

Zygotic genes that mediate torso receptor tyrosine kinase functions in the *Drosophila melanogaster* embryo

(terminal genes/insect evolution/maternal effect genes/pattern formation/morphogenesis)

TERESA R. STRECKER*, MAN LUN R. YIP, AND HOWARD D. LIPSHITZ†

Division of Biology 156-29, California Institute of Technology, Pasadena, CA 91125

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ABSTRACT The developmental signal that specifies the fates of cells at the anterior and posterior termini of the *Drosophila* embryo is transmitted by the torso receptor tyrosine kinase. This paper presents the results of a genetic interaction test for zygotic loci that act downstream of torso in the terminal genetic hierarchy. Tests of 26 zygotic mutants with defects in terminal development indicate that at least 14 reside in this hierarchy. The phenotypes associated with these genes fall into three classes, each of which represents a distinct aspect of terminal development and evolution. Four of the genes have been molecularly cloned and their products include an intercellular communication factor and three kinds of transcription factors.

The development of the anterior and posterior termini of the *Drosophila* embryo is programmed by a family of six maternally expressed genes collectively referred to as the “terminal” genes (1–5). One of these, torso (*tor*), encodes a transmembrane receptor tyrosine kinase (RTK) that is present throughout both terminal and central portions of the embryo (6, 7) but is activated only in the termini (2, 8, 9). The resulting signal is then transmitted through a cytoplasmic cascade that includes the D-raf serine/threonine kinase (5) into the nucleus where downstream zygotic loci are transcriptionally activated or repressed.

Previous studies of the embryonic termini have emphasized the role of the terminal genes in distinguishing terminal cell fates from central cell fates along the antero-posterior axis of the embryo. The development of the termini, however, entails a complex series of patterning and morphogenetic events, a large subset of which are programmed by torso (1, 8). We have reported (8) that loci mediating torso functions could be identified by their genetic interaction with a hypermorphic torso gain-of-function allele that behaves like a constitutively active RTK. This enabled us (8) and others (9) to demonstrate that the zygotic locus, tailless (*ttl*), acts downstream of torso in the terminal developmental pathway, a conclusion since verified molecularly (10). Although tailless clearly comprises one of the zygotic mediators of this terminal developmental signal, it directs only a subset of terminal development (11, 12).

Here we report that at least 13 additional zygotically active loci mediate the complex terminal developmental program. These mutants fall into three phenotypic classes that reflect distinct aspects of terminal development and evolution. The molecular biology of the terminal signal transduction pathway is considered in the light of these data.

MATERIALS AND METHODS

Loci Tested in This Study. torso (*tor*) (1, 8, 9); tailless (*ttl*) (11, 12); folded gastrulation (*fog*), twisted gastrulation (*tsg*)

(13); faint little ball (*flb*), hindsight (*hnt*), stardust (*sd*), u-shaped (*ush*) (14); hunchback (*hb*) (15); lines (*lin*), snail (*sna*), tailup (*tup*), twist (*twi*) (16); short gastrulation (*sog*) (17); giant (*gt*) (18); pointed (*pnt*), rhomboid (*rho*), Star (*S*) (19); decapentaplegic (*dpp*) (20); grain (*grn*), knickkopf (*knk*), krotzkopf verkherdt (*kkv*), tolloid (*tld*) (21); knirps (*kni*) (22); Krüppel (*Kr*) (23); spalt (*sal*) (24); and fork head (*fkh*) (25) were tested.

Screen for Suppression/Enhancement of *tor*⁴⁰²¹ and *tor*^{spic} by Zygotic Segmentation Mutants. Females that were (i) heterozygous for *tor*⁴⁰²¹, (ii) homozygous for *tor*^{spic}, or (iii) *tor*^{spic}/*tor*⁺; *Dp*(2;3) *P32*(*tor*⁺)/+ and also heterozygous for a given zygotic mutation were mated to males that were heterozygous for the same zygotic mutation. In each cross of type *i* >50 embryos were scored and in types *ii* and *iii* ≈100 embryos were scored with respect to their cuticle pattern 24 hr after fertilization. The change in the abdominal segment number was calculated relative to the wild-type controls. In crosses of types *ii* and *iii*, for zygotic mutations that result in a deletion of abdominal segments, the change in the average number of abdominal segments was normalized in light of the defined zygotic mutant phenotype. For example, *ttl*/*ttl* embryos lack abdominal segment 8, so the rescued embryos can exhibit a maximum of seven abdominal segments. This result was normalized to represent a net gain of one abdominal segment more than the actual average recorded. Similarly, *gt*, *hb*, and *lin* result in abdominal segment deletions (loss of three, one, and one segment, respectively). This was factored into the normalized change in the number of abdominal segments (respectively, the addition of 3, 1, and 1 to the observed average).

Demonstration of Suppression/Enhancement at the Cellular Blastoderm Stage. Females that were homozygous for *tor*^{spic} and heterozygous for a given zygotic mutation were mated to males that were heterozygous for the same zygotic mutation. Their embryos were allowed to develop at 25°C (suppressors) or 21°C (enhancers) for 1–4 hr (suppressors) or 2–5 hr (enhancers) and were then scored for fushi tarazu (*ftz*) stripes after visualization with anti-*ftz* antibody (26). In each case ≈100 embryos were scored.

Statistical Analysis. In all but 1 of the 29 comparisons listed in Table 1, the enhancement or suppression of the cuticular or *ftz* stripe phenotypes is significant at better than or equal to the $P = 0.02\%$ level using the nonparametric Wilcoxon rank sum test (8, 27). The exceptional case is *dpp* cuticle, which is significant at the $P = 0.06\%$ level. Where no effect is recorded, the differences are not significant ($P > 0.2\%$).

RESULTS

The torso Phenotype. Loss of maternal terminal gene function results in the deletion of the most anterior and posterior

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Abbreviation: RTK, receptor tyrosine kinase.

*Present address: Department of Biology, Pomona College, Claremont, CA 91711.

†To whom reprint requests should be addressed.

derivatives of the embryonic fate map and the respecification of the fates of cells in the terminal regions to form more central structures (1–5). Germ-band movement is also abnormal in loss-of-function torso embryos (1, 8).

We report here that, in addition, there is a ventralization of the fate map in the anterior and posterior terminal and subterminal regions of embryos from mothers homozygous for loss-of-function mutations in the maternal terminal genes torso and trunk. This is apparent from an examination of the number of denticles per abdominal denticle belt in wild-type embryos and embryos from maternal terminal mutant mothers. For example, in the first row of the sixth abdominal denticle belt from wild-type embryos, an average of 23.6 ± 2.5 denticles was found (range: 20–27; $n = 5$), whereas embryos from *tor^{PM51}/tor^{PM51}* mothers had an average of 39.1 ± 6.6 denticles (range: 28–47; $n = 10$) and those from *trk¹/trk¹*, mothers had an average of 33.8 ± 4.4 denticles (range: 29–41; $n = 5$). In embryos from *trk¹/trk¹* mothers, the loss of the supraesophageal ganglion and the dorsal shift in the position of the subesophageal ganglion (12) is consistent with a similar ventralization anteriorly. Thus, in addition to specifying antero-posterior pattern and morphogenesis, ma-

ternal terminal gene activity is required to specify dorsal cell fates in the termini of the embryo.

Identification of Zygotic Genes that Mediate torso RTK Functions. We have demonstrated (8, 28) that mutation of the tailless gene in embryos derived from constitutive hypermorphic *tor^{splc}* gain-of-function mothers restored central segments by interrupting the ectopically programmed terminal developmental pathway in the central region of the embryo. We have extended this genetic interaction screen by testing an additional 25 loci for suppression or enhancement of the central segment-loss phenotype of *tor* gain-of-function alleles (Table 1). Although mutations in these loci have been described as affecting terminal structures and/or morphogenesis (13–25), the loci have not been genetically associated with the terminal developmental hierarchy.

Mutations in 23 of these genes were initially tested for their effect on central development in embryos from females heterozygous for the most extreme dominant gain-of-function allele *tor⁴⁰²¹* (9). Embryos from *tor⁴⁰²¹* mothers can be classified into three phenotypic groups: 38% are empty sacs that exhibit remnants of the proventriculus; 56% form cuticle but no denticles; 6% form cuticle and some denticles

Table 1. Results of double-mutant analysis between torso gain-of-function mutations and zygotic mutants

Zygotic locus	Allele	<i>tor⁴⁰²¹/+</i>			<i>tor^{splc}/tor^{splc}</i>						<i>tor^{splc}/tor⁺; Dp tor⁺/+</i>		
		ES, no.	CO, no.	CD, no.	CA	Δ AS	AS	FA	Δ FS	FS	CA	Δ AS	AS
Wild type													
25°C	NA	38	56	6	NA	0.0	0.0 \pm 0.0	NA	0.0	2.3 \pm 1.1	NA	0.0	4.9 \pm 1.4
21°C	NA	—	—	—	NA	0.0	3.7 \pm 2.1	NA	0.0	5.4 \pm 1.2	NA	—	—
Suppressors: type A													
folded gastrulation (<i>fog</i>)	S4	0	75	25	*	—	—	Su	+1.0	3.1 \pm 1.6	*	—	—
hindsight (<i>hnt</i>)	E8	3	76	21	Su	+1.0	1.0 \pm 1.5	Su	+1.0	3.5 \pm 1.7	Su	+1.0	5.8 \pm 1.0
hunchback (<i>hb</i>)	14F21	4	70	26	†	—	—	—	—	—	Su	+0.5	4.5 \pm 0.6
lines (<i>lin</i>)	IIF103	6	78	16	—	—	—	—	—	—	Su	+2.0	5.9 \pm 1.1
short gastrulation (<i>sog</i>)	S6	4	56	41	Su	+1.0	0.9 \pm 1.4	Su	+1.5	3.7 \pm 1.8	—	—	—
tailless (<i>tll</i>)	L10	8	32	60	Su	+3.5	2.5 \pm 1.5	Su	+2.5	3.8 \pm 1.9	Su	+3.0	7.0 \pm 0.1
Suppressors: type B													
giant (<i>gt</i>)	A8	3	95	2	—	—	—	—	—	—	Su	+2.0	3.7 \pm 0.8
tailup (<i>tup</i>)	IIIB29	18	79	3	—	—	—	—	—	—	Su	+1.0	6.0 \pm 1.5
twisted gastrulation (<i>tsg</i>)	N9	1	96	3	Su	+1.5	1.6 \pm 1.4	Su	+1.0	3.2 \pm 1.2	—	—	—
u-shaped (<i>ush</i>)	IIA102	15	81	4	—	—	—	—	—	—	Su	+1.5	6.3 \pm 1.3
Enhancers													
decapentaplegic (<i>dpp</i>)	Hin-r4	95	5	0	—	—	—	—	—	—	En	-0.5	4.3 \pm 1.6
knickkopf (<i>knk</i>)	5C77	97	3	0	En [‡]	-2.0	1.6 \pm 1.4	En	-1.0	4.4 \pm 1.5	En [‡]	-2.5	2.4 \pm 0.9
pointed (<i>pnt</i>)	9J31	85	15	0	En [‡]	-1.0	2.7 \pm 1.6	—	—	—	En [‡]	-1.0	3.7 \pm 0.8
tolloid (<i>tld</i>)	9B66	92	8	0	En	-2.0	1.7 \pm 1.6	En	-2.0	3.5 \pm 1.3	En	-2.5	2.5 \pm 0.7
No effect													
faint little ball (<i>flb</i>)	IK35	30	66	4	—	—	—	—	—	—	—	—	—
fork head (<i>fkf</i>)	XT6	—	—	—	NE	0.0	0.0 \pm 0.2	—	—	—	NE	-0.5	4.4 \pm 1.2
grain (<i>grn</i>)	7L12	—	—	—	NE	0.0	0.0 \pm 0.1	—	—	—	—	—	—
knirps (<i>kni</i>)	5F107	28	70	2	—	—	—	—	—	—	—	—	—
krotzkopf verkhert (<i>kkv</i>)	14C73	68	32	0	—	—	—	—	—	—	—	—	—
Krüppel (<i>Kr</i>)	2	72	28	0	—	—	—	—	—	—	—	—	—
rhomboïd (<i>rho</i>)	7M43	50	50	0	—	—	—	—	—	—	—	—	—
snail (<i>sna</i>)	IIG05	56	42	0	—	—	—	—	—	—	—	—	—
spalt (<i>sal</i>)	IIA55	27	73	0	—	—	—	—	—	—	NE	0.0	5.0 \pm 1.3
Star (<i>S</i>)	IIN23	39	61	0	—	—	—	—	—	—	—	—	—
stardust (<i>sdt</i>)	N5	27	64	9	—	—	—	—	—	—	—	—	—
twist (<i>twi</i>)	ID96	35	65	0	—	—	—	—	—	—	—	—	—

Maternal torso genotypes are indicated. ES, empty sacs; CO, cuticle only; CD, cuticle plus denticles; CA, cuticle analysis; Δ AS, normalized change in number of abdominal segments; AS, actual number of abdominal segments (mean \pm SD); FA, *ftz* analysis; Δ FS, normalized change in number of *ftz* stripes; FS, actual number of *ftz* stripes (mean \pm SD); —, not tested in this particular assay; En, enhancer; NA, not applicable; NE, no effect; Su, suppressor.

**fog/fog* embryos consistently exhibit large holes in cuticle thereby preventing an accurate count of abdominal segments in the mature embryo.

†*hb/hb* embryos consistently exhibited a fusion or lawn of abdominal denticles that prevented an accurate count of abdominal segments in these mature embryos.

‡These numbers refer to the entire population rather than homozygotes and thus the enhancement is underestimated. In all other cuticle analyses listed, the homozygotes could be recognized and suppression/enhancement was assayed in that subpopulation only.

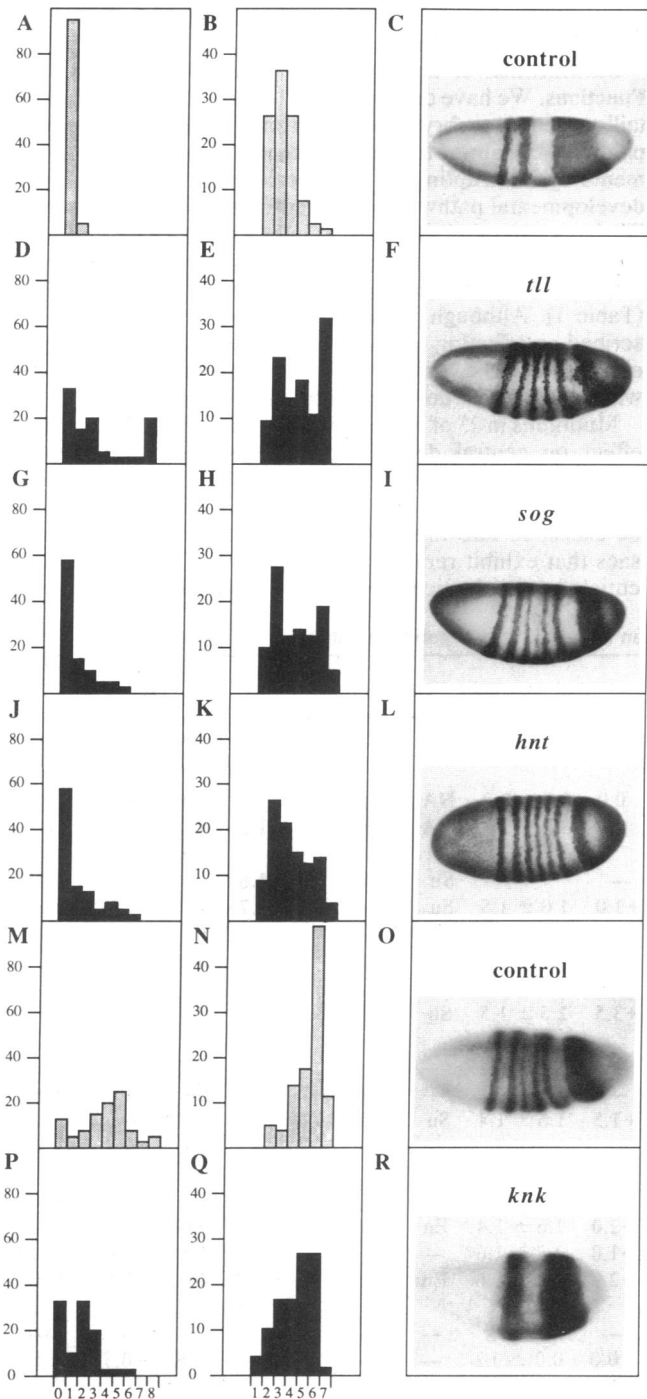


FIG. 1. Suppression or enhancement of central defects by zygotic terminal mutations in embryos derived from *tor^{spic}/tor^{spic}* females. (A, D, G, J, M, and P) Distribution of abdominal segment number in cuticle preparations of mature embryos. The percentage of embryos exhibiting a given number of abdominal segments (ordinate) is plotted against the abdominal segment number (abscissa). Shaded bars in A and M are the distributions in control embryos. Solid bars in D, G, J, and P are the distributions in progeny from crosses of *tor^{spic}/tor^{spic}*; mutation/+ mothers to mutation/+ fathers (or +/Y fathers in the case of X chromosome-linked loci). In D, G, and J, the embryos developed at 25°C; in P embryos developed at 21°C. (B, E, H, K, N, and Q) The distribution of *ftz* stripes in whole-mount preparations of cellular blastoderm stage embryos. Control distributions are shown as shaded bars and experimental distributions are shown as solid bars. (C, F, I, L, O, and R) Examples of expression patterns of *ftz* in whole-mount preparations of cellular blastoderm stage embryos, visualized with anti-*ftz* antibody and photographed under bright-field optics. (A–C) Control embryos developed at 25°C,

corresponding to the anal tuft and/or eighth abdominal denticle belt (Table 1). The zygotic genes could be classified into four categories (Table 1). (i) Type A suppressors are defined as those mutations that led to an increase in the proportion of embryos that formed cuticle with denticles ($29 \pm 16\%$ vs. 6% of control embryos) and a decrease in the fraction of embryos that formed empty sacs ($4 \pm 3\%$ vs. 38% of control embryos). (ii) A similar, but less dramatic, suppression was observed for type B suppressors that are defined as those mutations that led to a decrease in the proportion of embryos that formed empty sacs ($10 \pm 7\%$ vs. 38% of control embryos) and an increase in the proportion of embryos that formed cuticle (88 \pm 7% vs. 56% of control embryos). (iii) Enhancers are defined as mutations that led to a decrease in the proportion of embryos that formed cuticle or cuticle with denticles ($7 \pm 7\%$ vs. 62% of control embryos) and an increase in the proportion of embryos that formed empty sacs ($92 \pm 5\%$ vs. 38% of control embryos). (iv) The remaining 10 loci tested with *tor⁴⁰²¹* did not show significant shifts in phenotype and thus are classified as noninteracting.

Confirmation that the Zygotic Loci Mediate torso Functions.

To exclude the possibility that the observed interactions were allele-specific with respect to *tor⁴⁰²¹*, we repeated our tests using *tor^{spic}*, a weaker semidominant hypermorphic gain-of-function allele that results in embryos in which the loss or gain of segments *per se* can be assayed (8, 28). In the present study, mutations in *tll*, five additional suppressor loci and three enhancer loci identified in the *tor⁴⁰²¹* screen were retested in embryos from *tor^{spic}/tor^{spic}* females. Seven showed significant interaction (Table 1 and Fig. 1); for the other two the number of abdominal segments could not be determined accurately due to abnormal cuticle formation. For two of the interacting loci, additional alleles not listed in Table 1 (*hnt^{EH275a}* and *sog^{YP01}*) were tested to confirm that the interactions were not allele-specific (data not shown). Two added loci (*fkh* and *grn*) were tested with *tor^{spic}* and neither showed significant interaction (Table 1).

Further tests were conducted using a weak constitutive torso activity level [*tor^{spic}/tor⁺*; *Dp(2;3)P32 tor⁺/+* (8, 28)]. Seven suppressor, four enhancer, and two noninteracting loci were retested (Table 1). As expected, all of these loci retested as interacting with the exception of those two previously classified as noninteracting.

Hence, 13 of the 14 interacting loci identified using the high constitutive activity allele (*tor⁴⁰²¹*) retested positively in our cuticular analyses using intermediate (*tor^{spic}*) and low (*tor^{spic}/tor⁺*) torso activity levels. The remaining interacting locus *fog* retested positively in the fushi tarazu (*ftz*) assay (below).

The Zygotic Loci Act in the Terminal Pathway Prior to Cell Fate Determination. The analyses described to this point focused on alterations in the pattern of the late embryonic cuticle as a marker of interaction with *tor* gain-of-function alleles. However, it is known from genetic (8, 9, 28) and molecular (6, 7) analyses that torso RTK action occurs between 1 and 4 hr of embryogenesis. Thus interacting loci

from *tor^{spic}/tor^{spic}* females. In C three stripes are visible. (D–F) Embryos from *tor^{spic}/tor^{spic}*; *tll/+* females that were mated to *tll/TM3* males. The maximum number of segments is seven in D; the maximum number of stripes is six in E and F. (G–I) Embryos from *sog/FM7*; *tor^{spic}/tor^{spic}* females that were mated to *FM7/Y* males. In I, seven stripes are visible. (J–L) Embryos from *hnt/FM7*; *tor^{spic}/tor^{spic}* mothers that were mated to *FM7/Y* males. In L, seven stripes are present. (M–O) Control embryos developed at 21°C from *tor^{spic}/tor^{spic}* females. In O, six stripes are visible. (P–R) Embryos from *tor^{spic}/tor^{spic}*; *knk/+* females mated to *knk/TM3* males. In R, two stripes are visible. In all photographs, anterior is to the left. Views in C, F, I and R are lateral (dorsal toward the top of the page), the view in L is of the dorsal side of the embryo, and the view in O is ventro-lateral.

must also act during, or shortly after, these stages. This was confirmed for six of the interacting loci by analysis of an early marker of the embryonic fate map; namely, the expression pattern of the *ftz* segmentation gene (29) at the cellular blastoderm stage (Table 1 and Fig. 1). These results prove the action of these genes in the terminal developmental pathway prior to cell fate determination.

Interacting Zygotic Loci Identify Three Distinct Classes of Developmental Functions Associated with the Termini. The 14 interacting loci can be classified into three phenotypic classes that account for the torso loss-of-function phenotype.

Class I—terminal vs. central specification. Two loci—*tll* and *lin*—are required for the formation of anterior and posterior terminal structures and for specifying terminal as distinct from central cell fates along the antero-posterior embryonic axis (11, 12, 16). The latter conclusion is based on the observation that, with loss of terminal pattern in *tll* (11, 12) or *lin* embryos (this study), there is an expansion of the central pattern into the terminal regions. Thus, in these mutants, the terminal vs. central distinction is not maintained and terminally located cells are misspecified to be central in fate.

Class II—pattern specification within the termini. Three additional zygotic loci—*gt*, *hb*, and *knk*—are required for pattern specification along the antero-posterior axis of the termini (15, 18, 21) whereas three loci—*dpp*, *pnt*, and *tld*—are required for the establishment of dorso-ventral polarity in the termini of the embryo (19–21). Pattern defects occur without concomitant adoption of central fates by terminal cells; thus, these loci are not involved in the terminal vs. central cell fate decision.

Class III—morphogenesis of the termini. Two of the zygotic suppressor mutations—*sog* and *fog*—are required for the normal formation of the posterior midgut (endoderm) (13, 17). Four loci—*hnt*, *tsg*, *tup*, and *ush*—are required for normal terminal morphogenetic movements (13, 14, 16). Embryos mutant for *tsg* fail to extend their germ bands and lack some telson structures. In contrast, mutation of *hnt*, *tup*, or *ush* leads to the failure of germ-band retraction with no obvious pattern defects.

Genetic Interactions Among the Three Classes of Zygotic Loci. To document interactions among the three classes of terminal pathway genes, double-mutant combinations were made between *tll* (class I) and *lin* (class I), *sog* (class III, endoderm), or *hnt* (class III, germ band). Double mutants of *tll* with *sog* or *hnt* exhibited additive effects, suggesting spatial or biochemical independence of class I and III gene action. Although mutation of the *tll* gene results in the strongest known suppression of *tor*⁴⁰²¹, it does not completely suppress the effects (Table 1). The triple mutant combination of *tor*⁴⁰²¹ maternally with this strong zygotic suppressor (*tll*) and a weaker one (*hnt*) leads to even greater suppression of *tor*⁴⁰²¹ (data not shown). Again, this suggests additive effects of *tll* and *hnt*, emphasizing their separate functions in the terminal pathway.

In contrast, there is a synergistic interaction of the two class I genes *tll* and *lin*. Embryos mutant for both *lin* and *tll* consistently exhibited five to six abdominal segments, one to two fewer than the seven abdominal segments exhibited by each mutant separately. Furthermore, each abdominal segment appeared expanded along the antero-posterior axis, with partial fusions among these central segments. There was also evidence of a partial failure in germ-band retraction in these double-mutant embryos, a phenotype not observed in embryos singly mutant for either of these class I loci. The *tll*–*lin* synergism likely relates to the joint functions of these two genes in specifying terminal vs. central cell fates. The germ-band retraction phenotype suggests that there is likely to be cross-talk between the different parts of the terminal hierarchy.

DISCUSSION

Maternal torso Activity Is Mediated by Multiple Zygotic Genes. The torso loss-of-function phenotype suggested that several zygotic genes were likely to be involved in mediating torso functions in the termini of the *Drosophila* embryo. The first zygotic gene associated with the terminal hierarchy was *tailless* (8, 9, 12). Additional zygotic loci that reside in the terminal developmental hierarchy have been reported (30–32). Here we have identified a further 13 loci that mediate the torso developmental signal in the termini of the embryo.

Mutations in the 14 zygotic terminal pathway loci studied here differ in the extent to which they suppress or enhance the central abdominal deletions resulting from constitutive torso activity. In general, there is a positive correlation between the number of terminal structures/functions affected by mutations in a particular locus and the strength of its interactions with torso gain-of-function alleles. For example, *tll* and *tld*, respectively the strongest of the suppressors and enhancers, are required for the formation of the largest subdomains within the anterior and posterior termini.

The morphogenetic defects observed in embryos produced by *tor* loss-of-function mothers are less extreme than those observed in mutants for the zygotic loci that we have classified into class III. The most plausible explanation for this is that, whereas the class III genes (or their products) are regulated in part by the torso RTK-mediated pathway, they are also regulated by other intracellular signaling pathways (33). The localized focus in the posterior terminal region of the blastoderm fate map, for a “tail-up” phenotype (34) resembling that exhibited by three of the class III mutants (*hnt*, *tup*, and *ush*), is consistent with our conclusion that control of germ-band movement is, in part, a terminal pathway function.

Evolution of Terminal Functions in Insects and Arthropods. Among the developmental processes specified by the three classes of zygotic terminal genes presented above, the establishment of asegmental termini as separate from the central trunk region of the germ band is the most evolutionarily ancient (conserved in both annelids and arthropods) (35). We have shown here that this process is mediated through *tll* and *lin* in the *Drosophila* embryo. The termini of ancestral embryos are derived from the very anterior and posterior tips of the embryonic primordium. The regions of the *Drosophila* embryo most closely associated with these domains (acron anteriorly and telson posteriorly) have been shown to arise from the dorsal aspect of the anterior and posterior ends of the *Drosophila* blastoderm fate map (34, 36). Thus, it is not surprising that, during insect evolution, genes required to specify dorsal positional values in the *Drosophila* embryos, such as *tld* and *dpp*, have come under the regulation of the terminal developmental hierarchy. Interestingly, there are also interactions between maternal dorso-ventral pattern mutations and a torso gain-of-function allele (dorsal mutations suppress and deletion of *cactus* enhances the *tor*⁴⁰²¹ phenotype) (data not shown).

Another relatively ancient association has been between posterior midgut formation and the proctodeum (telson). In more primitive insects, the posterior midgut is formed from ventral invaginating cells that lie adjacent to the proctodeum (37). This ancestral mode of development may be reflected in *Drosophila* by the fact that the presumptive posterior midgut and proctodeum lie adjacent to one another on the blastoderm fate map (34, 36) and that *sog* gene activity is required in ventral tissues and in the region of the presumptive posterior midgut (17).

In contrast, germ-band retraction is a recent event in the evolution of insect development, since an analogous process does not occur during the development of primitive insects or other arthropod embryos. Consistent with this, mutations in

the *Drosophila* genes *hnt*, *tup*, and *ush* do not result in an alteration of pattern specification but specifically affect this particular morphogenetic movement.

Molecular Biology of the Zygotic Terminal Pathway Genes.

Four of the identified zygotic terminal pathway loci (*dpp*, *gt*, *hb*, and *tll*) have been molecularly cloned and their expression has been assayed in *tor* mutants (refs. 10, 38, and 39; R. Ray, K. Arora, C. Nüsslein-Volhard, and W. Gelbart, personal communication). In all four cases it has been shown that zygotic transcription of these loci in the termini of the embryo is activated or repressed in response to torso activity in the early embryo. This provides direct molecular evidence supporting our conclusions that these genes function in the terminal genetic hierarchy.

These gene products represent four distinct molecular species whose functions include intercellular communication by a transforming growth factor β -family homolog (*dpp*) (40) and transcriptional regulation (*gt*, *hb*, and *tll*). The latter represent three families of transcription factors: *gt* belongs to the leucine-zipper family (M. Capovilla, E. Eldon, and V. Pirrotta, personal communication), *hb* belongs to the zinc-finger family (41), and *tll* belongs to the steroid hormone receptor family (10). The three transcription factor genes are regulated in response to the torso signal and, in turn, control the expression of downstream genes that direct the detailed development of the termini. In contrast, *dpp* participates in cell-cell communication that is involved in refining terminal pattern.

The remaining loci are likely to encode additional components of the inter- or intracellular signaling pathways or the gene regulatory apparatus that programs terminal development. Molecular analyses of these genes will lead to insights into the mechanisms by which a transmembrane receptor tyrosine kinase and its multiple downstream genes convert a simple extracellular signal into a series of complex developmental processes.

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