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The Molecular Genetics of Tail Development in *Drosophila Melanogaster*

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Abstract. *The formation of the telson in the Drosophila embryo, which encompasses all structures posterior to abdominal segment 7, is under the control of the «terminal class» genes. These maternally expressed genes are organized in a signal transduction pathway which implicates cell-cell interactions between the germ cell derivatives (the nurse cells and oocyte) and the surrounding follicle cell epithelium. Activation of this localized signal transduction pathway at the termini of the embryo is believed to specify the domains of activation and repression of a set of zygotic genes whose interactions specify the various cell states required for the proper formation of tail structures.*

Introduction

Pattern formation in the *Drosophila* embryo requires the regulation of maternal and zygotic gene activities to establish and maintain cellular identities within progressively smaller developmental fields. Initially, embryonic polarity is set up along the anterior-posterior and dorsal-ventral egg axes by maternal gene products, which then permit the subsequent establishment of restricted domains of zygotic gene expression (see reviews in references 1,2, 3). It has been proposed that anterior-posterior polarity is determined by the generation of two gradients originating from opposite egg poles (4, 5). The maternal *bicoid* gene product is responsible for the morphogenetic gradient originating at the anterior egg pole (6, 7) while posteriorly localized *nanos* activity defines a region in which posterior body pattern can be specified by other morphogens (8, 9, 10).

In addition to these two gradients that operate along the anterior-posterior axis, an activity encoded by the «terminal class» of maternal effect genes is required for the establishment of cell fates at each of the embryonic poles (2, 11, 12, 13). Thus, in embryos lacking maternal «terminal class» function, both head and tail structures are deleted.

This review focuses on the maternal genes that are involved in defining the tail region and the zygotic genes which are responding to these maternal cues. A recent review of the genetic control of the other terminus of the embryo, the head region, which is under the control of both the *bicoid* and terminal class system can be found in Finkelstein and Perrimon (14). Here, we present a summary of the cascade of gene interactions that are involved in the formation of the telson and review the evidence for a gradient of «terminal class» activity.

Structure of the telson

The *Drosophila* larvae can be broadly divided into head, thorax, abdomen and tail or telson with each of these regions being specifically defined by characteristic cuticular landmarks. The telson encompasses all structures posterior to abdominal segment 7 (A7) (2). Since all of these «tail» structures can be deleted by mutations at any one of several loci, i.e., the maternal «terminal class» genes, this region of the *Drosophila* embryo constitutes a genetically defined developmental unit.

1. Cuticular tail structures. The major visible cuticular tail structures of a fyrst instar larvae are seen in figure 1B and schematized in figure 2A. The tail region is characterized by a lack of segmental reitiration of structures. A7, which is virtually indistinguishable from segments A2 through A6, will be used here as a comparison of segmented from non-segmented tail structures. The literature contains different terminologies for various tail structures; for this review the terminology, as well as much of the morphological description of the tail, is from Jurgens (15). A7 has well defined anterior and posterior segment borders. Ventrally the anterior cuticle of A7 bears 6 rows of darkly pigmented denticles which are followed posteriorly by a naked cuticular region. The dorsal surface is nearly covered by lightly pigmented hairs of varying thicknesses. Within this dorsal lawn are three

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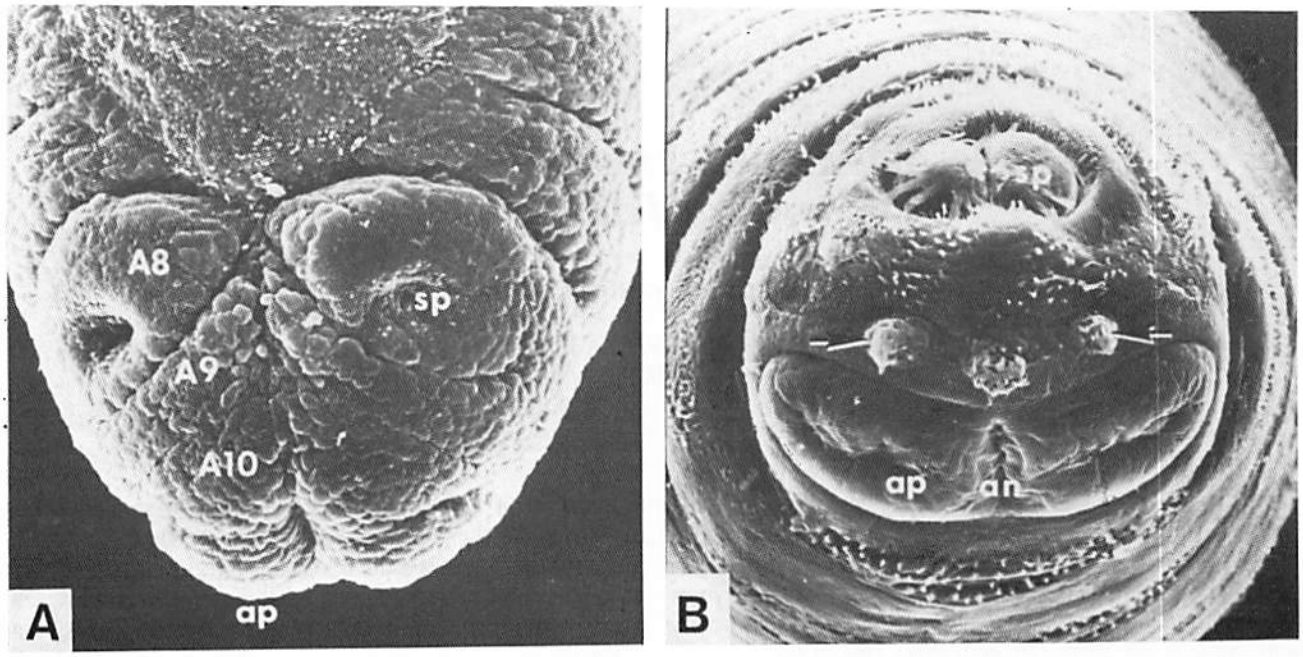

Figure 1. *Morphology of the telson. A and B are scanning electron micrographs of the posterior ends of a germ band shortened embryo and a fully mature embryo at hatching, respectively. From Turner and Mahowald (18). A. Complex morphogenetic movements have resulted in the placement of abdominal segment 8 (A8), from which the posterior spiracles (sp) develop, and rudimentary abdominal segment A10, from which the anal pads (ap) arise, in their approximate final positions. B. At hatching the posterior tip of the embryo is characterized by the prominant anal pads (ap) and the posterior spiracles (sp) with the anal sense organs (arrows) and anal tuft (just above the anus (an)) located between these structures.*

sensory hairs, the dorso-lateral (dh), lateral (lh) and ventro-lateral (vh) hairs.

In contrast, A8, while having a defined anterior segment border is lacking a recognizable posterior border. Ventrally, A8, like A7, bears a denticle band of thick, darkly pigmented denticles, however while in A7 there are 6 rows of denticles, A8 has only 4 rows of denticles. The denticles are not followed posteriorly by a naked region but by the large, convex anal pads (ap) which are separated ventro-medially by the anus (an). Just posterior to the anus, along the ventral midline is the anal tuft (at), a group of denticles resembling the ventral denticles seen more anteriorly. Dorsally, the tail ends with the posterior spiracles (ps), elongated protuberances, the base of which is covered with darkly pigmented hairs, the Fell. The apex of the posterior spiracles bear four groups of innervated hairs (sph) which encircle the spiracular openings (spo) and are continuous, via the cuticular specialization, the Filzkorper, with the dorsal longitudinal tracheal trunks. Sensory hairs, like those of A7, are not observed in the tail region; instead seven pairs of sensory protuberances or cones are observed (aso and numbers 1 through 6). Each sensory cone bears an innervated sensillium which is morphologically distinguishable into either a hair or peg type (see legend for figure 2A). The neural connections of the sensilla with the central nervous system is through the segmental nerves of either A8 or A9 (16).

2. The blastoderm fate map of the telson. Well before the appearance of the cuticular structures of the telson, extensive morphogenetic movements have taken place to organize individual terminal structures (Figure 1A). Turner and Mahowald (17, 18) used scanning electron microscopy to describe these complex cell movements. Various methods, UV-laser ablation or physical removal of small groups of cells and intracellular markers, have been utilized to trace the origins of specific structures or regions back to their presumptive cells of origin at the blastoderm stage (15, 19, 20, 21, 22, 23). For each of these methods small numbers of precisely localized cells of a blastoderm stage embryo are destroyed, removed or labeled. To determine the presumptive fates of the «altered» cells, development is allowed to proceed and the locations of the subsequent defects or labeled cells are scored in mature embryos or first instar larvae. Many experiments are required to achieve a detailed fate map for the myriad of external and internal structures. For each method utilized the generated fate maps, for the large part, confirm the results from the other methods.

Figure 2B is a presumptive fate map for many of the tail structures described in figures 1B and 2A. At the blastoderm stage the anterior limit of the tail anlage is defined to be at 20% egg length (EL; egg length is measured from the posterior tip of the blastoderm embryo, 0%, to the anterior tip, 100%) since irradiation or cell removal at 20% EL, in addition to tail defects causes defects in the posterior half of A7 (15, 21, 33). As demonstrated by cell killing and labeling experiments the posterior 10% of the blastoderm stage

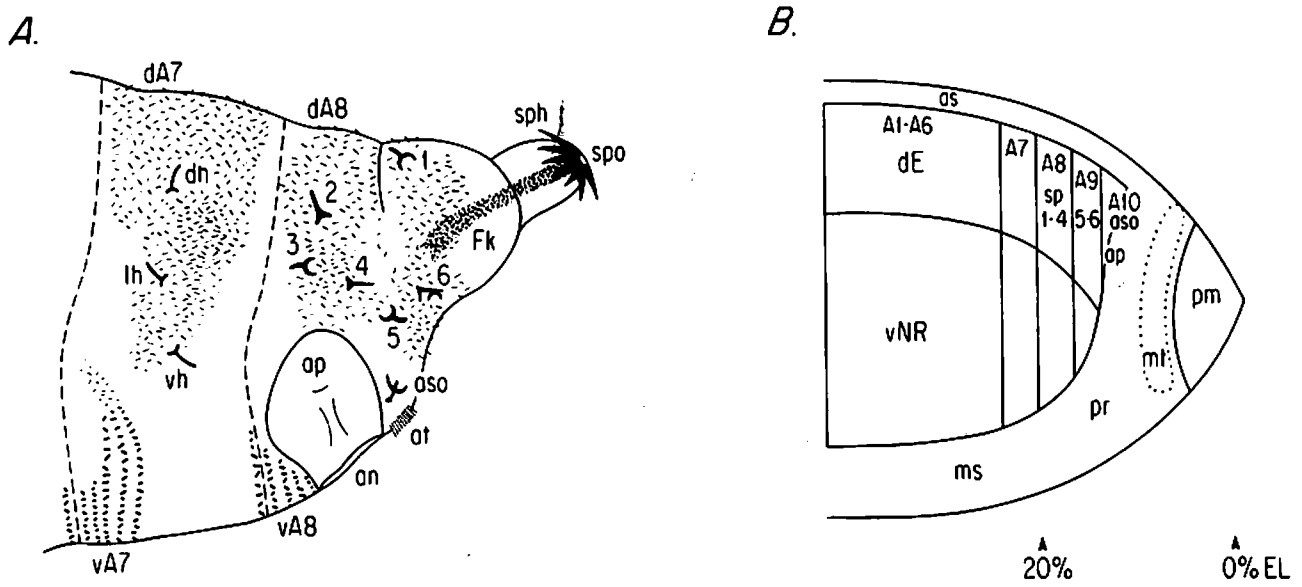

Figure 2. *Cuticular elements and fate map of the telson. A. Schematic drawing of the cuticular structures at the posterior end of a Drosophila larvae. All structures posterior to abdominal segment 7 (A7) are part of the tail region or telson. B. Presumptive blastoderm fate map of the structures of the telson. In both panels, anterior is to the left and dorsal is up. Modified from Hartenstein and Campos-Ortega (20).*

Abbreviations: an, anus; ap, anal pads; as, amnioserosa; aso, anal sense organs; at, anal tuft; A1-A10, abdominal segments 1 through 10; dE, dorsal epidermis; dA7 and dA8, hairy dorsal surface of abdominal segments 7 and 8; dh, dorsal hair; EL, egg length; Fk, Filzkorper; lh, lateral hair; ms, mesoderm; mt, Malpighian tubules; pm, posterior midgut; pr, proctodeum; sp, spiracles; sph, spiracular hairs; spo, spiracular opening; vA7 and vA8, ventral denticel bands of abdominal segments 7 and 8; vh, ventral hair; vNR, ventral neurogenic region. Numbers 1 through 6 represent sense organs: 1 and 2 are the dorso-medial; 3 and 4 are the anterior lateral; and 5 and 6 are the posterior lateral sense organs. Sence organs 1, 3 and 5 are peg types and 2 and 4 are hair types. The anal sense organ and sense organ 6 contain both hair and peg sensillum (15).

embryo develops exclusively into internalized structures, specifically the posterior midgut (pm), the Malpighian tubules (mt) and the hindgut (hg) and proctodeum (pr) (Figures 2B and 3). No cuticular defects were found when cells between 0 and 10% EL were destroyed following irradiation (15). Similarly, when horseradish peroxidase-labeled cells were transplanted into this region, their progeny were found to be incorporated into internalized structures (22) (Figure 3).

«Terminal class» maternal genes

During embryogenesis specification of telson, as well as acron (head), cellular fates requires the maternal activity of at least six genes collectively known as the «terminal class» genes. These are: *fs(1)Nasrat* (12), *D-raf* or *l(1)pole hole* (24), *fs(1)pole hole* (25), *fs(2)trunk*, *fs(2)torso* (13), and *fs(3)torso-like* (2). When any one of these gene products are absent in the female germ line similar maternal effect defects on embryonic development are observed, i.e., embryos with both anterior and posterior deletions (see review in reference 26). Posteriorly, these deletions encompass from 0-20% egg length;Wi.e., the entire telson, which includes the abdominal 8 segment, anal pads, posterior spiracles, hindgut, Malpighian tubules and posterior midgut. The deletion of the telson occurs by a reorganization of the cellular fates along the anterior-posterior axis. Posteriorly located blastoderm cells now adopt more anterior cellular fates (27, 28) and thus fail to differentiate the tail structures. Interestingly, all six maternal members of the «terminal class» genes have been reported to have a «pole hole» phenotype which consists in the absence of blastoderm cell formation underneath the pole cells. This defect is readily visualized following staining of mutant embryos with a nuclear stain (12, 13, 29). The basis for this phenotype is not understood.

fs(2)torso (tor). The *tor* gene has been well characterized genetically and molecularly. Two kinds of *tor* mutations exist: *tor* loss-of-function alleles which lack all terminal structures; and, hyperactive, gain-of-function *tor* alleles which produce a «spliced» phenotype, where embryos lack thoracic and abdominal structures, but retain terminal structures (30, 31). Thus, the «spliced» embryonic phenotype is the reverse of the loss-of-function phenotype. The *tor* gene encodes a putative transmembrane receptor tyrosine kinase and neither its RNA or protein products are spatially restricted to the embryonic poles (32, 33). That the *tor* protein is not spatially restricted suggests that its kinase activity is modulated such that it is only activated at the embryonic poles. The mere existence of constitutively active gain-of-function *tor* alleles that redirect

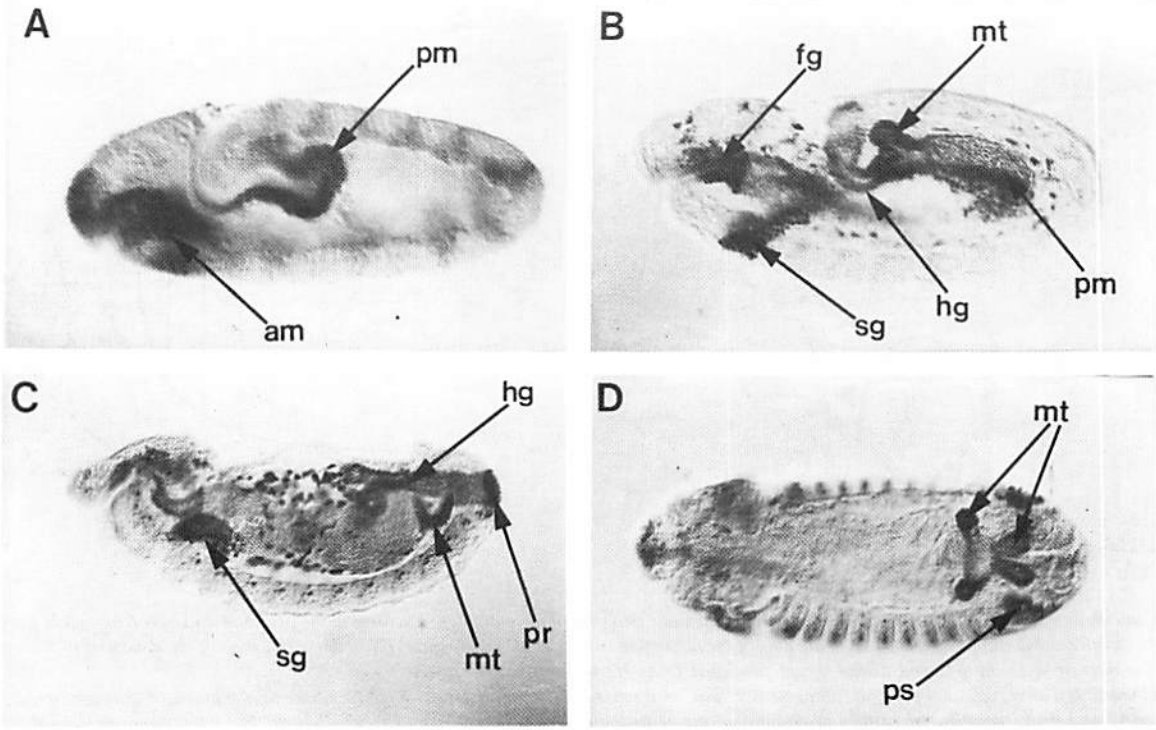

Figure 3. *Development of the internalized telson structures. To follow the wildtype development of the structures originating from the posterior 10% of blastoderm stage embryos, the expression pattern of the lacZ insertion strain 1A121 (75) (A) and the protein expression patterns of forkhead (B,C) and cut (D) were used. Stages are according to Campos-Ortega and Hartenstein (16). In all panels anterior is to the left. Dorsal is up in A, B and C. D is a dorsal view. A. Stage 11. By the time the germ band is fully extended, the posterior-most blastoderm cells have invaginated to form the posterior midgut (pm). B. Stage 12. The posterior midgut continues its invagination and the Malpighian tubule anlage (mt), as well as the hindgut (hg) and proctodeal anlage also become internalized. C. Early stage 13. With germ band shortening, the hindgut assumes its final position respective to the proctodeum (pr). The Malpighian tubules have begun to elongate. D. Late stage 13. The tracheal tree has begun to form and the «pits» destined to become incorporated into the posterior spiracles (ps) are clearly visible. Other abbreviations: am, anterior midgut; fg, foregut; sg, salivary glands.*

thoracic and abdominal regions into terminal fates suggests that in wildtype embryos the *tor* protein is activated only locally at the embryonic termini. The existence of the «spliced» phenotype also allows the conclusion that, aside from the putative *tor* ligand, each of the components of the terminal system must be present throughout the embryo in order for terminal structures to replace more central body elements (33).

l(1)pole hole l(1)ph or *D-raf*. The *l(1)ph* gene is maternally required to achieve appropriate embryonic terminal cell fates (24, 29). However, unlike mutations in the other maternal «terminal class» loci, $l(1)ph^+$ activity is also necessary at other stages of development. The *l(1)ph* gene was first identified as a zygotic lethal mutation causing death at the larval-pupal transition stage of *l(1)ph* hemi- or homozygous progeny derived from heterozygous females (29). The proliferation of imaginal cells fails to occur in these third instar larvae, resulting in small, undifferentiated imaginal discs. Thus, zygotic synthesis of $l(1)ph^+$ product is essential for imaginal disc development, but it is not required for the growth of non-dividing polyploid larval cells. Since all imaginal tissues are equally affected by *l(1)ph* mutations it seems likely that expression of this gene plays a fundamental role in dividing cells.

The *D-raf* RNA is evenly distributed throughout the embryo (34). The *D-raf* gene shows 45% homology at the amino-acid level to the human *raf-1* gene, with as much as 65% homology at the C-terminus encompassing the protein kinase domain (35, 36). The serine/threonine kinase domain of *D-raf* is well conserved suggesting that in flies the protein also behaves as a protein kinase.

fs(1)Nasrat (fs(1)N), fs(1)pole hole (fs(1)ph), fs(2)trunk (trk) and fs(3)torso-like (tsl). Less is known about the function of these «terminal class» genes. The maternal effect phenotypes of these three genes are virtually identical but in addition, for both *fs(1)N* and *fs(1)ph*, there are alleles in which females lay only collapsed, flaccid eggs (12, 15) indicating a role for these genes in egg envelop formation. Of special interest is the *tsl* gene which has been shown by mosaic analysis to function in the follicle cell epithelium during oogenesis (37). This property makes *tsl* unique among all other known maternal members of the terminal class which

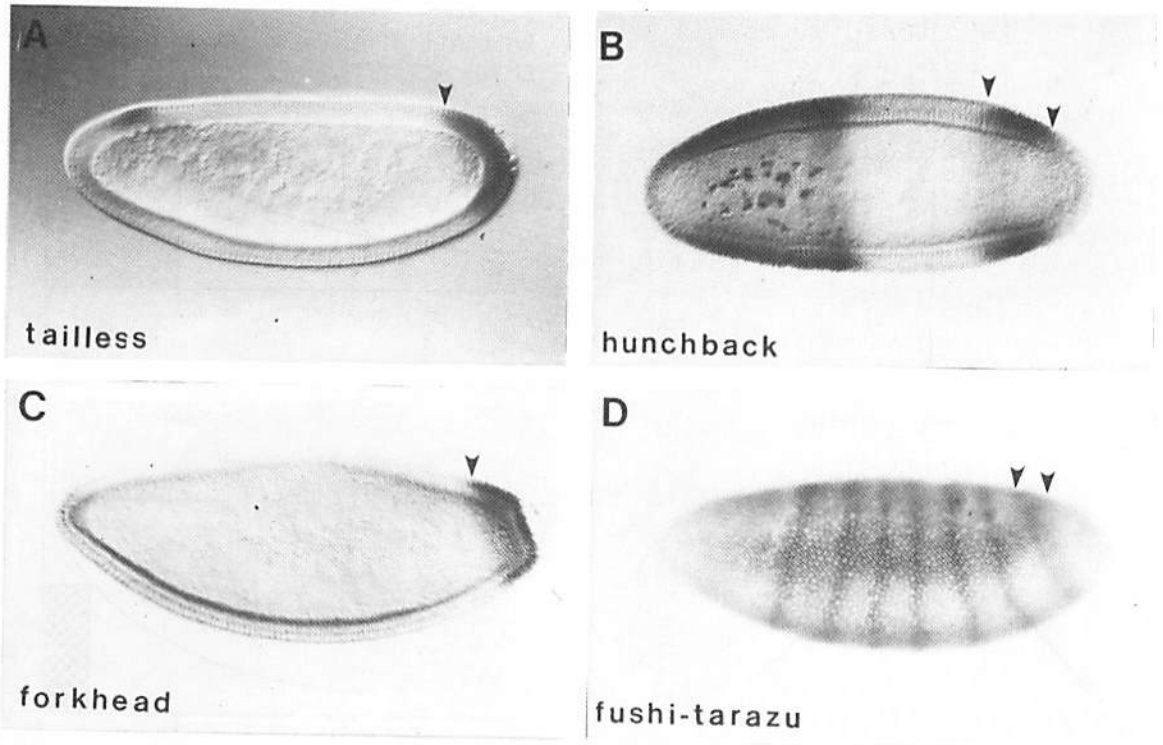

Figure 4. *Blastoderm expression patterns of zygotic telson genes. Wildtype blastoderm stage embryos with (A) tailless, (B) hunchback, (C) forkhead and (D) fushi-tarazu expression patterns. The expression patterns were revealed by non-radioactive in situ hybridization (A), antibody staining (B,C) and using a ftz-lacZ construct (76). Arrowheads indicate the anterior and posterior limits of staining at the posterior ends of the embryos. Note that tailless, at this stage, is expressed at a higher level in the posterior domain. In all panels anterior is to the left and dorsal is up in all panels.*

by mosaic analysis have been shown to function exclusively in the germ line (37, 38). The cloning of these genes has not yet been reported.

«Terminal class» primary zygotic genes

Posteriorly, two zygotic genes *tailless (tll)* and *huckebein (hkb)*, are believed to be the first zygotic genes that respond to the «terminal class» maternal activity (39, 40). Their domains of expression likely overlap but their resulting developmental lesions are complementary.

tailless (tll). Deleted in *tll* mutant embryos are structures from about 7-20% egg length, including the abdominal 8 segmenq, anal pads, posterior spiracles, hindgut, and Malpighian tubules. The posterior midgut is also reduced in size in severe *tll* alleles. In wildtype, at the syncitial blastoderm stage, *tll* transcripts are initially expressed symmetrically from 0-20% and 80-100% egg lengths. However, by the cellular blastoderm stage, *tll* expression (Figure 4A) resolves into smaller domains at both termini with the posterior domain extending from 0-15% egg length (41). The *tll* gene product, a member of the steroid receptor superfamily (41), may function as a transcription factor to activate terminal-specific genes.

huckebein (hkb). In *hkb* mutant embryos only the posterior midgut is deleted (40), which is the only posterior structure not completely deleted in *tll* mutant embryos. *hkb* encodes a putative transcription factor with multiple zinc fingers that is expressed in both the anterior and posterior midgut anlagen (G. Bronner and H. Jackle, personal communication).

The «terminal class» signal transduction pathway

The phenomenon of epistasis is when the action of one gene can wholly or partially obscure the effect of a different gene. Genetic epistasis experiments allow the ordering of genes, with different mutant phenotypes, within the same or different pathways of action. For example, if in embryos doubly mutant for genes A and B both mutant phenotypes are observed, then these genes do not function in the same pathway of action. However, if in embryos doubly mutant for genes A and B only the phenotype of gene B is observed, then gene B is «epistatic» to gene A and therefore may function downstream of gene A in a biochemical pathway.

Often times genes with indistinguishable mutant phenotypes belong to the same functional pathway and in these cases epistasis experiments may be uninformative. However, if for one of these genes gain-of-function alleles exist that produce

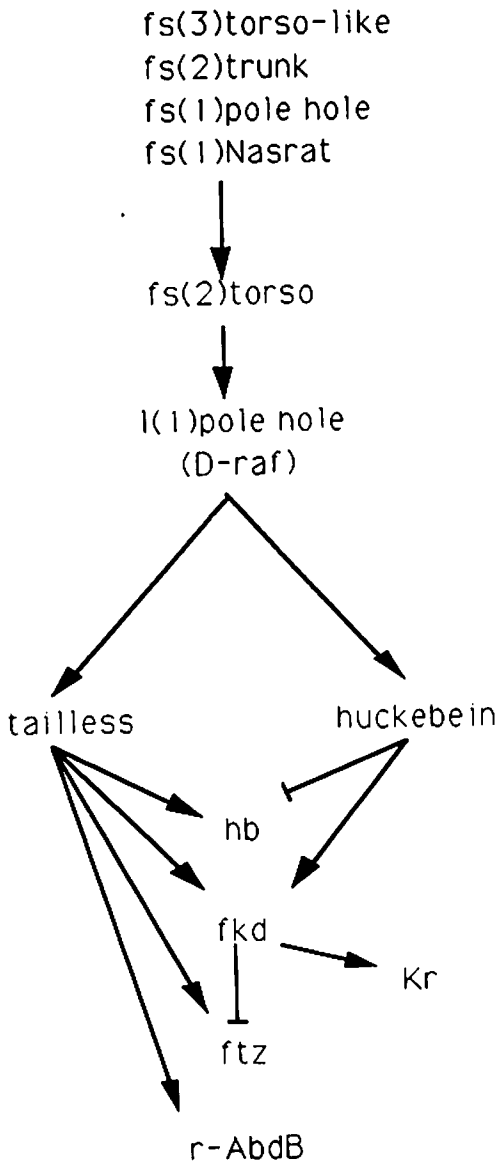

Figure 5. *Model of the control of gene activities during telson formation. This model depicts the interactions between the known maternal and zygotic members of the terminal class signal transduction pathway. This model is based on both genetic epistasis experiments and expression patterns of specific genes in various mutant backgrounds (see text). All interactions need not be direct. Activations are indicated by arrows and repressions by blocks.*

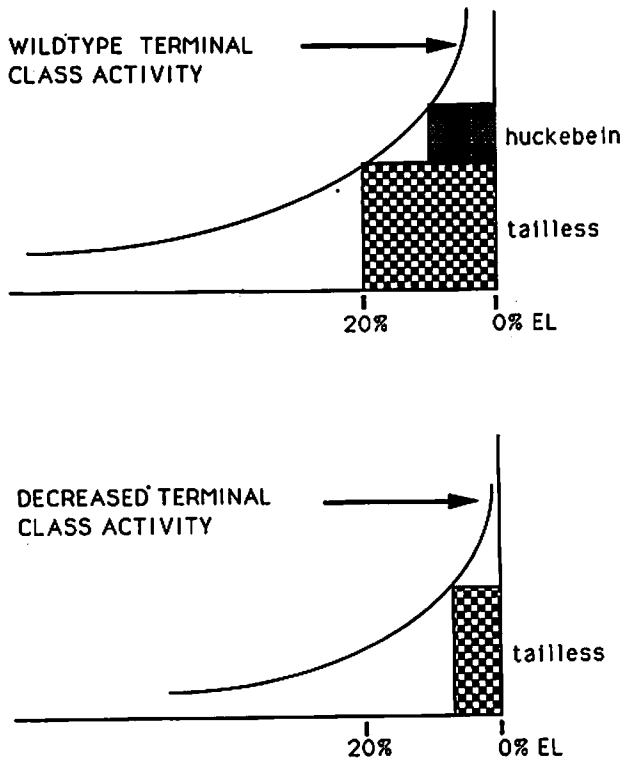

Figure 6. *A gradient of terminal class activity? This model postulates that in wildtype (top) a gradient of terminal class activity is established at the posterior pole with hkb being expressed at higher levels of terminal activity and tll expressed at lower levels. If this terminal activity is absent, as it is in mutations from torso, D-raf and the other maternal members of the pathway, neither tll or hkb are expressed (see text). However, if the terminal class activity is decreased, but not eliminated (bottom), it is predicted that tll would be expressed in a smaller domain and hkb may fail to be expressed at all.*

a phenotype different than the loss-of-function phenotype, then epistasis experiments can be carried out. As described above, loss-of-function mutations from maternal members of the terminal class, as well as *tll* and *tll, hkb* double mutants, do not develop the most terminal cuticular elements. Hyperactive *tor* alleles give the reverse phenotype, where embryos lack thoracic and abdominal structures, but retain enlarged terminal structures; the «spliced» phenotype (30, 31, 34). The effect of the hyperactive *tor* dominant alleles can be negated by mutations in *D-raf, tll* and both *tll* and *hkb* genes but not by mutations in the remaining maternal members of the terminal system (30, 31, 34, 40). This result supports a model whereby *tor* activates *D-raf, tll* and *hkb* since the effect of an excessively active *tor* protein can be suppressed by reducing the number of molecules of *D-raf*/ *tll* and both *tll* and *hkb* in the egg (see also 26).

This model is supported by the molecular characterization of both the *tor* and *D-raf* genes which turn out to encode molecules involved in signal transduction. *tor* encodes a putative transmembrane tyrosine protein kinase and *D-raf* encodes a putative serine/threonine protein kinase. Since receptor tyrosine kinases are activated when they become bound to their ligands and transmit this extracellular signal to the cytoplasm by phosphorylating intracellular proteins a current working model (Figure 5) implies that the receptor tyrosine kinase *torso*, activated by an extracellular ligand, would activate a phosphorylation cascade mediated through the serine/threonine protein kinase *D-raf*. This phosphorylation cascade would ultimately define the domains of trans-

criptional regulation of the zygotic trancription factors *tll* and *hkb*. Both the co-extensive nature of the *tll* and *hkb* mutant phenotypes and the observation that *tll, hkb* double mutant embryos are phenotypically similar to *torso* mutant embryos suggests that posteriorly *tll* and *hkb* are sufficient to mediate the maternal activity of *torso* (40).

Neither the RNA or protein products generated by the *tor* gene are spatially restricted to the embryonic poles (32, 33). Additionally, loss-of-function mutations in *tsl, trk, fs(1)ph* or *fs(1)N* block the activation of *torso* protein without altering its normal distribution (33). It therefore appears that *tor* protein depends on these four genes to become locally activated. Unique among these genes is *tsl* whose function is required in the follicle cells (37) making it a good candidate for the *tor* ligand, or at least required for the production of the ligand.

A gradient of terminal class activity

It is apparent that the *tor* activity is organized into a gradient allowing for the formation of discrete terminal structures at various levels on *tor* activity (33). The temperature sensitive, gain-of-function *tor* allele, *RL3*, is more active at 29°C than at 25°C, and more active at 25°C than at 18°C. This allele is used to generate incremental changes in the level of *tor* protein activity and in double mutant combination with *trk* the females give rise to embryos where the only *tor* activity is attributable to the constitutively active mutant protein. The cuticles of larvae derived from these mutant females at 29°C reveal near normal complements of terminal structures. As the temperature of the females is lowered the resulting larvae show progressively fewer terminal structures suggesting that different levels of *tor* activity specifies distinct terminal pattern elements (33).

It is likely that in wildtype a gradient of terminal class activity is established at the posterior pole (Figure 6, top) with *hkb* being expressed at higher levels of terminal class activity and *tll* expressed at lower levels. As described above this terminal activity is absent from *tor, D-raf* and the other maternal «terminal class» members of the pathway where neither *tll* or *hkb* are expressed (30, 40, G, Bronner and H. Jackle, personal communication). However, when this gradient is decreased, but not eliminated (Figure 6, bottom) we postulate that *tll* will be expressed in a smaller domain and *hkb* may fail to be expressed at all. The testing of these predictions is dependent on genetic means to decrease, but not eliminate, the terminal class activity perhaps through the use of hypomorphic alleles of genes downstream of *tor* or by the isolation of new loci which when mutated alters the terminal activity.

«Terminal class» secondary downstream zygotic genes

Other genes that are known to be under the control of the maternal terminal system and whose expression are mediated by the primary terminal zygotic gap genes, *tll* and *hkb*, are the gap gene *hunchback (hb)* (42), the homeotic gene *fork head (fkd)* (43, 44), the pair-rule gene *fushi-tarazu (ftz)* (27), the regulatory element of the homeotic gene *Abdominal B* (r-AbdB)(45), the gap gene *Kruppel* (60) and the homeotic gene *caudal* (65). As described below each of these genes are expressed in the posterior 20% of the developing embryo and the posterior expression patterns of each is variably affected in embryos mutant for maternal and primary zygotic members of the «terminal class».

fork head (fkh). In *fkd* mutant embryos, the posterior tail structures (anal pads, Malpighian tubules and hindgut) have been homeotically replaced by more anterior tail and posterior abdominal structures (anal sense organs, dorsal hairs and denticles). Additionally, the posterior midgut degenerates (44, 46). The embryonic *fkd* expression pattern is complex. In widtype blastoderm embryos *fkd* is expressed from both 0-13% and 94-100% egg lengths (Figure 4C). At gastrulation, *fkd* protein in widtype embryos is seen lining the posterior midgut invagitation. Then, at germ band elongation and throughout later stages of embryogenesis *fkd* protein is observed in the foregut, hindgut, Malphghian tubules and salivary glands (43, 44) (Figure 3B, C). Molecular analysis of the *fkd* gene reveals an highly conserved, novel DNA binding motif that is also present in the rat transcription factor HNF-3a (44, 47, 48). Consistent with the idea that *fkd* acts to regulate transcription is the finding that its protein product localizes to the nucleus (44).

In wildtype embryos *fkd* is known to be under the control of the terminal system since in embryos lacking *torso* activity, or doubly mutant for both *tll* and *hkb*, the posterior *fkd* domain is completely lacking, while in either *tll* or *hkb* mutant embryos it is merely reduced, but not missing (40). *fkd* also functions to define the posterior boundary of *ftz* expression (see below), since in *fkd* mutant embryos the 7th *ftz* stripe is expanded posteriorly (49).

hunchback (hb). Posteriorly, homozygous mutant embryos derived from strong *hb* alleles have deleted A8 and posterior parts of A7 (50). At the blastoderm stage in wildtype embryos, the gap gene *hb* is expressed in both an anterior and posterior domain. The posterior expression begins as a cap which subsequently is repressed at the posterior pole and resolves into a single stripe from 10-20% egg length (42, 50, 51) (Figure 4B). Sequence analysis of the *hb* gene reveals the presence of two putative DNA binding finger domains in the *hb* protein (51) suggesting that *hb* functions as a transcription factor.

Posterior *hb* expression is under the control of the terminal system since in embryos lacking *tor* activity, or doubly mutant for both *tll* and *hkb*, the posterior *hb* domain is completely lacking. (42, 49). The evolution of the *hb* polar cap of expression into a posterior stripe is due to repression by *hkb*, since in *hkb* mutant embryos *hb* remains expressed as a polar cap (49). The boundaries, then, of the posterior *hb* stripe are defined by *tll* activation and *hkb* repression.

fushi-tarazu (ftz). Homozygous *ftz* mutant embryos die late in embryogenesis with cuticles bearing only half the number of segments of wildtype embryos. Also, the posterior spiracles are mispositioned laterally and appear poorly differentiated (52). In wildtype cellular blastoderm embryos the pair-rule gene *ftz* is expressed in seven evenly spaced stripes of cells along the anterior-posterior axis with the 7th stripe positioned between 10 and 20% EL (53) (Figure 4D). Molecular analysis of the *ftz* gene reveals that it contains an homeodomain of the *Antennapedia* type and functions as a transcription factor (reviewed in reference 54).

In embryos lacking *torso* activity, or mutant for *tll*, the 7th *ftz* stripe is entirely deleted and the 6th stripe is expanded posteriorly, while in *hkb* mutant embryos the 7th *ftz* stripe is present but expanded posteriorly (40, 55). In wildtype the 7th *ftz* stripe likely appears at a given threshold of *tll* activity since analysis of this stripe in various hypomorphic *tll* alleles reveals various degrees of stripe formation (49). The posterior boundary of the 7th *ftz* stripe coincides with the anterior boundary of the posterior *fkd* domain; however in *fkd* mutant embryos the 7th *ftz* stripe extends further posterior, indicating that *fkd* expression represses the posterior expansion of the 7th *ftz* stripe (49).

r-AbdB. r-AbdB is required to specify the identity of parasegment 14 (PS14), corresponding to posterior A8 and much of A9 and possibly PS15 (45). Not unexpectedly, it is expressed, beginning at germ band extension stages, in PS14 and PS15 (56). In *r-AbdB* mutant animals structures derived from PS14 are partially transformed into PS13 derived structures (45, 56). Molecular analysis of the *AbdB* gene reveals a transcriptionally complex locus encoding homeobox containing proteins which regulate two distinct functions, either specification of PS10-15 *(m-AbdB)* or specification of PS14-15 *(r-AbdB)* (57, 58, 59).

r-AbdB expression is deleted in embryos lacking *torso* activity and in *tll* null mutants; however in *tll* hypomorphs varying levels or *r-AbdB* is observed (49). Ectopic expression of *r-AbdB* is also observed in embryos derived from *torso* gain-of-function mutant mothers (49); again suggesting that *r-AbdB* is under the control of the terminal system.

Kruppel (Kr). In addition to the well known central domain of expression, the gap gene *Kr* is also expressed in a cap at the posterior pole in the anlagen of the posterior midgut and Malpighian tubules (60, 61). Consistent with this finding is the observation that in *Kr* mutant embryos the Malpighian tubules are missing (62). In these mutants it is known that the cells which normally give rise to the Malpighian tubules do not die but instead are integrated into the hindgut suggesting that *Kr* also has homeotic properties (63). *Kr* likely functions as a transcription factor since it encodes a protein with multiple putative DNA-binding fingers (64).

Kr expression in the central segmentation domain is repressed by *tll* and *hkb;* however both of these genes activate *Kr* in the posterior Malphighian tubule domain. Further, this posterior activation is mediated through the *fkd* gene since in *fkd* mutant embryos the posterior *Kr* domain is deleted (40, 61). As described below, posterior *Kr* expression is also required for *caudal (cad)* expression in the Malpighian tubule anlage (61).

caudal (cad). Homozygous *cad* mutant embryos obtained from heterozygous mothers give rise to larvae which lack several posterior cuticular structures, the anal tuft, parts of the anal pads and the terminal sense organs (65). The *cad* gene produces two transcripts, one expressed maternally and the other zygotically. Both encode identical proteins (66). The maternal product is expressed in a concentration gradient along the anterior-posterior axis, highest at the posterior pole, after fertilization from nuclear cycle 12 through the onset of gastrulation. The zygotic product appears during the cellular blastoderm stage as a stripe between 13 and 19% egg length and continues to be expressed throughout embryogenesis in cells of the posterior midgut, the Malpighian tubules, the hindgut and cells in posterior epidermal structures (65, 66, 67). *cad* encodes an homeobox containing protein and likely functions as a transcription factor (67, 68).

Zygotic *cad* expression is dependent on *Kr.* In *Kr* mutant embryos the cells destined to form the Malpighian tubules are incorporated into the hindgut and in these embryos no *cad* expression is seen in the hindgut (61). However, *cad* is not necessary for differentiation of the Malpighian tubules since in late *cad* mutant embryos the Malpighian tubules, visualized with antibodies to *fkd,* appear like wildtype (61).

Putative «terminal class» secondary downstream zygotic genes

Two genes, *spalt* and *cut* have expression patterns and mutant phenotypes which suggest that they are most likely regulated by the terminal system. However, this has not yet been determined.

spalt (sal). sal functions in two regions near each pole of the embryo to promote head and tail as opposed to trunk development. Specifically, in *sal* mutant embryos posterior head segments are transformed into anterior thoracic structures and anterior tail segments are transformed into posterior abdominal structures. Posteriorly *sal* incompletely transforms A9 and A10 into A8 (69). Another homeotic gene, *Abd-B* (see above), also functions in this domain; however, embryos doubly mutant for *sal* and *Abd-B* mutations simply modify their respective mutant phenotypes in PS14 and 15 suggesting that these two genes act independently (69). Transcripts from the homeotic gene *sal* first accumulate at syncytial blastoderm stages ventro-laterally along the entire length of embryo, but by cellular blastoderm high levels are observed anteriorly (60 to 70% EL) and posteriorly (0 to 15% EL). During later embryonic stages *sal* is expressed posteriorly in the hindgut, but also in regions where no requirement has been observed (70). The putative *sal* gene encodes a small mRNA of 0.8 kb which is under the control of over 15 kb of upstream sequences. The putative *sal* protein contains internal repeats and other repeated motifs but no homeobox (70).

cut (ct). The *cut* locus is genetically complex. The alleles

fall into five complementation groups, two of which result in embryonic lethality. In these dead embryos the Malpighian tubules are missing (71, 72). In wildtype embryos antibodies to the *cut* protein label the peripheral nervous system (PNS) as well as many cells in the central nervous system (CNS), the Malpighian tubules and cells surrounding both the anterior and posterior spiracles (72). The *cut* protein contains a homeodomain and likely functions as a transcription factor (73).

Perspectives

Genetic and molecular evidence suggests that during embryogenesis the specification of the telson cellular fates involves a signal transduction pathway. The components of this signal transduction pathway are set up during oogenesis. Cellular interactions between germ cells and follicular cells triggers a localized phosphorylation cascade that culminates in the local activation of transcription factors at the embryonic poles.

In the next few years intensive studies along three major lines of research will certainly be emphasized.

First, it will be of extreme interest to understand the mechanism by which the *tor* receptor tyrosine kinase becomes locally activated. Obviously, good candidates for genes involved in this local activation are the maternal «terminal class» genes that act upstream of *torso*. Molecular characterization of these genes will certainly help in understanding this process.

A second set of studies will focus on the understanding of the mechanisms by which information is transduced from the *tor* receptor tyrosine kinase to the nuclear transcription factors. Genetic and molecular analyses support a model by which the putative serine/threonine protein kinase *D-raf* is the main signal transducer in this pathway. Therefore, it will be important to understand whether *D-raf* is able to either directly modify a transcription factor(s) that defines the domains of activity of both *tailless* and *huckebein* or whether *D-raf* acts on other intermediate factors which in turn regulate a transcription factor(s). Screens for second site modifiers to identify loci that interact with *torso* or *D-raf* might help to identify the interacting proteins.

Third, studies on the «terminal class» system will allow a detailed analysis of the zygotic gene hierarchy involved in tail formation. Already a number of components have been identified (see the section on secondary «terminal class» genes). Here the use of the «enhancer trap technique» (reviewed in reference 74) may play an important role in identifying genes that are regulated by either tailless and/or *huckebein*.

In conclusion, the formation of the *Drosophila* tail involves the use of a complex system of cell-cell interactions and transcriptional gene regulation that operates during oogenesis and early embryonic development. The formation of the tail provides an exciting developmental system to further our understanding of the control of gene activity during pattern formation.

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References

1 Akam M: The molecular basis for metameric pattern in the *Drosophila* embryo. Development *101:* 1-22, 1987.
2 Nusslein-Volhard C, Frohnhofer HG and Lehmann R: Determination of anteroposterior polarity in *Drosophila*. Science *238:* 1675-1681, 1987.
3 Inhgam PW: The molecular genetics of embryonic pattern formation in *Drosophila*. Nature *335:* 25-33, 1988.
4 Lehmann R and Nusslein-Volhard C: Abdominal segmentation, pole cell formation, and embryonic polarity require the localized activity of *oskar* a material gene in *Drosophila*. Cell *47:* 141-152, 1986.
5 Frohnhofer HG and Nusslein-Volhard C: Organization of anterior pattern in the *Drosophila* embryo by the maternal gene *bicoid*. Nature *324,* 120-125, 1986.
6 Driever W and Nusslein-Volhard C: A gradient of *bicoid* protein in *Drosophila* embryos. Cell *54:* 83-93, 1988a.
7 Driever W and Nusslein-Volhard C: The *bicoid* protein determines position in the *Drosophila* embryo in a concentration-dependant manner. Cell *54:* 95-104, 1988b.
8 Hulskamp M, Schroder C, Pfeifle C, Jackle H and Tautz D: Posterior segmentation of the *Drosophila* embryo in the absence of a maternal posterior organizer gene. Nature *338:* 629-631, 1989.
9 Struhl G: Differing strategies for organizing anterior and posterior body pattern in *Drosophila* embryos. Nature *338:* 741-744, 1989.
10 Irish V, Lehmann R and Akam M: The *Drosophila* posterior-group gene *nanos* functions by repressing *hunchback* activity. Nature *338:* 646-648, 1989.
11 Nusslein-Volhard C, Wieschaus E and Jurgens G: Segmentierung bei *Drosophila:* Eine genetische analyse. *In:* Verh. Dtsh. Zool. Ges. (Gustav Fisher Verlag, Stuttgart). pp. 91-104, 1982.
12 Degelmann A, Hardy PA, Perrimon N and Mahowald AP: Developmental analysis of the torso-like phenotype in *Drosophila* produced by a maternal-effect locus. Dev Biol *115:* 479-489, 1986.
13 Schupbach T and Wieschaus E: Maternal effect mutations affecting the segmental pattern of *Drosophila*. Roux's Arch Dev Biol *195:* 302-307, 1986.
14 Finkelstein R and Perrimon N: The molecular genetics of head development in *Drosophila melanogaster*. Development, in press, 1991.
15 Jurgens G: Segmental organisation of the tail region in the embryo of *Drosophila melanogaster*. Roux's Arch Dev Biol *196:* 141-157, 1987.
16 Campos-Ortega JA and Hartenstein V: »The Embryonic Development of *Drosophila melanogaster*«. Springer-Verlag Press, New York, 1985.
17 Turner FR and Mahowald AP: Scanning electron microscopy of *Drosophila melanogaster* embryogenesis: I. Gastrulation and segmentation. Dev Biol *57:* 403-416, 1977.
18 Turner FR and Mahowald AP: Scanning electron microscopy of *Drosophila melanogaster* embryogenesis: III. Formation of the head and caudal segments. Dev Biol *68:* 96-109, 1979.
19 Jurgens G, Lehmann R, Schardin M and Nusslein-Volhard C: The segmental organisation of the head in the embryo of *Drosophila melanogaster*. A blastoderm fate map of the cuticle structures of the larval head. Roux's Arch Dev Biol *195:* 359-377, 1986.
20 Hartehstein V, Technau GM and Campos-Ortega JA: Fate-mapping in wild-type *Drosophila melanogaster*. III. A fate map of the blastoderm. Roux's Arch Dev Biol *194:* 213-216, 1985.
21 Lohs-Schardin M, Cremer C and Nusslein-Volhard C: A fate map for the larval epidermis of *Drosophila melanogaster:* Localized cuticle defects following irradiation of the blastoderm with an ultraviolet laser microbeam. Dev Biol *73:* 239-255, 1979.
22 Technau GM and Campos-Ortega JA: Fate-mapping in wild-type *Drosophila melanogaster*. II. Injections of horseradish peroxidase in cells of the early gastrula stage. Roux's Arch Dev Biol *194:* 196-212, 1985.
23 Underwood EM, Turner FR and Mahowald AP: Analysis of cell

movements and fate mapping during early embryogenesis in *Drosophila melanogaster*. Dev Biol *74:* 286-301, 1980.
24 Ambrosio L, Mahowald AP and Perrimon N: *H(1)pole hole* is required maternally for pattern formation in the terminal regions of the embryo. Development *106:* 145-158, 1989.
25 Perrimon N, Mohler JD, Engstrom L and Mahowald AP: *X*-linked female sterile loci in *Drosophila melanogaster*. Genetics *113:* 695-712, 1986.
26 Siegfried E, Ambrosio L and Perrimon N: Serine/threonine protein kinases in *Drosophila*. TIGS *6(11):* 357-362, 1990.
27 Mlodzik M, DeMOntrion CM, Hiromi Y, Krause HM and Gehring WJ: The influence on the blastoderm fate map of maternal-effect genes that affect the antero-posterior pattern in *Drosophila*. Genes and Dev *1:* 603-614, 1987.
28 Winslow GM, Carroll SB and Scott MP: Maternal-effect genes that alter the fate map of the *Drosophila* blastoderm embryo. Dev Biol *129:* 72-83, 1988.
29 Perrimon N, Engstrom L and Mahowald AP: A pupal lethal mutation with a paternally influenced maternal effect on embryonic development in *Drosophila melanogasteer*. Dev Biol *110:* 480-495, 1985.
30 Klingler M, Erdelyi M, Szabad J and Nusslein-Volhard C: Function of *torso* in determining the terminal anlagen of the *Drosophila* embryo. Nature *335:* 275-277, 1988.
31 Strecker TR, Halsell SR, Fisher WW and Lipshitz HD: Reciprocal effects of hyper- and hypoactivity mutations in the *Drosophila* pattern gene *torso*. Science *243:* 1062-1066, 1989.
32 Sprenger F, Stevens LM and Nusslein-Volhard C: The *Drosophila* gene *torso* encodes a putative receptor tyrosine kinase. Nature *338:* 478-483, 1989.
33 Casanova J and Stuhl G: Localized surface activity of *torso*, a receptor tyrosine kinase, specifies terminal body pattern in *Drosophila*. Genes and Development *3:* 2025-2038, 1989.
34 Ambrosio L, Mahowald AP and Perrimon N: Requirement of the *Drosophila* raf homologue for *torso* function. Nature *342:* 288-291, 1989.
35 Mark GE, MacIntyre RJ, Digan ME, Ambrosio L and Perrimon N: *Drosophila melanogaster* homologs of the *raf* oncogene. Mol Cell Biol *7:* 2134-2140, 1987.
36 Nishida Y, Hata M, Ayaki T, Ryo H, Yamagata M, Shimizu K and Nishizuka Y: Proliferation of both somatic and germ cells is affected in the *Drosophila* mutants of *raf* proto-oncogene. EMBO J *7:* 775-781, 1988.
37 Stevens LM, Frohnhofer HG, Klingler M and Nusslein-Volhard C: Localized requirement for *torso-like* expression in follicle cells for development of terminal anlagen of the *Drosophila* embryo. Nature *346:* 660-663, 1990.
38 Schupbach T and Wieschaus E: Germline autonomy of maternal-effect mutations aftering the embryonic body pattern of *Drosophila*. Dev Biol *113:* 443-448, 1986.
39 Strecker TR, Merriam JR and Lengyel JA: Graded requirements for the zygotic terminal gene, *tailless*, in the brain and tail region of the *Drosophila* embryo. Development *102:* 721-734, 1988.
40 Weigel D, Jurgens G, Klinger M and Jackle H: Two gap genes mediate maternal pattern information in *Drosophila*. Science *248:* 495-498, 1990.
41 Pignoni F, Baldarelli RM, Steingrimsson E, Dias RJ, Patapoutian A, Merrian JR and Lengyel JA: The *Dorsophila* gene *tailless* is expressed at the embryonic termini and is a member of the steroid receptor superfamily. Cell *62:* 151-163, 1990.
42 Tautz D: Regulation of the *Drosophila* segmentation gene *hunchback* by two maternal morphogenetic centers. Nature *332:* 281-284, 1988.
43 Weigel D, Bellen HJ, Jurgens G and Jackle H: Primordium specific requirement of the homeotic gene *fork head* in the developing gut of the *Drosophila* embryo. Roux's Arch Dev Biol *198:* 201-210, 1989.
44 Weigel D, Jurgens G, Kuttner F, Seifert E and Jackle H: The homeotic gene *fork head* encodes a nuclear protein and is expressed in the terminal regions of the *Drosophila* embryo. Cell *57:* 645-658, 1989.
45 Casanova J, Sanchez-Herrero E and Morata G: Identification and characterization of a parasegment specific regulatory element of the *Abdominal-B* gene of *Drosophila*. Cell *47:* 627-638, 1986.
46 Jurgens G and Weigel D: Terminal versus segmental development in the *Drosophila* embryo: The role of the homeotic gene *fork head*. Roux's Arch Dev Biol *197:* 345-354, 1988.
47 Weigel D and Jackle H: The *fork head* domain: A novel DNA binding motif of eukaryotic transcription factors? Cell *63:* 455-456, 1990.
48 Lai E, Prezioso VR, Tao W, Chen WS and Darnell JE Jr: Hepatocyte nuclear factor 3a belongs to a gene family in mammals that is homologous to the *Drosophila* homeotic gene *fork head*. Genes and Dev *5:* 416-427, 1991.
49 Casanova J: Pattern formation under the control of the terminal system in the *Drosophila* embryo. Development *110:* 621-628, 1990.
50 Lehmann R and Nusslein-Volhard C: *hunchback*, A gene required for segmentation of an anterior and posterior region of the *Drosophila* embryo. Dev Biol *119:* 402-417, 1987.
51 Tautz D, Lehmann R, Schnurch H, Schuh R, Siefert E, Kienlin K and Jackle H: Finger protein of novel structure encoded by *hunchback* a second member of the gap class of *Drosophila* segmentation genes. Nature *327:* 383-389, 1987.
52 Wakimoto BT and Kaufman TC: Analysis of larval segmentation in lethal genotypes associated with the *Antennapedia* gene complex in *Drosophila melanogaster*. Dev Biol *81:* 51-064, 1981.
53 Hafen E, Kuroiwa A and Gehring WJ: Spatial distribution of transcripts from the segmentation gene *fushi tarazu* during *Drosophila* embryonic development. Cell *37:* 833-841, 1984.
54 Gehring WJ and Hiromi Y: Homeotic genes and the homeobox. Ann Rev Genet *20:* 147-173, 1986.
55 Mahoney PA and Lengyel JA: The zygotic segmentation mutant *tailless* alters the blastoderm fate map of the *Drosophila* embryo. Dev Biol *122:* 464-470, 1987.
56 Sanchez-Herrero E and Crosby M: The *Abdominal-B* gene of *Drosophila melanogater:* overlapping transcripts exhibit two different spatial distributions. EMBO J *7:* 2163-2173, 1988.
57 Celniker SE, Keelan DJ and Lewis EB: The molecular genetics of the *bithorax* complex of *Drosophila:* Characterization of the products of the *Abdominal-B* domain: Genes and Dev *3:* 1424-1436, 1989.
58 Boulet AM, Lloyd A and Sakonju S: Molecular definition of the morphogenetic and regulatory functions and the cis-regulatory elements of the *Drosophila Abd-B* homeotic gene. Development *111:* 393-405, 1991.
59 Sanchez-Herrero E: Control of the expression of the *bithorax* complex genes *abdominal-A* and *Abdominal-B* by cis-regulatory regions in *Drosophila* embryos. Development *111:* 437-449, 1991.
60 Knipple DC, Seifert E, Rosenberg UB, Preiss A and Jackle H: Spatial and temporal patterns of *Kruppel* gene expression in early *Drosophila* embryos. Nature *317:* 40-45, 1985.
61 Gaul U and Weigel D: Regulation of *Kruppel* expression in the anlage of the Malpighian tubles in the *Drosophila* embryo. Mech Dev *33:* 57-68, 1991.
62 Gloor H: Schadigungsmuster eines Letalfaktors (*Kr*) von *Drosophila melanogaster*. Arch Julius Klaus Stift *25:* 38-44, 1950.
63 Harbecke R and Janning W: The segmentation gene *Kruppel* of *Drosophila melanogaster* has homeotic properties. Genes and Dev *3:* 114-122, 1989.
64 Rosenberg UB, Schroder C, Preiss A, Kienlin A, Cote S, Riede I and Jackle H: Structural homology of the product of the *Drosophila Kruppel* gene with *Xenopus* transcription factor IIA. Nature *319:* 336-339, 1986.
65 MacDonald PM and Struhl G: A molecular gradient in early *Drosophila* embryos and its role in specifying the body pattern. Nature *324:* 537-545, 1986.
66 Mlodzik M and Gehring WJ: Expression of the *caudal* gene in the germ line of *Drosophila:* Formation of an RNA and protein gradient during early embryogenesis. Cell *48:* 465-478, 1987.
67 Mlodzik M, Fjose A and Gehring WJ: Isolation of *caudal*, a *Drosophila* homeobox-containing gene with maternal expression, whose transcripts form a concentration gradient at the preblastoderm stage. EMBO J *4:* 2961-2969, 1985.
68 Hoey T, Doyle HJ, Harding K, Wedeen C and Levine M: Homeobox gene expression in anterior and posterior regions of the *Drosophila* embryo. Proc Natl Acad Sci USA *83:* 4809-4813, 1986.
69 Jurgens G: Head and tail development of the *Drosophila* embryo involved *spalt* a novel homeotic gene. EMBO J *7:* 189-196, 1988.
70 Frei E, Schuh R, Baumgartner S, Burri M, Noll M, Jurgens G, Seifert E, Nauber U and Jackle H: Molecular characterization of *spalt*, a homeotic gene required for head and tail development in the *Drosophila* embryo. EMBO J *7:* 197-204, 1988.
71 Jack J: Molecular organizaation of the *cut* locus of *Drosophila melanogaster*. Cell *42:* 869-876, 1985.
72 Blochlinger K, Bodmer R, Jan LY and Jan YN: Patterns of expression of *cut*, a protein required for external sensory organ development in wild-type and *cut* mutant *Drosophila* embryos. Genes and Dev *4:* 1322-1331, 1990.
73 Blochlinger K, Bodmer R, Jack J, Jan LY and Jan YN: Primary structure and expression of a product from *cut*, a locus involved in

specifying sensory organ identity in *Drosophila*. Nature *333:* 629-635, 1988.
74 Wilson C, Bellen HJ and Gehring WJ: Position effects on eukaryotic gene expression. Annu Rev Cell Biol *6:* 679-714, 1990.
75 Perrinon N, Noll E, McCall K and Brand A: Generating lineage-specific markers to study *Drosophila* development. Dev Genet *12:* 238-252, 1991.
76 Hiromi Y and Gehring WJ: Regulation and function of the *Drosophila* segmentation gene *fushi tarazu*. Cell *50:* 963-974, 1987.

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