

Introduction

The establishment of the segmentation pattern in the *Drosophila* embryo requires both maternal and zygotic gene products during early embryogenesis. Maternally active genes establish the overall anterior–posterior axis of the embryo and large regions of the pattern along this axis (Nüsslein-Volhard, 1977, 1979; Schüpbach & Wieschaus, 1986; Nüsslein-Volhard *et al.* 1987). Zygotic genes are required for the formation of more localized regions of the segmentation pattern. The zygotic *tailless* (*tll*) gene (Jürgens *et al.* 1984; Strecker *et al.* 1986) is required for the formation of the anterior and posterior ectodermal regions that border the aggregate domains affected by the zygotic gap mutations (Wieschaus *et al.* 1984; Lehmann & Nüssslein-Volhard, 1987; Bender *et al.* 1987; Petschek *et al.* 1987).

Defects observed in the mature embryonic cuticle of *tll* embryos can be traced to defects in the 9 h embryo (Strecker *et al.* 1986). Anteriorly, an abnormal dorsal cephalopharyngeal skeleton can be traced to a reduced clypeolabrum and procephalic lobe. Posteriorly, the absence of the tail region [term used by Jürgens (1987) to refer to the larval epidermis behind the posterior boundary of the seventh abdominal segment] can be traced to an absence of the most posterior abdominal segments. Despite the absence of the tail region, *tailless* embryos are of normal body length due to an expansion of the remaining abdominal segments (Strecker *et al.* 1986). This alteration in the remaining positional values has been shown to occur by the cellular blastoderm stage (Mahoney *et al.* 1986; Mahoney & Lengyel, 1987), consistent with the hypothesis that the *tailless* gene has a fundamental role in establishing and/or maintaining positional information along the anterior–posterior axis at the time of cell determination.

We present here a detailed genetic and phenotypic investigation of the *tll* gene and its relationship to the

maternal terminal gene family (defined by Nüsslein-Volhard *et al.* 1987). The isolation of simple, overlapping deficiencies of *tll* facilitated the characterization of null and hypomorphic genotypes. We have determined the elements of the embryo most sensitive to *tll* gene activity and have correlated the degree of anterior and posterior phenotypic severity in the *tll* allelic series. Using anti-horseradish peroxidase staining, we tested the effect of a lack of *tll* gene activity on the brain. Characterization of double mutants establishes the relationship between *tll*, maternal terminal and zygotic gap genes.

Materials and methods

Stocks

The original *tll* allele (tll^1 = L10.22) was isolated on a *st e* marked chromosome in an EMS mutagenesis screen (Jürgens *et al.* 1984). Gene and chromosome symbols are described by Lindsley & Grell (1968) and Lindsley & Zimm (1985, 1986, 1987). All stocks were maintained on standard *Drosophila* media (Ashburner & Thompson, 1978).

The deficiency and duplication chromosomes used to determine the location of *tll* are shown in Fig. 1. The terminal 3R duplications, *Dp(3;1)*s *34*, *152P*, *93*, *150P*, *79*, *1A* and *48*, were provided by Dr R. MacIntyre and have been previously described (Frisardi & MacIntyre, 1984; Kongsuwan *et al.* 1986; Strecker, 1987). The terminal 3R deficiencies, *Df(3R)*s *J55*, *R97*, *A113* and *L129*, have also been previously described (Lindsley *et al.* 1972; Kongsuwan *et al.* 1986; Strecker, 1987).

Screen for X-ray-induced tll *alleles*

2- to 5-day-old males homozygous for the recessive marker *ca* (Lindsley & Grell, 1968) were irradiated with 3600 rad and crossed to untreated H^{D1} *cd ca/In(3R)C, Tb Sb cd ca* virgin females at 25°C (50 males:200 females per bottle). After 2–3 days, males were transferred to fresh bottles and given additional untreated H^{D1} *cd ca/In(3R)C, Tb Sb cd ca* virgin females. Males were discarded 5 days after irradiation. The heterozygous *(ca)*/In(3R)C* F_1 progeny were then screened for induced *tll* mutations as follows. Single *(ca)*/In(3R)C* F_1 males were crossed to three to five tll^1*/In(3R)C, Tb Sb cd ca* virgin females. Each test mating was kept in a vial for 12 to 15 days at 25°C after which it was screened for the absence of the *(ca)*/*tll^1 F_2 progeny class. Since only the latter will have normally shaped pupal cases, while the remaining progeny carry the *Tb* (*Tubby*) pupal marker on the *In(3R)C* balancer chromosome, the absence of wildtype pupae was used as a selection criterion for lines containing putative *tll* alleles. In addition, virgin *(ca)*/In(3R)C* F_1 females were mated en masse to tll^1*/In(3R)C* males for two days, after which fertilized females were placed individually in vials and kept at 25°C for 12 to 15 days. Each vial was then screened as described above. From matings that exhibited an absence of wildtype pupae, *(ca)*/In(3R)C* males and virgin females were crossed *inter se* to establish a balanced stock.

Analysis of structures in larval cuticle

The cuticle of unhatched mature embryos was prepared for examination by the procedure of van der Meer (1977), modified as described previously by Strecker *et al.* (1986). Anteriorly, structures of the cephalopharyngeal skeleton (described by Jürgens *et al.* 1986) were examined in detail, namely the dorsal arms, dorsal bridge, vertical plates, ventral arms, median tooth (labrum) and dorsal sac (Fig. 3A). (Note that the region termed the pharyngeal ridge in our original description of the *tll* mutant phenotype [Strecker *et al.* 1986] is what we now refer to as the dorsal cephalopharyngeal skeleton.) Posteriorly, *tll* embryos were examined for the presence of cuticular structures of the tail region as defined by Jürgens (1987). In addition to the ventral denticle belts and dorsal hairs of the seventh and eighth abdominal segments, the presence or absence of the dorsal straight spinules (or Fell), Filzkörper, posterior spiracles, anal tuft and anal pads was noted (Fig. 3I; Lohs-Schardin *et al.* 1979; Sato & Denell, 1986; Whittle *et al.* 1986; Jürgens, 1987).

Analysis of early embryos using scanning electron microscopy (SEM)

Staged embryos were collected and prepared for SEM according to the protocol of Mahowald & Turner (1978) modified as described by Strecker *et al.* (1986). Anteriorly, 9 h *tll* embryos were examined for the degree of reduction of the clypeolabrum, procephalic lobe and optic plaques (Fig. 4A; Turner & Mahowald, 1979; Schoeller, 1964). (Note that in our original description of the *tll* mutant phenotype [Strecker *et al.* 1986] the optic plaques were incorrectly termed optic lobes.)

Horseradish peroxidase staining of the central nervous system

The supra- and suboesophageal ganglia were visualized by staining with anti-horseradish peroxidase antibody (Jan & Jan, 1982; Hartenstein & Campos-Ortega, 1986) using modifications suggested by J. Campos-Ortega (personal communication). Embryos were collected and aged to 16–18 h at 22°C at which time they were dechorionated with 50 % bleach and transferred to heptane:4 % paraformaldehyde/PBS (phosphate-buffered saline: 130 mM-NaCl, 7 mM-Na_2HPO_4, 3 mM-NaH_2PO_4) pH 7·2 (1:1) and rotated for 20 min. The fixative was removed and embryos were devitellinized by shaking vigorously in 100 % methanol for 1 min. Then embryos were washed in PBS and postfixed for 40 min in PBS containing 4 % paraformaldehyde. Embryos were washed 20 min each in PBS, PBS containing 0·2 % BSA (PBS/BSA) and PBS containing 0·2 % BSA and 0·2 % Triton X-100 (PBT) and then incubated for 20 min in 10 % goat serum (Sigma) in PBT. Following addition of rabbit anti-horseradish peroxidase (HRP; Cappel) to a final dilution of 1:4000, the embryos were incubated overnight at room temperature with gentle agitation. Embryos were then washed in PBT, preblocked by a 30 min incubation in 10 % goat serum in a volume appropriate for diluting the second antibody, then incubated for 3 h at room temperature with goat anti-rabbit IgG conjugated to HRP (Sigma) added to a final dilution of 1:50. Embryos were washed

20 min each in PBT, PBS/BSA and PBS, and were then stained for HRP activity using diaminobenzidine (1 mg ml^{-1}, 0·01 % H_2O_2; Polysciences). The reaction was monitored under a dissecting microscope and stopped by diluting with PBS. Stained embryos were stored in PBS containing 0·02 % NaN_3 and were mounted in glycerol for microscopic examination and photography using differential interference contrast optics. Embryos were scored for the presence of the following brain structures; supra- and suboesophageal ganglia, anterior and posterior trunks of the supraoesophageal neuropile and the frontal commissure (Campos-Ortega & Hartenstein, 1985; Fig. 3E).

Double-mutant constructions

Mutant alleles of various genes were placed in combination with *tll*. The alleles used and their sources were *trunk* [*trk*^{RA41}, Schüpbach & Wieschaus, 1986] from T. Schüpbach and E. Wieschaus; *fs(1)Nasrat* [*fs(1)N*^{211}, Degelmann *et al.* 1986] from A. P. Mahowald; *Krüppel* [*Kr*^{1}, Wieschaus *et al.* 1984] and *hunchback* [*hb*^{14F}, Lehmann & Nüsslein-Volhard, 1987; Bender *et al.* 1987] from Bowling Green Stock Center; *extra sex combs* [*esc*^{2} and *esc*^{5}, Struhl, 1981] from G. Struhl. Double-mutant embryos were obtained utilizing standard genetic crosses; the mature cuticle of these embryos was examined as described above.

Results

Generation of tll *alleles and cytological mapping of* tll *locus*

In the EMS mutagenesis screen in which it was identified (Jürgens *et al.* 1984), only one *tll* allele was recovered. We wished to obtain additional *tll* alleles to determine the null phenotype, the regions of the pattern most sensitive to loss of *tll* gene function and whether anterior and posterior regions of the embryo are affected to a similar degree by different *tll* alleles. From 10 100 X-ray mutagenized chromosomes, we identified six additional *tll* alleles. Two of the alleles, *tll*^{a} and *tll*^{c}, are cytologically normal. The cytology of the three recovered deletions, *tll*^{e}, *tll*^{f} and *tll*^{g}, places the *tll* locus in the chromosomal region 100A1,2-100B4,5 (Fig. 1). The localization of *tll* was further refined with the recovery of the *tll*^{2} allele, an inversion with a distal breakpoint between bands 100A5,6 and 100B1,2. Additional confirmation of this localization was obtained from the observation that the combination of the proximal part of *T(Y;3)A113* with *Dp(3;1)150P*, which creates a synthetic deletion of the region between 100A5,6 and 100B1,2 (Kongsuwan *et al.* 1986), uncovers the *tll* mutant phenotype (Fig. 1). Embryos carrying this synthetic deficiency, and *tll*^{1}, *tll*^{2}, *tll*^{a} or *Df(3R)tll*^{e}, die and exhibit the embryonic *tll* phenotype. All the evidence is thus consistent with the localization of the *tll* gene within 100A5,6-100B1,2.

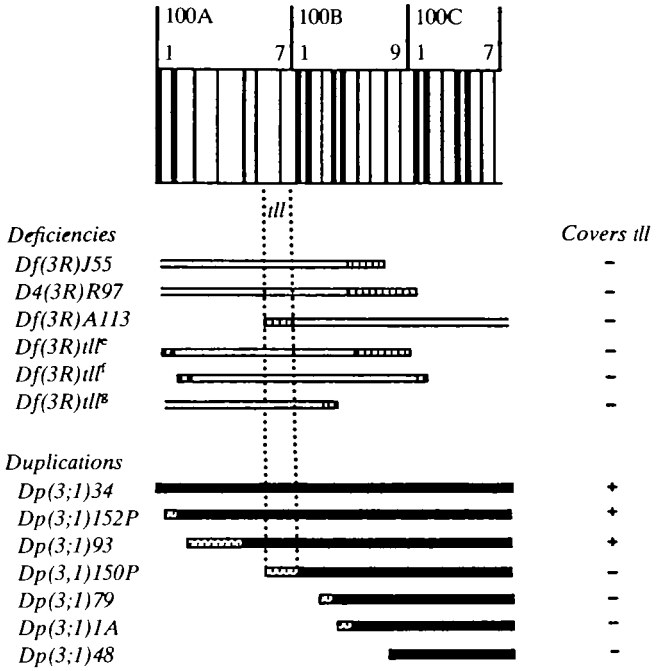


Fig. 1. Mapping of the *tailless* gene to the 100A5,6 to 100B1,2 interval. Bars represent the cytological extents of the deficiencies (open) and the duplications (solid). The dotted boxes represent the uncertainty in the limits of the cytological breakpoints. The results of mapping the *tailless* mutation are summarized in the right column: '−' represents a deficiency that uncovers or a duplication that fails to cover the *tll* mutation, while '+' represents a deficiency that does not uncover or a duplication that covers the *tll* mutation. *Df(3R)L129* (not shown) is deficient for the 100D,E-telomere region and does not uncover the *tll* locus. Although, due to the limits of the cytological analysis, the uncertainties in the breakpoints of *Df(3R)A113* and *Dp(3;1)150P* overlap in the figure, genetic analysis shows that the combination of *Df(3R)A113* and *Dp(3;1)150P* generates a small deficiency which uncovers *tll* (see text).

tailless *phenotypic series*

The *tll* alleles were ordered into a graded phenotypic series on the basis of the following description of the development of the anterior and posterior regions of the embryo. Anteriorly, the major elements of the dorsal cephalopharyngeal skeleton are the dorsal bridge, dorsal arms and vertical plates (Fig. 3A); fate mapping traces the origin of these structures to the procephalic lobe of the early segmented embryo, and earlier, to the acronal anlage of the cellular blastoderm (Jürgens *et al.* 1986). Posteriorly, fate mapping indicates that the cuticular elements of the tail region arise from the prospective abdominal segments in the cellular blastoderm as follows: anal pads (A11 and A10), anal tuft (telson, which is dorsal to A9/A10), dorsal straight spinules or Fell (anterodorsal A8), spiracles and Filzkörper (posterodorsal A8) and the

eighth abdominal denticle belt (anteroventral A8) (Fig. 3I; Jürgens, 1987; Sato & Denell, 1986; Whittle *et al.* 1986).

Recovery of the *Df(3R)tll*^{e} and *Df(3R)tll*^{g} overlapping deficiencies (Fig. 1) permitted the construction of trans-heterozygotes which contain a small deficiency of the region including the *tll* gene and which give rise to the *tll* null phenotype (Fig. 2E). Anteriorly, *tll*^{e}/*tll*^{g} (=*tll* null) embryos have normal derivatives of the gnathal (mandibular, maxillary and labial) segments, i.e. antennomaxillary sense organs, lateralgräten, ventral arms, H-piece, mouthhooks, cirri and labial sense organs. The most obvious anterior cuticle defect is an abnormal dorsal cephalopharyngeal apparatus; the contralateral homologues of the dorsal bridge fail to fuse and the dorsal arms are reduced in length (Fig. 3C). Although the median tooth (labrum) is formed, it is located in a more posterior position along the dorsal sac; additionally, scleritized material lies within the dorsal sac just posterior to the labrum ('dorsal pouch' syndrome described by Jürgens *et al.* 1986). Posteriorly, these embryos lack all derivatives of the tail region, as well as the posteroventral cuticle of A7. In the most extreme embryos (a small fraction of the total), the denticle belt and dorsal hairs of A7 are also deleted (Fig. 3K). Although all cuticular structures of the tail region are missing in *tll* null embryos, the anal opening and proctodeum are always present.

We describe below the phenotypes of several *tll* alleles, which exhibit weaker phenotypes than that of *tll* null embryos. Each of these alleles, when in trans to *Df(3R)tll*^{e}, give rise to embryos which exhibit a slightly more extreme distribution of mutant phenotypes than that observed when the allele is homozygous; this was determined by scoring the phenotypic range of 50 embryos of each genotype. Based on these observations, *tll*^{1}, *tll*^{a} and *tll*^{2} are considered hypomorphs.

Embryos that are *tll*^{1}/*tll*^{1} or *tll*^{1}/*Df(3R)tll*^{e} (Fig. 2D) are indistinguishable from *tll* null embryos in the anterior, but show a weaker phenotype in the posterior: while the ventral denticle belt of A8, dorsal straight spinules, Filzkörper, posterior spiracles, anal tuft and anal pads are missing, a small tuft of A8 dorsal hairs and all cuticular structures of A7 are present (Fig. 3J). In a few less extreme cases, *tll*^{1}/*tll*^{1} embryos display a cluster of denticles, probably a remnant of the A8 denticle belt, in addition to dorsal hairs at the posterior end of the embryo.

Homozygous *tll*^{a} or *tll*^{a}/*Df(3R)tll*^{e} embryos show a weaker phenotype (Fig. 2C) than *tll* null and *tll*^{1} embryos. Anteriorly, *tll*^{a}/*tll*^{a} embryos have a continuous dorsal bridge but reduced dorsal arms (Fig. 3B); the median tooth is positioned just posterior to the mouthhooks as in wildtype embryos. There is little, if any, scleritized material in the dorsal sac. Posteriorly, *tll*^{a}/*tll*^{a} embryos are indistinguishable from *tll*^{1} embryos (Fig. 3J).

Homozygous *tll*^{2} embryos exhibit the weakest mutant phenotype. Approximately half of these embryos hatch from the chorion and die as first instar larvae, showing the same phenotype as the lethal embryos (Fig. 2B). Anteriorly, the cephalopharyngeal skeleton is normal in *tll*^{2} homozygous or *tll*^{2}/*Df(3R)tll*^{e} embryos. Posteriorly, the majority of *tll*^{2}/*tll*^{2} and *tll*^{2}/*Df(3R)tll*^{e} embryos and larvae have a slightly reduced eighth abdominal denticle belt and partial Filzkörper. These embryos frequently have only one posterior spiracle and an anal tuft in an abnormal position, adjacent to the dorsal straight spinules. The most extreme *tll*^{2}/*Df(3R)tll*^{e} embryos have a reduced A8 ventral denticle belt and lack the dorsal straight spinules in addition to the remaining elements of the tail region, while the least extreme *tll*^{2}/*tll*^{2} embryos exhibit all tail derivatives except the anal pads (Fig. 2B).

Effect of tailless *on the supraoesophageal ganglion*

Structures of the cephalopharyngeal skeleton that are reduced or missing in mature *tll* embryos (dorsal bridge and arms) have been fate mapped to a position in the blastoderm embryo adjacent to the presumptive brain (supraoesophageal ganglion; Jürgens *et al.* 1986). Furthermore, during germband extension, elements of the cephalopharyngeal skeleton map to the surface of the procephalic lobe while the supraoesophageal ganglion is formed from the procephalic and optic lobes (Fig. 4B; Jürgens *et al.* 1986; Campos-Ortega & Hartenstein, 1985).

To test the hypothesis that *tll* also results in the reduction of the adjacent supraoesophageal ganglion anlage, we used antibody to horseradish peroxidase, which stains the central nervous system (Jan & Jan, 1982). In *tll* null embryos, the central nervous system is truncated abruptly at the anterior end of the suboesophageal ganglion (Fig. 3G). Both the anterior trunk of the supraoesophageal neuropile (*at*) and the posterior trunk of the supraoesophageal commissure (*pt*) are missing. Consistent with this observation, the posterior half of the procephalic lobe (*p*) and the adjacent optic plaque (*o*) [described in *Calliphora* by Schoeller (1964)] are deleted in *tll* null embryos at the completion of germ band retraction (Fig. 4C, arrow). The optic plaque borders the optic lobe invagination and has been proposed to be the primordium of Bolwig's organ, the larval visual organ (Bolwig, 1946; Schoeller, 1964). The hypomorphic *tll*^{a}/*tll*^{a} embryos exhibit a consistently less extreme reduction of the brain region (Fig. 3F), lacking only the portions

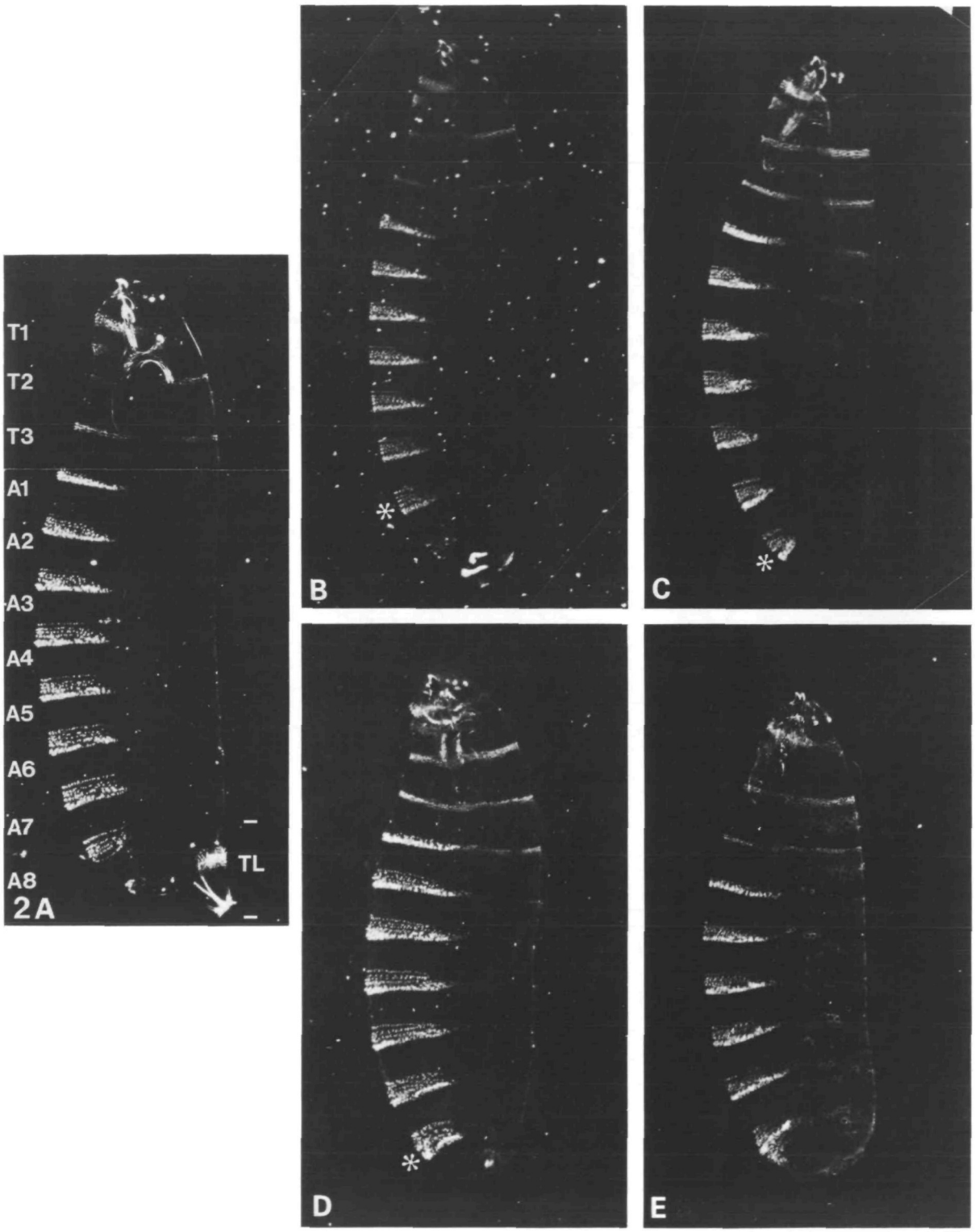
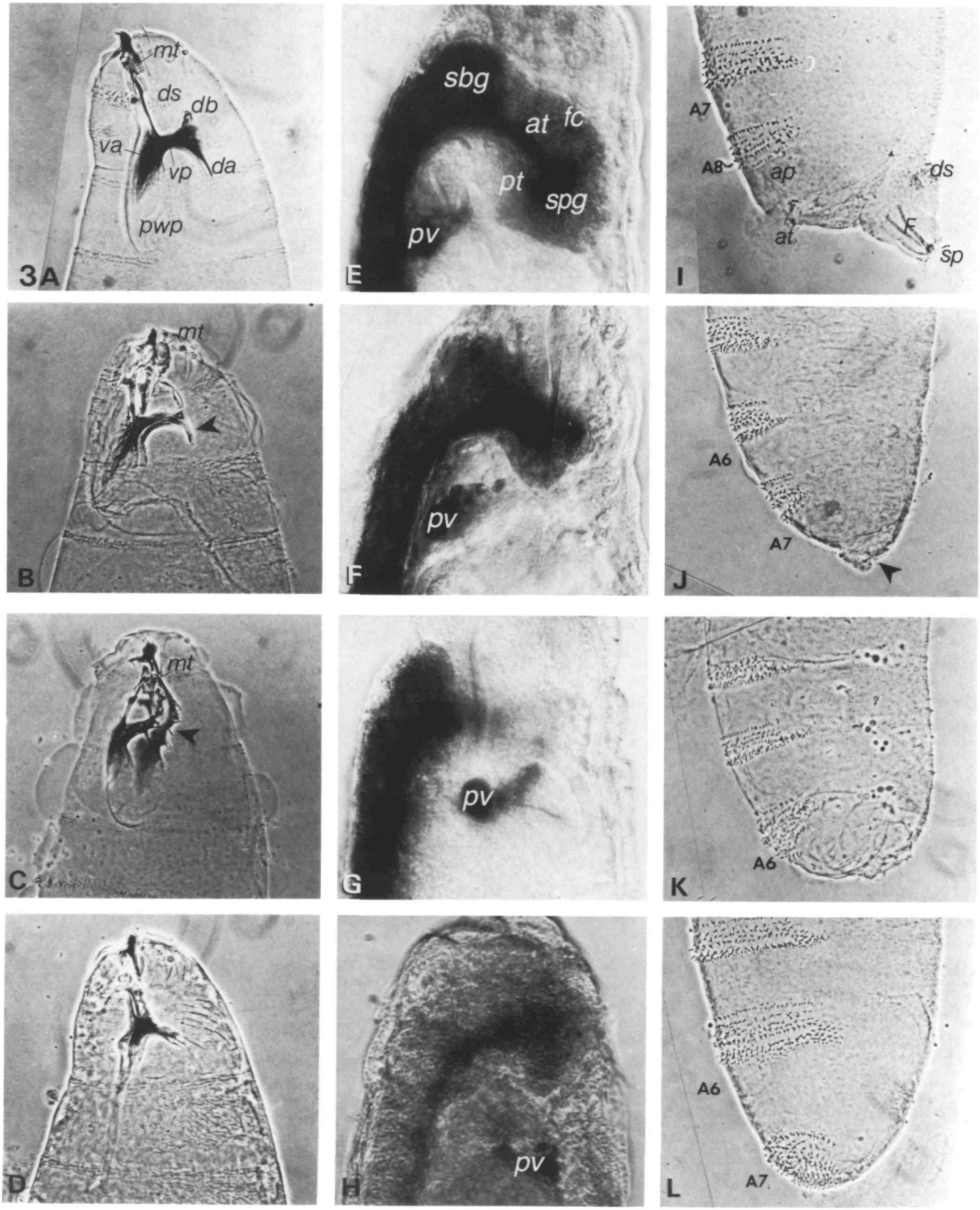


Fig. 2. Cuticular phenotypes of null and hypomorphic *tailless* alleles. Dark-field photomicrographs of lateral views of unhatched mature embryos are shown and are ordered from weakest to most extreme mutant phenotypes. The seventh abdominal segment is marked with an asterisk. (A) Wildtype, note the long dorsal arms and tail region. (B) $tll^2/Df(3R)tll^e$; anteriorly the cephalopharyngeal skeleton is normal; posteriorly, only the anal pads are missing. (C) tll^a/tll^a; anteriorly, the dorsal arms are shortened; posteriorly, the eighth abdominal denticle belt and tail region are absent. (D) $tll^1/Df(3R)tll^e$; anteriorly, the dorsal arms are shortened and the dorsal bridge is missing; posteriorly, the eighth abdominal denticle belt and tail region are absent. (E) $Df(3R)tll^e/Df(3R)tll^8$; anteriorly, the dorsal arms and dorsal bridge are missing; posteriorly, the seventh and eighth abdominal denticle belts and tail region are lacking. T1–T3, thoracic segments; A1–A8, abdominal segments; TL, tail region.

posterior to the posterior trunk of the supraoesophageal ganglion (*pt*), consistent with the hypomorphic effect of the tll^a allele on the cephalopharyngeal skeleton (see above). Furthermore, the more hypomorphic $tll^2/Df(3R)tll^e$ embryos have a normally formed supraoesophageal ganglion, consistent with



the normal cephalopharyngeal skeleton in tll^2 embryos.

Lack of tll *gene activity results in the expansion of subterminal positional values*

Due to the internalization of regions adjacent to the anterior domain (acron) affected by the *tll* mutation, an alteration in the anterior segmentation pattern of the mature mutant embryo is difficult to assess. However, with the complete deletion of acronal derivatives in *tll* null embryos there is a substantial increase in segment length, not only posteriorly in segments A2 through A6, but also anteriorly in T1 (Fig. 5). Consistent with the increase of T1 length in these embryos, there is an increase in the size of the dorsal ridge, the dorsal component of the labial segment (Fig. 4C; Campos-Ortega & Hartenstein, 1985). Thus, concomitant with the deletion of the acron and tail regions in *tll* embryos, there is an expansion of the remaining segmentation pattern toward the opposite ends of the embryo, consistent with previous observations (Strecker *et al.* 1986; Mahoney & Lengyel, 1987). The increase in segment length is correlated with the severity of the mutant phenotypes, such that *tll* null embryos exhibit the greatest increase in length of their anterior and posterior body segments, while hypomorphic *tll* embryos ($tll^1/Df(3R)tll^e$, $tll^a/Df(3R)tll^e$ and $tll^2/Df(3R)tll^e$) exhibit proportionately smaller increases in segment length (data not shown; Strecker, 1987).

Fig. 3. Effect of *tll* and *trk* mutations on the head and tail regions.

(A–D) Phase-contrast photomicrographs of the cephalopharyngeal skeleton in cuticle preparations. (A) Wildtype, note the long dorsal arms, dorsal bridge, vertical plates, median tooth and posterior wall of the pharynx. (B) tll^a/tll^a, note the shortened dorsal arms and intact dorsal bridge (arrow). (C) $Df(3R)tll^e/Df(3R)tll^8$, note the shortened dorsal arms and absent dorsal bridge (arrow). (D) trk^1/trk^1, note that, in addition to a reduced dorsal bridge and dorsal arms, this embryo lacks a median tooth and has a reduced posterior wall of the pharynx [*da*, dorsal arms; *db*, dorsal bridge; *vp*, vertical plates; *va*, ventral arms; *ds*, dorsal sac; *mt*, median tooth; *pwp*, posterior wall of pharynx].

(E–H) Phase-contrast photomicrographs of the supraoesophageal ganglion in fixed embryos stained with anti-horseradish peroxidase. (E) Wildtype, note the large supraoesophageal ganglion and the anterior and posterior trunks of the supraeosophageal neuropile. (F) tll^a/tll^a, note the absence of the posterior region of the supraoesophageal ganglion. (G) $Df(3R)tll^e/Df(3R)tll^8$, note the central nervous system ends abruptly with the formation of the suboesophageal ganglion and the dorsal shift of the proventriculus. (H) trk^1/trk^1, note the reduced size of the supraoesophageal region and dorsal shift of the proventriculus [*sbg*, suboesophageal ganglion; *spg*, supraoesophageal ganglion; *at*, anterior trunk of supraoesophageal neuropile; *fc*, frontal commissure; *pt*, posterior trunk of supraoesophageal commissure; *pv*, proventriculus].

(I–L) Phase-contrast photomicrographs of the tail region in cuticular preparations. (I) Wildtype, note the anal pads, anal tuft, Filzkörper with spiracles, dorsal straight spinules, eighth abdominal denticle belt and seventh abdominal segment with denticle belt. (J) tll^a/tll^a, note that this embryo ends with a few dorsal straight spinules at the posterior end (arrow; tll^1 embryos exhibit this same posterior mutant phenotype).
(K) $Df(3R)tll^e/Df(3R)tll^8$; this embryo ends at the sixth abdominal segment. (L) trk^1/trk^1, note that this embryo ends in the denticle belt of the seventh abdominal segment. *ap*, anal pads; *at*, anal tuft; *F*, Filzkörper; *sp*, posterior spiracles; *ds*, dorsal straight spinules; A6–A8, abdominal segments.

Double-mutant combinations of tll *with other pattern genes*

What is the position of *tll* in the hierarchy of maternal and zygotic loci that determine the body pattern in the *Drosophila* embryo? The phenotypes of two classes of pattern mutants, i.e. the maternal terminal (*torso*, *trunk*, *fs(1)Nasrat*; originally referred to as 'torso-like' genes; Schüpbach & Wieschaus, 1987; Degelmann *et al.* 1987; Nüsslein-Volhard *et al.* 1987) and the zygotic gap genes (Nüsslein-Volhard & Wieschaus, 1980; *Krüppel*, Wieschaus *et al.* 1984; *hunchback*, Lehmann & Nüsslein-Volhard, 1987 and Bender *et al.* 1987) suggest that they may be involved in functions related to that of the *tll* gene. The relationship between *tll* and these pattern mutants was addressed through double mutant and phenotypic analysis. To determine if the *tll* gene acts independently of the segmental identity of the subregion it affects, *tll* was combined with the homeotic mutant, *extra sex combs* (*esc*).

tll *and the maternal terminal mutations*

The segmental domains deleted by the *tll* mutation overlap with those regions deleted by the maternal effect terminal mutations (Fig. 7). A phenotypic comparison of the anterior and posterior cuticle defects in *trk* and *tll* embryos reveals the common pattern elements deleted by these mutations. In the anterior, *tll* embryos lack only acronal derivatives (dorsal arms, dorsal bridge), while *trk* embryos lack both these and the labral derivative: the median tooth (Fig. 3D; Schüpbach & Wieschaus, 1986; Jürgens *et al.* 1986). In addition, like *tll* embryos, *trk* embryos exhibit a significant reduction in the size of the supraoesophageal ganglion (Fig. 3H). In the posterior, *tll* affects only the ectodermal tail region and A7, while *trk* embryos lack both this ectodermal region and endodermal structures as well, i.e. the

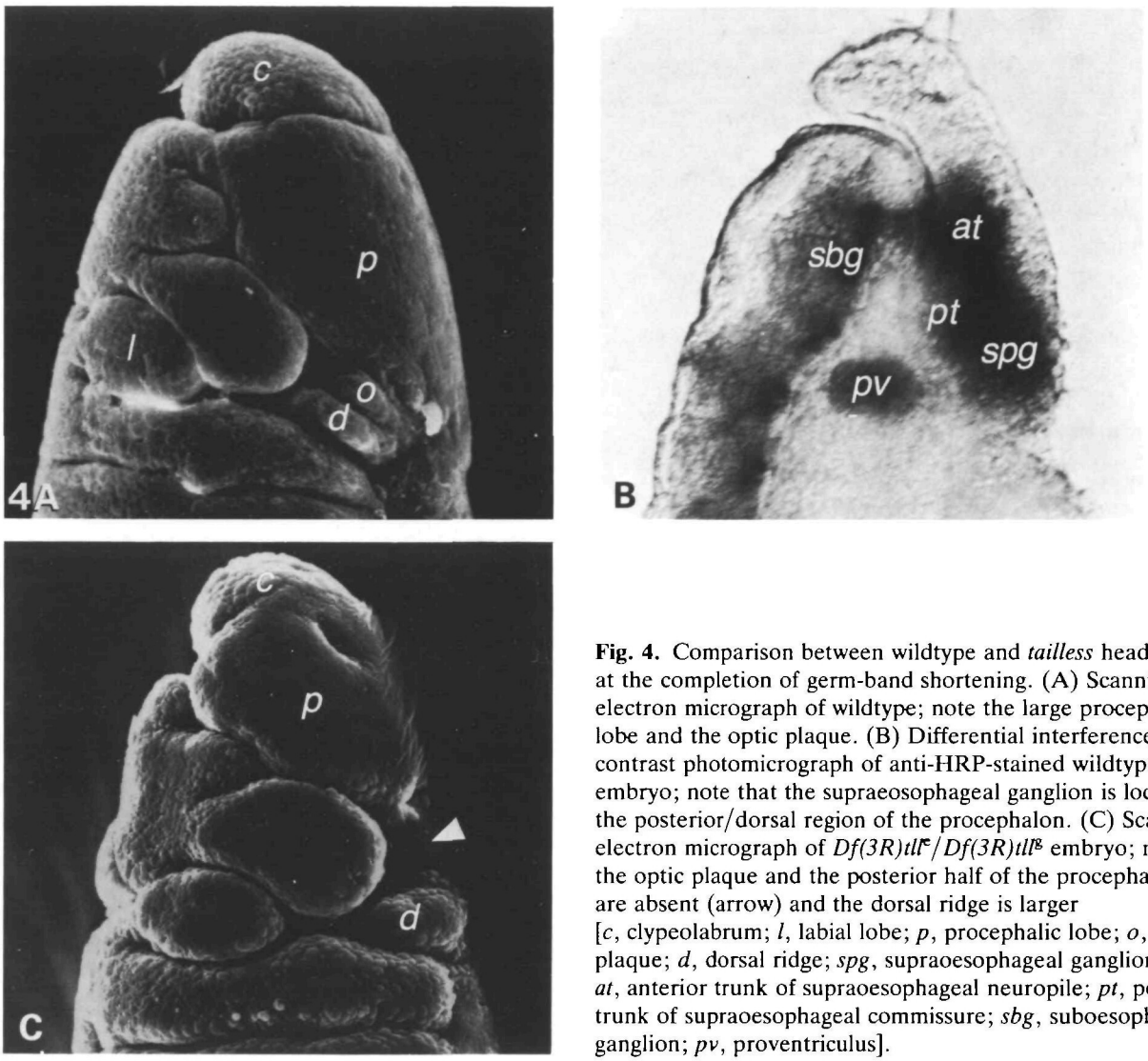


Fig. 4. Comparison between wildtype and *tailless* head regions at the completion of germ-band shortening. (A) Scanning electron micrograph of wildtype; note the large procephalic lobe and the optic plaque. (B) Differential interference contrast photomicrograph of anti-HRP-stained wildtype embryo; note that the supraeosophageal ganglion is located in the posterior/dorsal region of the procephalon. (C) Scanning electron micrograph of $Df(3R)tll^{e}/Df(3R)tll^{g}$ embryo; note that the optic plaque and the posterior half of the procephalic lobe are absent (arrow) and the dorsal ridge is larger [*c*, clypeolabrum; *l*, labial lobe; *p*, procephalic lobe; *o*, optic plaque; *d*, dorsal ridge; *spg*, supraoesophageal ganglion; *at*, anterior trunk of supraoesophageal neuropile; *pt*, posterior trunk of supraoesophageal commissure; *sbg*, suboesophageal ganglion; *pv*, proventriculus].

posterior midgut and proctodeum (Fig. 3L; Schüpbach & Wieschaus, 1986). Finally, as we have described above for *tll*, the remaining positional values in maternal terminal embryos are expanded to replace those which are deleted (Schüpbach & Wieschaus, 1986; Degelmann *et al.* 1986).

Using double-mutant constructs, we investigated how the embryo responds to an alteration in anterior–posterior positional values caused by both the maternal effect terminal and zygotic *tailless* mutations. The larval cuticle of 50 embryos from a cross of $trk^{1}/trk^{1};tll^{a}/In(3R)C$ females to $+/+;Df(3R)tll^{e}/In(3R)C$ males was scored (Fig. 6A). Although 25% (12 embryos) of these should have been tll^{a}/Df and thus lacked tll^{+} gene activity, all 50 embryos appeared identical to *trk* embryos and exhibited the most extreme *trk* phenotype (Fig. 6A). [In contrast, 18% (9/50) of the tll^{+} embryos from *trk/trk* mothers exhibited weaker posterior phenotypes, namely a complete seventh abdominal segment and reduced eighth abdominal denticle belt.] Similar results were obtained in a double-mutant combination of $fs(1)N^{211}$ and tll^{a} (Fig. 6B). Thus the loss of both *tll* and *trk* (or *fs(1)N*) does not result in a more extreme mutant phenotype than for *trk* (or *fs(1)N*) alone, but does result more frequently in embryos exhibiting the most extreme *trk* (or *fs(1)N*) phenotype. These results are consistent with the idea that *trk*, *fs(1)N* and *tll* are involved in a common process.

tll *and the gap mutations*

Like *tll*, the gap mutations also affect large, contiguous, aperiodic regions of the segmentation pattern. We placed *tll* in combination with the gap mutants *Kr* and *hb* to determine whether these zygotic genes interact. The tll^{1},hb^{14F} double mutant produced a cuticular phenotype which is the sum of the *tll* and *hb* mutant phenotypes (data not shown; Strecker, 1987). As expected, the tll^{1},Kr^{1} embryo lacks T1 through A5 as well as the telson and most of A8; A6 and A7 are

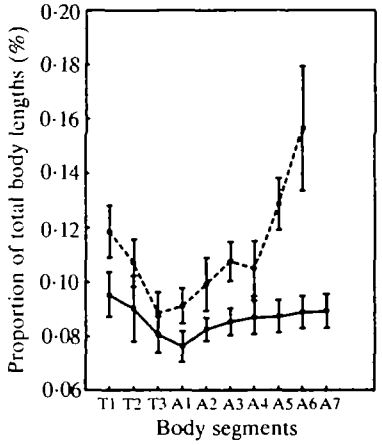


Fig. 5. Expansion of anterior and posterior pattern in *tll* embryos. The proportion that each body segment contributed to the total length of the mature embryo was determined by measuring the mature cuticle as previously described (Strecker *et al.* 1986). The average proportion each body segment contributes to the total body length of $Df(3R)tll^{e}/Df(3R)tll^{8}$ ($n = 19$, dashed line) and wildtype ($n = 30$, solid line) embryos is shown; vertical bar = standard error.

present. The *tll,Kr* double-mutant phenotype is consistently more extreme, however, than the predicted sum of the two phenotypes: there are additional denticles in the region between A6 and A7, which lack normal polarity and point toward the ventral midline of the embryo. Furthermore, there is a partial fusion between all the ventral denticle belts (Fig. 6D). This is similar to the 'lawn' phenotype observed in *hb,kni* and *h,ftz* double-mutant embryos, where there is a fusion and loss in polarity of the ventral denticle belts, which is interpreted as a failure of the embryo to form segments (Nüsslein-Volhard *et al.* 1985). A common characteristic among these three double-mutant combinations is that the mutant loci that were combined have complementary phenotypes with respect to one another (Nüsslein-Volhard *et al.* 1985; Strecker & Merriam, 1986). Presumably the deletion of large, complementary regions of the segmentation pattern, found for the *tll,Kr*, *hb,kn* and *ftz,h* double mutants, results in the remaining segment boundaries becoming fused or failing to form.

tll *combined with a maternal homeotic mutation*

The maternal effect gene, *esc*, is required during development for the correct specification of segment identity; the body segments in embryos from *esc/esc* females mated to *esc/esc* males develop like A8 (Struhl, 1981). To determine whether *tll* acts independently of the segment identity of the region it is required to specify, embryos from the cross of $esc^{2}/esc^{5};tll^{1}/+$ females to $esc^{2}/esc^{5};tll^{1}/+$ males were examined (Fig. 6F). Approximately one quarter of these embryos, while exhibiting the characteristic A8 transformation of the gnathal, thoracic and abdominal segments, also lacked the telson and had one fewer A8 denticle belt. These embryos were presumed to be doubly mutant for *esc* and *tll* gene functions. Although these embryos lacked derivatives of the three most-posterior abdominal segments, they were not shorter in length, due to an increase in the width of the remaining body segments. The transformation of most of the body segments to A8 by the *esc* mutation did not alter the effect of the *tll* mutation. This is perhaps not surprising, given that *esc* appears to act toward the end, and *tll* toward the beginning, of nuclear cycle 14 (Struhl & Brower, 1982; Mahoney & Lengyel, 1987). We conclude that the *tll* gene acts independently of segment identity.

Test of maternal tll *activity*

As the zygotic *tll* and maternal terminal genes appear to be involved in the same process (see above), we tested for maternal tll^{+} expression by asking whether extra maternal copies of the tll^{+} gene result in a less extreme *tll* mutant phenotype. This approach has been shown to be consistent with results obtained using germline clones (Wieschaus & Noell, 1986).

Flies that are *Dp(3;1)93/X;Df(3R)A113/TM6* have two functional copies of the tll^{+} gene, while those that are $tll^{1}/TM3$ have only one. If these two genotypes are reciprocally crossed to each other, a proportion of the F_1 progeny that are $tll^{1}/Df(3R)A113$ will not inherit *Dp(3;1)93* and hence will have no functional *tll* gene (Fig. 1). If the tll^{+} gene is expressed maternally as well as zygotically, a difference in phenotypic severity should appear among the resultant $tll^{1}/Df(3R)A113$ progeny from these reciprocal crosses. 50 embryos from each reciprocal cross were scored with respect to the mature anterior and posterior cuticular phenotypes and were found to be indistinguishable from one another. Furthermore, embryos from reciprocal crosses using *Dp(3;1)34* and *Dp(3;1)150P* (Fig. 1) were also observed to be phenotypically indistinguishable. These results indicate that wildtype maternal levels of the tll^{+} gene do not lessen the *tll* mutant phenotype. Although gene dosage experiments do not provide as conclusive evidence as pole cell transplantation, the results reported above are consistent with the proposal that tll^{+} is not maternally expressed.

Discussion

We have shown that in *tll* embryos the supraoesophageal ganglion (brain) is deleted, the procephalic lobe is significantly reduced and, in the cephalopharyngeal skeleton of the mature cuticle, the dorsal arms and dorsal bridge are missing. The anlagen for these

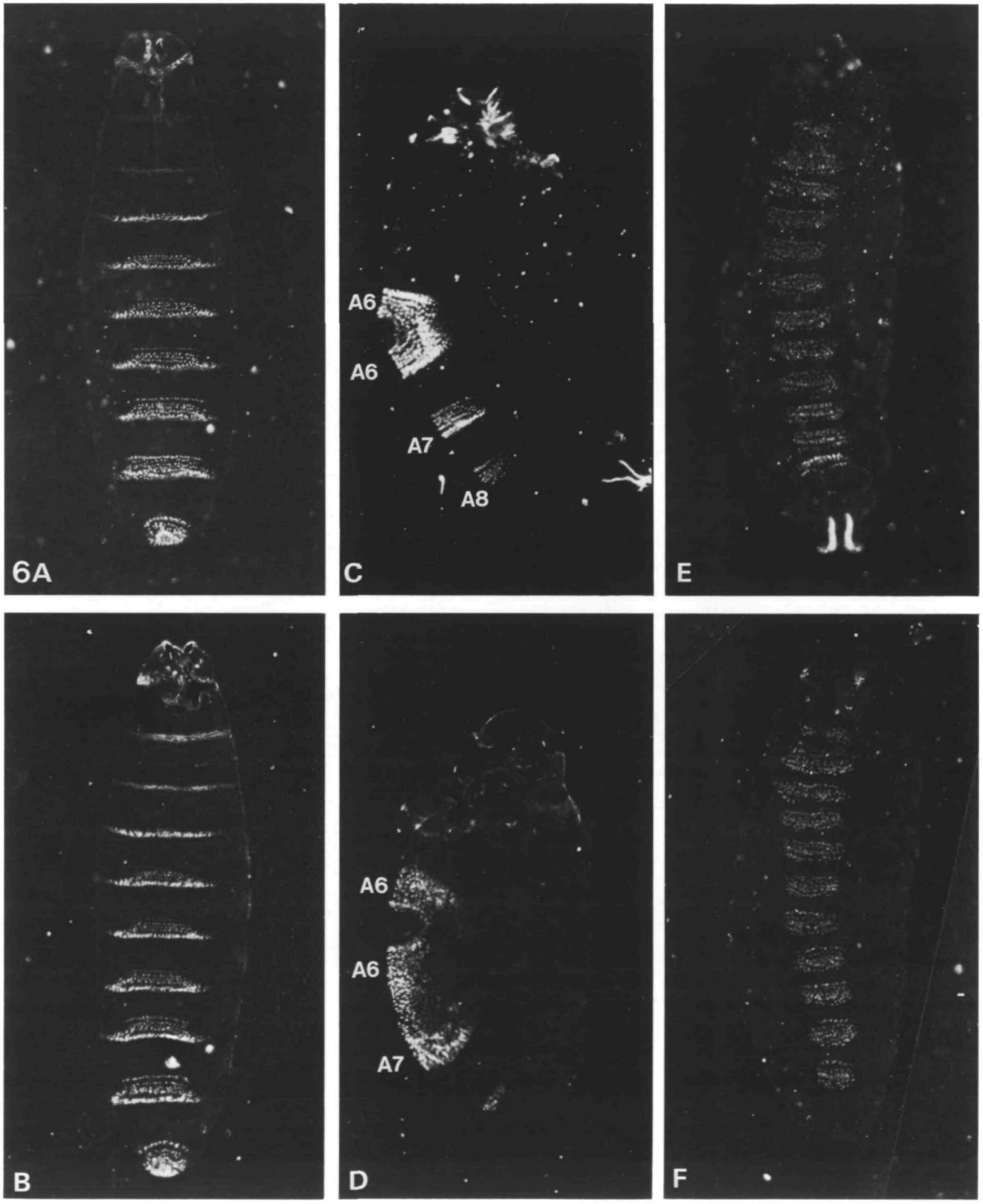


Fig. 6. Double-mutant constructs of *tll* with maternal and zygotic pattern mutants. Ventral views of the larval cuticle of representative embryos from the crosses of (A) trk^1/trk^1;$tll^a/In(3R)C$ females to +/+;$Df(3R)tll^e/In(3R)C$ males, and (B) $fs(1)N^{211}/fs(1)N^{211}$;$tll^a/In(3R)C$ females to +/+;$Df(3R)tll^e/In(3R)C$ males. Lateral views of (C) Kr^1/Kr^1 and (D) tll^1/tll^1;Kr^1/Kr^1 embryos. Ventral views of (E) embryo from esc^2/esc^5 females and (F) tll^1/tll^1 embryo from an esc^2/esc^5 female.

structures have been fate mapped to the acron in the cellular blastoderm (see fig. 9 in Jürgens *et al.* 1986). In addition, *tll* embryos lack derivatives of the seventh abdominal segment and tail region, which has been defined as comprised of segments A8–A11 and an unsegmented telson (see fig. 7 in Jürgens, 1987). Based on these observations, we conclude that the *tll* gene is required for the formation of the acron and tail region in the cellular blastoderm. The anlagen for the acron and tail occupy approximately the same area at opposite ends of the blastoderm fate map and are equidistant from the midpoint (50 % egg length) along the anterior–posterior axis (Hartenstein *et al.* 1985; Jürgens *et al.* 1986; Jürgens, 1987). The requirement for the *tll* gene by these two distant regions of the early embryo suggests that there is an underlying developmental relationship between the acron and tail regions in *Drosophila*.

There is a graded requirement for tll *in anterior–posterior ectodermal domains*

The *tll* alleles were ordered in a continuous graded series ranging from the most extreme to the weakest allele: $Df(3R)tll^{e}, Df(3R)tll^{g} > tll^{1} > tll^{a} > tll^{2}$ (data summarized in Table 1). There is a reasonably good correlation between the severity of the anterior and posterior mutant phenotypes, i.e. the weakest posterior phenotype is observed in tll^{2} embryos which have a normal cephalopharyngeal skeleton, while the most severe posterior reduction in *tll* null embryos is correlated with the most extreme deletions of structures in the dorsal cephalopharyngeal apparatus. This correlation between anterior and posterior phenotypic severities suggests that the anterior and posterior functions of the *tll* gene are not separable.

The allelic series further revealed the differential requirements of elements of the segmentation pattern for *tll* gene activity (Table 1). In the posterior, the most hypomorphic *tll* embryos lack only the anal pads, which have been fate mapped to the presumptive A10 and A11 region in the cellular blastoderm and which constitute the most posterior structure of the tail region (Jürgens, 1987). With increasing phenotypic severity, we observed deletion of the anal tuft, which arises from a more anterior blastoderm position, the telson region, dorsal to the presumptive A9/A10 boundary. Less sensitive structures are the posterior spiracles (posterodorsal A8), followed by the Filzkörper (posterodorsal A8) and the eighth abdominal denticle belt (anteroventral A8). Finally, in the most extreme *tll* phenotypes, the anterior half of A8 and posterior half of A7 are deleted. These observations suggest that there is a graded, posterior-to-anterior, requirement for the *tll* gene product in the posterior ectodermal region of the embryo; the peak of this requirement is in the presumptive A11 region (~12 % egg length).

The effect of different *tll* alleles on the formation of the supraoesophageal ganglion suggests that there is a similar graded requirement for the *tll* gene product in the anterior (Table 1). Hypomorphic tll^{a} embryos lack the most posterior region of the supraoesophageal ganglion; this region includes, but may not be

Table 1. *Structures affected in* tll *phenotypic series*

	Structure	Origin*	Alleles			
			null†	tll^{1}	tll^{a}	tll^{2}
(A) Anterior						
	Brain	Acron	–	r	r	+
	Optic plaque	Acron	–	r	r	+
	Procephalic lobe	Acron	r	r	r	+
	Dorsal arms	Acron	–	–	r	+
	Dorsal bridge	Acron	–/r	–/r	r	+
(B) Posterior						
	Anal pads	A10/A11	–	–	–	–
	Anal tuft	Telson (adjacent to A9/A10)	–	–	–	r
	Filzkörper/spiracles	A8	–	–	–	–/r
	Denticle belt	A8	–	–/r	–/r	r/+
	Dorsal hairs	A8	–	r	r	r/+
	Denticle belt	A7	r	+	+	+
	Dorsal hairs	A7	r/+	+	+	+

–, missing; r, reduced in size and/or abnormal in morphology; +, present and normal.
* Based on recent fate mapping of head and tail regions (Jürgens *et al.* 1986; Jürgens, 1987).
† $Df(3R)tll^{e}/Df(3R)tll^{g}$ embryos.

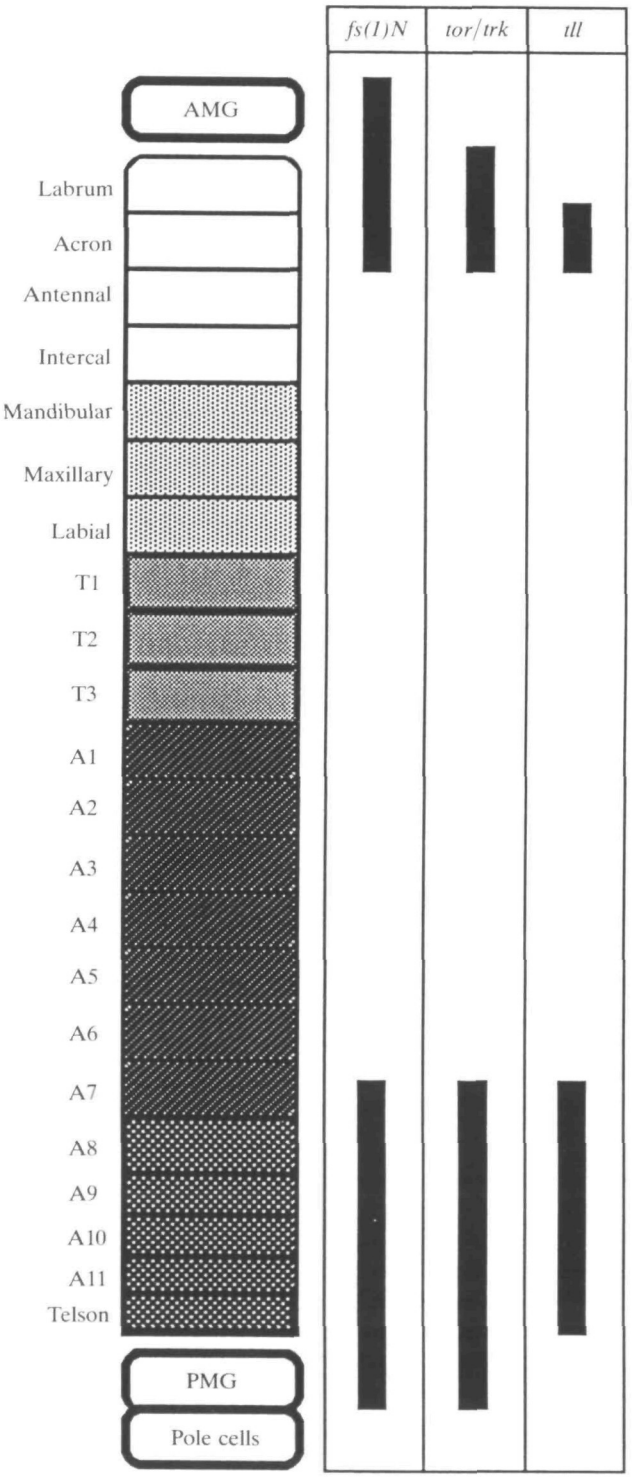


Fig. 7. Domains affected by maternal terminal and zygotic *tll* mutations. The terminal mutation *fs(1)N* deletes the endodermal and ectodermal derivatives of the anterior and posterior ends of the embryo (Degelmann *et al.* 1986), while *tor* and *trk* delete anterior ectodermal and posterior endodermal and ectodermal derivatives (Schüpbach & Wieschaus, 1986). The *tll* mutation results in the deletion of smaller regions within the pattern domains affected by these maternal terminal genes.

limited to, the optic lobe (Campos-Ortega & Hartenstein, 1985). An absence of the optic lobe in hypomorphic *tll* embryos is consistent with the abnormal rounded shape of the optic plaque (Strecker *et al.* 1986), which is presumably related to the failure of the optic plaque to become internalized just prior to the advance of the dorsal fold (Strecker, 1987). *tll* null embryos are missing not only the posterior, optic lobe-containing portion of the brain, but also the anterior portion of the brain (Fig. 3, *at*, *pt*) and, at an earlier stage, the optic plaques. These observations suggest that there is a graded requirement for the *tll* gene product in the primordia of the supraoesophageal ganglion; the peak of this requirement is probably in the presumptive optic lobe.

Although *tll* embryos lack acronal derivatives and the tail region, they are of normal body length due to the increase in the size of the remaining body segments, particularly those segments immediately adjacent to the deleted regions. This alteration of the remaining segmentation pattern correlates with the severity of the anterior and posterior mutant phenotypes observed in *tailless* embryos. With the deletion of a larger fraction of the anterior and posterior domains, there is a greater expansion of the remaining segmentation pattern. This can be traced back to an altered fate map in the blastoderm embryo, detected from altered expression patterns of *fushi tarazu* (*ftz*) and *hairy* (*h*) genes (Mahoney & Lengyel, 1987). These observations, coupled with the smooth posterior–anterior progression of deleted abdominal structures in the *tll* allelic series, support our previous suggestion that the *tailless* gene is required for the establishment and/or maintenance of graded positional information along the anterior–posterior axis (Mahoney & Lengyel, 1987).

Tailless *is a zygotic terminal gene*

Among the existing mutations known to affect the body pattern of the embryo, those in the maternal terminal family, i.e. *torso*, *trunk* and *fs(1)Nasrat* (Schüpbach & Wieschaus, 1986; Degelmann *et al.* 1986), result in a phenotype most closely resembling that of *tll*. The terminal phenotype consists of the deletion of derivatives of the labrum and acron, anteriorly, and the tail region and endodermal derivatives, posteriorly. The pattern elements deleted by the *tll* mutation, the acron and tail, are a subset of those affected by mutants in the terminal family (Fig. 7).

In addition to deleting structures from the same anterior and posterior domains, both *tll* and the terminal mutants result in an expansion of the remaining pattern, rather than the death of improperly

patterned cells. This constitutes a fundamental distinction between *tll* and the gap gene mutants. Anteriorly, the pattern expansion is observed as an increase in the segment width of T1 and the dorsal ridge in *tll* embryos (Fig. 4C), and an anterior shift of the cephalic furrow and anterior midgut invagination in *torso* and *trunk* embryos (Schüpbach & Wieschaus, 1986). Posteriorly, the pattern expansion is observed as an increase in abdominal segment width (A5, A6) in *tll* embryos, and a posteriorward shift of subterminal embryonic structures in *torso* and *trunk* embryos (Schüpbach & Wieschaus, 1986). The posteriorward shift in pattern can be traced to changes in the blastoderm fate map. As assessed by *h* and *ftz* gene expression, similar shifts are seen in *fs(1)N*, *tll*, *tor* and *trk* embryos (Degelmann *et al.* 1986; Mahoney *et al.* 1986; Mahoney & Lengyel, 1987; Mlodzik *et al.* 1987). These results suggest that the zygotic *tailless* gene is required to maintain the positional information initially established by the action of the maternal terminal genes.

This idea is supported by the results of double-mutant analysis. The same structures are missing from embryos deficient for *tll* and *trk* (or *fs(1)N*) as from embryos deficient only for *trk* (or *fs(1)N*); furthermore, *trk,tll* double-mutant embryos more frequently exhibit a more extreme *trk* phenotype than do *trk,tll*$^{+}$ embryos. These results are consistent with those obtained with *tor,trk* double mutants (Schüpbach & Wieschaus, 1986). [In contrast to *tll*, the gap mutant *giant* does not recognize the alteration in positional values resulting from the *fs(1)N*^{211} mutation (Petschek *et al.* 1987).] We interpret our double-mutant results as suggesting that *trk*, *fs(1)N*, *tor* and *tll* are involved in a common pathway required to establish and maintain positional information at the anterior and posterior ends of the embryo. Specifically, we propose that *tll* mediates the establishment of the acron and tail regions within the larger domains affected by the maternal terminal genes.

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