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Reciprocal Effects of Hyper- and Hypoactivity Mutations in the *Drosophila* Pattern Gene *torso*

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In *Drosophila*, five "terminal" polarity genes must be active in females in order for them to produce embryos with normal anterior and posterior ends. Hypoactivity mutations in one such gene, *torso*, result in the loss of the most posterior domain of *fushi tarazu* expression and the terminal cuticular structures. In contrast, a *torso* hyperactivity mutation causes the loss of central *fushi tarazu* expression and central cuticular structures. Cytoplasmic leakage, transplantation, and temperature-shift experiments suggest that the latter effect is caused by abnormal persistence of the *torso* product in the central region of the embryo during early development. Thus, the amount and timing of *torso* activity is key to distinguishing the central and terminal regions of the embryo. Mutations in the *tailless* terminal gene act as dominant maternal suppressors of the hyperactive *torso* allele, indicating that the *torso* product acts through, or in concert with, the *tailless* product.

THREE CLASSES OF MATERNAL EFFECT pattern genes specify the anterior-posterior axis of the *Drosophila* embryo (*1, 2*). When females carry "anterior" mutations, their progeny lack acronal, gnathal, and thoracic structures. Loss of the abdominal region (and the germ cells) occurs in offspring of the "posterior" or grandchildless-*knirps* class mutants. The "terminal" or *torso*-like genes comprise six loci that are involved in specifying the asegmental anterior (acron) and posterior (telson) structures of the embryo (Fig. 1, A and B) (*1–4*). Five of these are maternal effect genes [*torso* (*1*), *trunk* (*1*), *torsolike* (*5*), *fs* (*1*) *Nasrat* (*3*), and *fs* (*1*) *polehole* (*3*)] and one is zygotic in action [*tailless* (*4*)]. Here we focus on the mechanism of *torso* (*tor*) gene action. We show that hyper- and hypoactivity mutations in *tor* lead to reciprocal pattern defects. We investigate the basis for these effects and discuss their implications for the role of *tor* in establishing embryonic pattern.

Mothers homozygous for the $spliced^{RL3}$ mutation (*6*) produce embryos that lack thoracic and abdominal structures and have only an acron and a telson (Fig. 1E and Table 1B). The *spliced* mutation is semidominant: at 25°C heterozygous females produce offspring lacking up to three abdominal segments, with the second and third abdominal segments showing the greatest sensitivity (Table 1D). The *spliced* phenotype is complementary to that produced in progeny of females homozygous for hypoactivity mutations in the terminal class genes (compare in Fig. 1, B with C through E) (*1–4*). We have used chromosomal deficiencies and a duplication for the *tor* region to demonstrate that the *spliced* mutation maps to the same cytological region as *tor* (43C3-43E7) (*1*), and that it is a hypermorphic (7) (hyperactivity) *tor* allele (Table 1). Specifically, when a *spliced* homozygous or heterozygous mother carries an extra dose of the wild-type tor^+ gene, the embryos exhibit a more extreme phenotype, with a consistent loss of an additional one to two abdominal segments (compare in Table 1, A with B and C with D). In contrast, reduction of the number of copies of the *spliced* gene in *spliced* hemizygous mothers rescues the central region defects, resulting in the presence of the wild-type number of abdominal segments (compare in Table 1, B and G). Further, hypoactivity mutations in the *tor* gene also rescue the abdominal defects of *spliced* individuals (compare in Table 1, D with E and F). These genetic tests are consistent with the conclusion that *spliced* is a hyperactivity allele of *tor*, which we, therefore, designate as tor^{splc} (*8*). Although hypoactivity of *tor* causes a loss of the acron and telson and respecification of these regions to form central structures (*1*), hyperactivity of *tor* has no effect on the termini and causes loss of the thorax and abdomen (*9*).

The tor^{splc} allele is heat-sensitive. This fact enabled us to observe a progressive deletion of central embryonic regions due to increased *tor* expression in embryos from tor^{splc} homozygous mothers (Fig. 1, C through E). The cuticular defects correlated well with alterations in the expression of the zygotic pair-rule segmentation gene, *fushi tarazu* (*ftz*), an earlier, molecular marker of embryonic pattern (Fig. 2, A through D) (*10–13*). In wild-type embryos *ftz* is expressed early in embryogenesis in a pattern of seven stripes (Fig. 2A) (*10–12*). In embryos (from tor^{splc} females) raised at low temperatures (18° to 19°C), zero to three of the abdominal segments are missing, with A2 and A3 exhibiting the greatest sensitivity (Fig. 1C and Table 1B). This correlates with a reduction, or complete absence, of *ftz* stripe 4 (Fig. 2B). At intermediate temperatures (21° to 23°C) most of the eight abdominal segments are absent, whereas acronal, gnathal, and thoracic structures develop normally anteriorly, and A7, A8, and the telson develop normally posteriorly (Fig. 1D and Table 1B). At these temperatures, there is a compression and fusion of *ftz* stripes 2, 3, and 5 with a concomitant broadening of stripe 7 (Fig. 2C). At high temperatures (25°C) the progeny of homozygous mothers develop as bags of cuticle with acronal and gnathal structures, such as mouth hooks and pharynx anteriorly and a telson with filzkörper posteriorly (Fig. 1E and Table 1B). Only *ftz* stripe 1 and the broad posterior stripe remain in these embryos (Fig. 2D). About 25% of the embryos that are allowed to develop at 25°C show no activation of *ftz* expression. The embryonic fate map of embryos from tor^{splc} females, reflected by the pattern of *ftz* expression, thus suggests that the absence of stripes 2 to 5 likely corresponds to loss of segments T1 through A5. The presence of stripe 1 and the most posterior stripe (the expanded and fused stripes 6 and 7) are consistent with the presence of acronal, gnathal, and telson structures. The alterations in *ftz* expression and the loss of central structures caused by hyperactivity of *tor* contrasts with the loss of *ftz* stripe 7 and terminal structures caused by hypoactivity alleles of *tor* (Figs. 1B and 2G) (*11, 14*).

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Taken together, these data suggest that the *tor* gene product has dual functions in early embryogenesis: to promote the development of the acron and telson in the terminal regions of the embryo and to suppress the development of central structures (thorax and abdomen) at the termini. This explains why hyperactivity of *tor* has no effect on the termini of the embryo and yet causes loss of the central structures.

To test whether the loss of thoracic and abdominal structures in progeny of tor^{splc} mothers is a direct consequence of active *tor* gene product in the central part of the embryo, cytoplasmic leakage and transplantation experiments were carried out (Table 2 and Fig. 2, E through H) (*15*). We assayed directly for the rescue of the pattern of *ftz* expression at a time corresponding to the germ-band extended stage, 4 to 5 hours after leakage or transplantation. Leakage of cytoplasm from the central region of early tor^{splc} embryos [stages 1 to 2, 0 to 80 min after fertilization (*16*)] does not rescue the abnormalities in *ftz* expression (Table 2 and Fig. 2E). However, leakage from slightly older embryos [stages 3 to 4, 80 to 150 min after fertilization (*16*)] results in restoration of up to four stripes of *ftz* expression in the central region of 15% of the embryos (Table 2 and Fig. 2F). This result is consistent with a model in which *tor* activity is normally present in the central region during stages 1 to 2 (hence leakage of excess *tor* product has no effect) but must disappear from this region by stages 3 to 4 for normal central development to ensue. The tor^{splc} mutation could result in the production of either a constitutively active *tor* product or a *tor* product with increased stability, resulting in the presence of *tor* activity centrally at stages 3 to 4. Removal of the *tor* product by leakage from the central region at these stages thus rescues the central defect in embryos from tor^{splc} females.

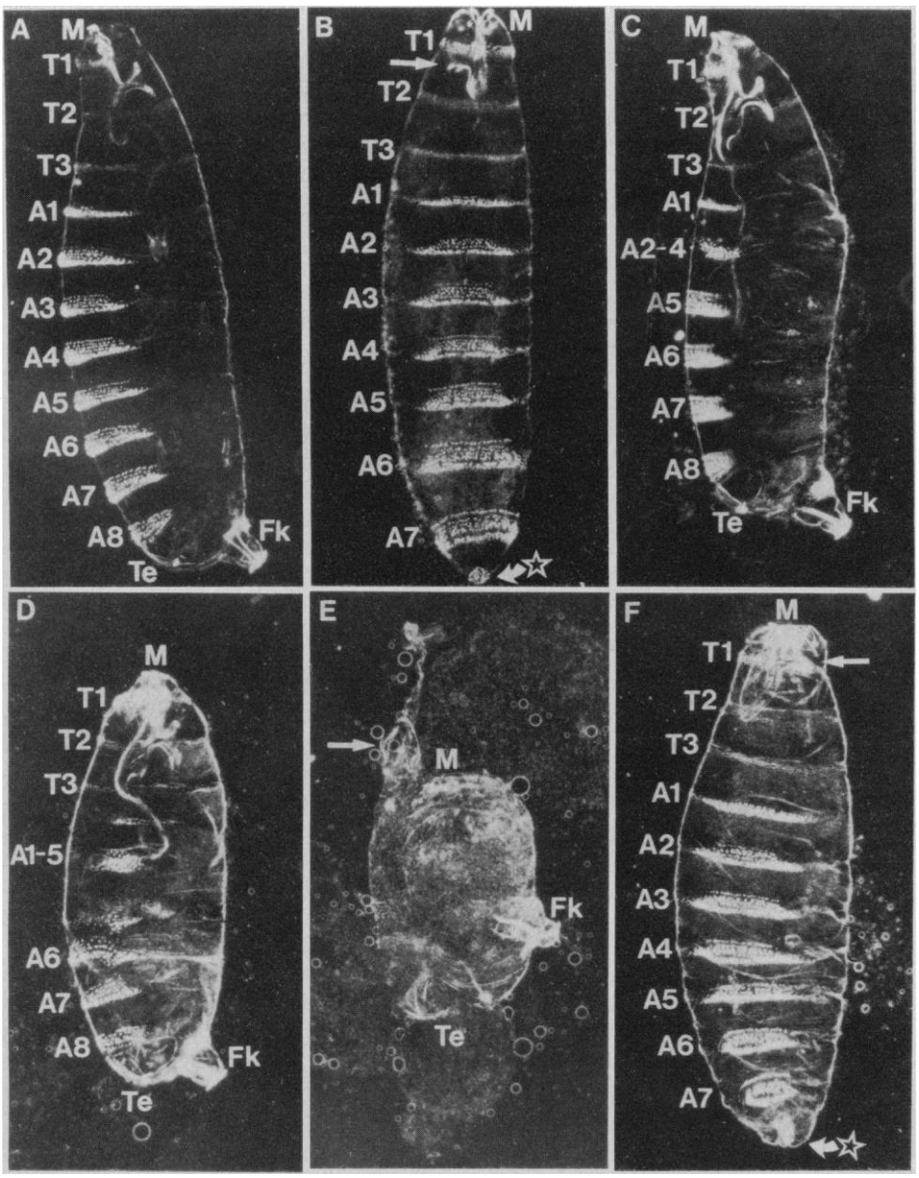

Fig. 1. Phenotypes of wild-type and mutant embryos shown in dark field. (**A**) Wild type. For a detailed description of the cuticular pattern, see (*23*). M, mouthhooks; T1 to T3, thoracic segments; A1 to A8, abdominal segments; Te, telson; Fk, filzkörper. (**B**) Null *torso* phenotype (maternal genotype: tor^1/tor^1). Note the reduction in pharyngeal head skeleton anteriorly (arrow). Posteriorly, the embryo ends with a patch of denticles corresponding to A8 (arrow with star), and lacks the telson. See (*1*) for detailed description. (**C**) Weak tor^{splc} phenotype (maternal genotype: tor^{splc}/tor^{splc}) seen in embryos that develop at 19°C. Note the reduction or loss of abdominal segments 2, 3, and 4. (**D**) Intermediate tor^{splc} phenotype [maternal genotype as in (C)] seen when embryos develop at 22.5°C. Note absence or reduction of abdominal segments 1 through 5. This embryo exhibits more segments than the average for the intermediate class. (**E**) Strong tor^{splc} phenotype [maternal genotype as in (C)] produced when embryos develop at 25°C. Note undifferentiated cuticle bag lacking T1 through A8 segments but showing mouthhook material and uninvoluted pharynx (arrow) anteriorly and telson with filzkörper posteriorly. (**F**) Rescue of tor^{splc} phenotype by reduction of tll^+ to single dose in mother (maternal genotype: tor^{splc}/tor^{splc}; $tll^1/+$) and to zero doses in the embryo (embryonic genotype: $tor^{splc}/+$; tll^1/tll^1). Note the absence of terminal structures [symbols as in (B)] but complete rescue of thorax and abdomen. Embryo developed at 22.5°C. In (A) to (F) anterior is toward the top of the page; in (A), (C), (D), and E) ventral is to the left, and in (B) and (F) the entire view is of the ventral side. Methods were as in (*16*). For quantitative data and statistical analysis see Table 1.

To confirm that *tor* activity is present centrally at stages 3 to 4 in embryos produced by tor^{splc} mothers, but not in wild-type embryos, we transplanted cytoplasm from the central region into the posterior pole of embryos from tor^- mothers. We assayed for the reappearance of the seventh *ftz* stripe that is missing in tor^- embryos (*11*). Cytoplasm from the tor^{splc} embryos rescued the seventh stripe in 23% of the tor^- embryos (Table 2 and Fig. 2H), but cytoplasm from wild-type embryos was unable to exert any rescuing effect (Table 2 and Fig. 2G). These data are consistent with the suggestion that the loss of central structures in embryos from tor^{splc} females is a consequence of abnormal persistence of *tor* activity in the central region beyond stages 1 to 2.

The temperature sensitivity of the tor^{splc} mutation allowed us to carry out temperature shift experiments to determine, by an independent method, when the tor^{splc} gene product acts to cause the loss of central embryonic structures (*17*). The temperature-sensitive period includes stages 3 and 4 (1 to 2 hours after fertilization at 29°C, Fig. 3), consistent with our hypothesis that it is the presence of *tor* product in the central region of progeny of tor^{splc} females at these stages that results in the loss of central structures.

We consider two of the models that can

explain the reciprocal phenotypic effects of hyper- and hypoactivity of the tor^+ product, and our rescue of these defects by cytoplasmic leakage and transplantation. In one, the tor^+ product is normally uniformly distributed in the egg or embryo but only activated in the termini, and the tor^{splc} defect causes constitutive activation throughout the embryo. Alternatively, the tor^+ product is normally active throughout the embryo early in development (stages 1 to 2), but must subsequently disappear from the central region, remaining active only at the poles (stages 3 to 4) (*18*). If the tor^{splc} gene product is more stable than the wild-type product or is constitutively active, it could persist in the central region beyond stages 1 to 2, causing defects there. Duncan (*19*) has proposed a similar model to explain the effects of the hypermorphic ftz^{Ual} mutations.

To examine possible regulatory interactions among terminal class genes, we determined whether a reduction in activity of other terminal genes might rescue the pattern defects caused by *tor* hyperactivity. Reduction of the dosage of the terminal gene, *fs (1) Nasrat* (*3*), from the normal two doses to a single dose, did not rescue the central embryonic defects in progeny of females that are homozygous for tor^{splc}. However, reducing the number of copies of the *tailless* (*tll*) terminal gene (*4*) had striking effects on the tor^{splc} phenotype. First, reduction to a single dose of the wild-type tll^+ gene in the mother (genotype: tor^{splc}/tor^{splc}; $tll^1/+$) was sufficient to substantially rescue the central region in her offspring [in Table 1, compare B and J (i)], indicating that loss-of-function mutations in *tll* can act as dominant maternal suppressors of tor^{splc} (*20*). This is the first evidence that the *tll* gene has a maternal function in addition to its zygotic function (*21*), analogous to the dual maternal-zygotic activity of the gap gene *hunchback* (*22*). More remarkably, when tor^{splc}/tor^{splc}; $tll^1/+$ mothers were mated to $tll^1/+$ fathers, there was complete rescue of the central region in the 25% of the embryos that were homozygous for the tll^1 mutation [Fig. 1F and Table 1, J (ii)], suggesting that a further reduction in *tll* gene function zygotically could completely rescue the central defects caused by hyperactivity of the *tor* gene product. This provides direct evidence that the *tor* gene product acts through, or in concert with, *tll* to promote terminal development and repress central development, as was suggested previously (*4*).

We have shown that the *tor* gene product has both positive and negative functions in establishing the anterior-posterior embryonic pattern: it promotes terminal development and represses central development. Initially, it is likely to be present throughout the embryo, but its activity must be restricted to the termini early in development in order that the development of central structures not be repressed. The *tll* gene product mediates both the positive and negative *tor* functions. When *tll* is ectopically activated in the central region (as in tor^{splc} embryos) loss

Table 1. Phenotypic and gene dosage analysis. The number of abdominal segments is expressed to the nearest half-segment. In all pairwise comparisons cited in the text, the differences are significant at better than or equal to the $P = 0.02\%$ level by using the nonparametric Wilcoxon rank sum test (*24*). Rearrangement breakpoints in the *tor* region are as follows: *Dp(2;3)P32:*41A-44C/D (*25*). *Df(2R)CA58:*43A3/4-43F8 (*26*). In (J), part (i) presents data for the $tll^1/+$ and +/+ embryos (75%) and part (ii) for tll^1/tll^1 embryos (25%) that were recognized by the absence of termini.

Female genotype		Doses tor^{splc}	Doses tor^+	Temperature (°C)	No. of abdominal segments (± SD)	*n*
A. tor^{splc}/tor^{splc}; *Dp(2;3)P32*/+		2	1	25	0 (±0.0)	37
				22–23	0 (±1.0)	26
				18–19	5 (±1.5)	25
B. tor^{splc}/tor^{splc}		2	0	25	0 (±0.0)	34
				22–23	1.5 (±1.5)	51
				18–19	6.5 (±2.0)	22
C. tor^{splc}/+; *Dp(2;3)P32*/+		1	2	25	5 (±1.5)	30
				22–23	6.5 (±1.0)	16
				18–19	7.5 (±1.0)	13
D. tor^{splc}/+		1	1	25	7 (±1.5)	30
				22–23	8 (±0.0)	24
				18–19	8 (±0.5)	22
E. tor^{splc}/tor^1		1	0	25	8 (±0.0)	24
				22–23	8 (±0.0)	29
				18–19	8 (±0.0)	22
F. tor^{splc}/tor^{PM51}		1	0	25	8 (±0.0)	41
G. tor^{splc}/*Df(2R)CA58*		1	0	25	8 (±0.5)	28
				22–23	8 (±0.0)	24
				18–19	8 (±0.0)	18
H. +/+; *Dp(2;3)P32*/+		0	3	25	8 (±0.0)	25
I. +/*Df(2R)CA58*		0	1	25	8 (±0.0)	32
				18–19	8 (±0.0)	39
J. tor^{splc}/tor^{splc}; $tll^1/+$	(i)	2	0	25	2.5 (±1.5)	35
				22–23	3 (±2.0)	56
	(ii)	2	0	22–23	7 (±0.0)	19

Table 2. Results of cytoplasmic leakage and transplantation experiments. Leakage and transplantation were as described in (*15*). However, instead of analyzing the embryonic cuticle, the embryos were allowed to develop for 4 to 5 hours at 25°C after treatment. They were then assayed for *ftz*-β-galactosidase expression (*13*). *n*, number of stained embryos examined. —, not applicable. ND, not done.

Treatment	Host			Donor			*n*	*Rescue*
	Maternal genotype	Developmental stage	Position (% egg length)	Maternal genotype	Developmental stage	Position (% egg length)		
None	tor^{splc}	1, 2	—	—	—	—	76	None
		3, 4	—	—	—	—	77	None
Leakage	tor^{splc}	1, 2	90–100	—	—	—	54	None*
		3, 4	90–100	—	—	—	ND	ND
Leakage	tor^{splc}	1, 2	40–60	—	—	—	77	None
		3, 4	40–60	—	—	—	73	11 (15%)†
Leakage	tor^{splc}	1, 2	0–10	—	—	—	130	None
		3, 4	0–10	—	—	—	39	None
Transplant	tor^{PM51}	1, 2	0–10	Wild type	3, 4	40–60	29	None
Transplant	tor^{PM51}	1, 2	0–10	tor^{splc}	3, 4	40–60	30	7 (23%)‡

*Stripe 1 was shifted anteriorly, as expected (*2, 15*). †Stripes 2 through 5 were partially or completely restored. ‡Stripe 7 was partially or completely restored.

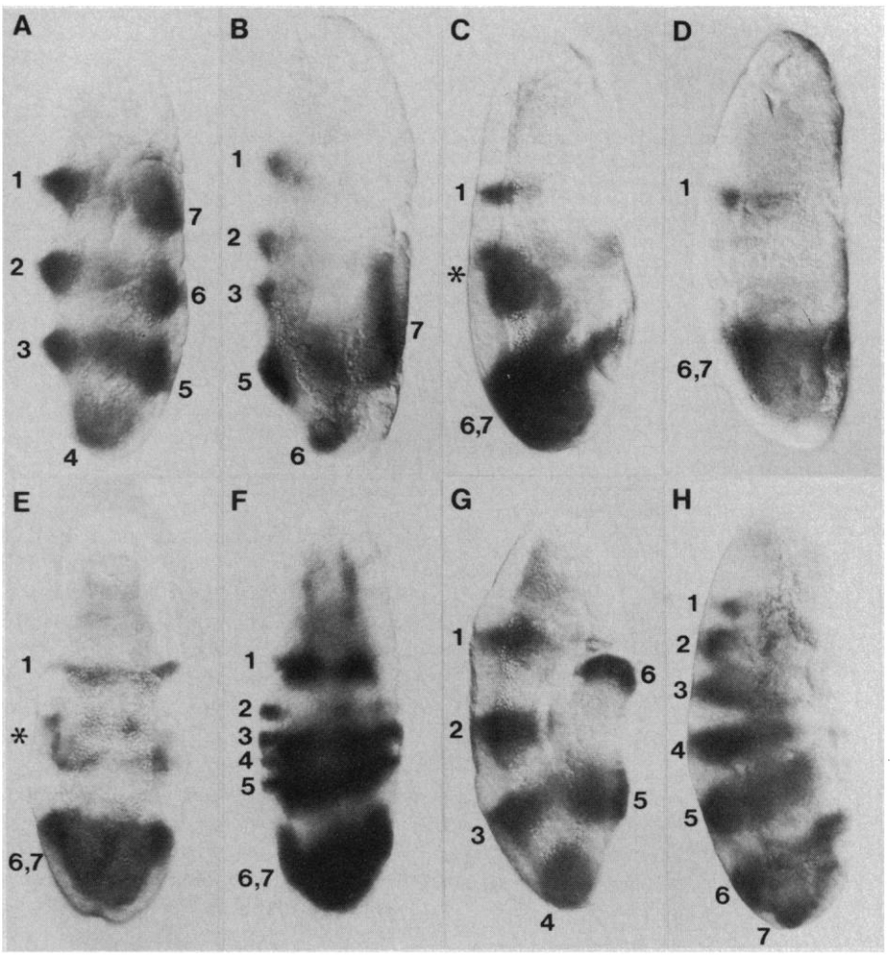

Fig. 2. Expression of a *ftz*-β-galactosidase construct (*13*) visualized with differential interference contrast optics in embryos produced by wild-type and tor^{splc} mothers, and in rescued embryos produced by mutant females. The *ftz* stripes are numbered from anterior to posterior. Embryos in (A) to (G) are at the germ band extended stage, while embryo in (H) has a retracted germ band. (**A**) Wild type. Seven stripes are present at the extended germ band stage, with the seventh stripe broader than the anterior six (*10–12*). (**B**) Embryo produced by homozygous tor^{splc} females. This embryo was raised at 19°C and shows a loss of stripe 4 and a slight broadening of stripe 7. (**C**) Embryo raised at 22.5°C that shows compression of stripes 2, 3, and 5 (asterisk) and a broad posterior stained region, presumably due to the expansion of stripe 7 and its fusion with stripe 6. (**D**) Embryo raised at 25°C showing stripe 1, absence of stripes 2 to 5, and a wide posterior band of expression. (**E**) Control embryo for the cytoplasmic leakage experiment, raised as in (D). The asterisk marks the remains of the compressed stripes 2, 3, and 5. (**F**) Embryo from which cytoplasm was leaked from between 40 and 60% egg length ventrally. Note the restoration of distinct stripes 2, 3, 4, and 5 in the central region, something never seen in unleaked controls. Crosses in (B) to (F) were tor^{splc}/tor^{splc}; *EΔ-669/EΔ-669* females to *EΔ-669/EΔ-669* males. Note that germ band extension is often incomplete in embryos produced by tor^{splc} females. (**G**) Embryo produced by tor^{PM51}/tor^{PM51}; *EΔ-669/EΔ-669* mother, into the posterior pole of which stage 4 wild-type central cytoplasm was injected. Stripe 7 is missing and stripes 4 through 6 are spaced further apart than in wild type [compare (A)]. Germ band retraction does not occur normally in embryos produced by tor^{-} mothers. (**H**) Embryo from mother as in (G), but into the posterior pole of which central cytoplasm from tor^{splc}/tor^{splc}; *EΔ-669/EΔ-669* stage 4 embryos was injected. Note the appearance of stripe 7, the correct spacing of the more anterior stripes, and the restoration of the ability to undergo normal germ band retraction. In (A) to (D) and (G) and (H) the view is a sagittal optical section with ventral to the left and anterior toward the top of the page, in (E) and (F) the view is a horizontal optical section with anterior toward the top of the page.

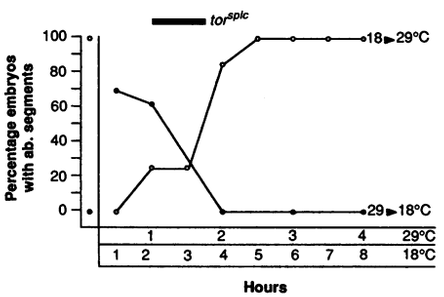

Fig. 3. Temperature-sensitive period of tor^{splc}. Each point on the ordinate represents the percentage of the embryos (produced by homozygous tor^{splc} mothers) showing one or more abdominal segments. (○), Shifts from 18° to 29°C; (●), shifts from 29° to 18°C. For each point, an average of 39 embryos was scored (range, 15 to 80). Embryos were shifted at the age indicated on the abscissa. The control values for unshifted embryos that were allowed to develop continuously at 18° or 29°C are shown in the column to the left of the shift curves. Cellularization occurs at approximately 2 hours at 29°C (4 hours at 18°C). As indicated by the filled bar, the temperature-sensitive period of tor^{splc} can be seen to largely span stages 3 and 4 (*16*). Experiments were performed as in (*1*).

of the thorax and abdomen occurs. When functional *tll* product is eliminated from embryos produced by tor^{splc} mothers, the persistent *tor* product cannot exert its repressive effect in the central region and normal thoracic and abdominal development ensues. The absence of tll^{+} function from the termini of these centrally rescued embryos prevents the positive action of *tor* in terminal development, resulting in the absence of terminal structures.

Note added in proof: M. Klingler *et al.* (*27*) have recently shown that reversion of tor^{splc} with EMS (*6*) results in the tor^{-} phenotype. They refer to tor^{splc} as tor^{RL3}. In contrast to their results, however, we show here that tor^{splc} is semidominant and that *tll* can act as a dominant maternal suppressor of tor^{splc}.

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9. Three doses of tor^{+} do not result in an abnormal segmentation pattern (Table 1H). The *tor* locus is not haplo-insufficient since embryos from hemizygous mothers retain a normal segmentation pattern (Table 1I).
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13. The construct used comprises the upstream enhancer element and zebra stripe element of the *ftz* regulatory region (*12*) as well as the transcribed but untranslated *ftz* leader region, fused to the *Escherichia coli lacZ* gene at codon number two of the *ftz* open reading frame. The code name of the construct is *EΔ-669*. It was constructed and inserted on the third chromosome via P element–mediated transformation by C. Dearolf, J. Topol, and C. Parker (*Genes Dev.*, in press). Staining for β-galactosidase activity was as in (*12*) with minor modifications.
14. In addition to deleting complementary regions of the fate map, hypoactive and hyperactive *tor* mutations have opposite effects on the spatial distribution of positional values along the embryonic anterior-posterior axis. Whereas the remaining segmentation pattern in progeny of *tor* hypomorphs is expanded toward the anterior and posterior poles of the embryo, hypermorphic mothers produce embryos in which the *ftz* stripes corresponding to the central thoracic and abdominal region appear compressed toward the center, consistent with the loss of the central structures.
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18. In either case, because tor^{splc} behaves as a hypermorphic allele upon addition or removal of doses of the wild-type tor^{+} gene, the tor^{+} gene product must be present at stages 1 to 2 in the central region of embryos produced by wild-type mothers. If the tor^{+} gene product was not normally present centrally in wild type, but was ectopically expressed there in progeny of tor^{splc} mothers, then tor^{splc} should behave as a neomorphic mutation rather than a hypermorphic one. The simplest molecular model explaining how addition or removal of the wild-type tor^{+} product causes a change in the central region of tor^{splc} embryos, proposes that autoactivation of tor^{+} normally occurs. Thus, the presence of constitutive or hyperstable tor^{splc} product centrally would increase the activity or stability of the tor^{+} gene product in this region.
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20. That this suppression is maternal is shown by the fact that we see no rescue of the central defect in embryos produced by tor^{splc}/tor^{splc}; +/+ females mated to tll^{1}/+ males. This dominant maternal suppression of the tor^{splc} allele provides us with a selective screen (maternal fertility) for transposon tagging of *tor*, *tll*, and any other locus that can be mutated to suppress tor^{splc}.
21. The previous test for maternal *tll* gene activity (*4*) addressed whether extra maternal doses of tll^{+} could rescue the mutant phenotype of tll^{-} homozygous zygotes. Since the results were negative, they could not exclude the possibility that *tll* is both maternally and zygotically active. Together with the present results, these data suggest that two maternal doses of tll^{+} might have been insufficient to raise the amount of activity in the zygote to a level capable of rescuing the tll^{-} zygotic phenotype. Alternatively, there may be functional differences between the maternal and zygotic *tll* activities.
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Macrophage Inflammatory Protein–1: A Prostaglandin-Independent Endogenous Pyrogen

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Macrophage inflammatory protein–1 (MIP-1) produced a monophasic fever of rapid onset whose magnitude was equal to or greater than that of fevers produced with either recombinant human cachectin (or tumor necrosis factor) or recombinant human interleukin-1. However, in contrast to these two endogenous pyrogens, the fever induced by MIP-1 was not inhibited by the cyclooxygenase inhibitor ibuprofen. Thus, MIP-1 may participate in the febrile response that is not mediated through prostaglandin synthesis and clinically cannot be ablated by cyclooxygenase inhibitors.

FEVER IS A HIGHLY COMPLEX RESPONSE in mammalian host defense that results in a temporary rise in basal temperature and appears to be governed through the action of cytokines. Originally, this proposal was based on the ability of stimulated peripheral leukocytes to release a soluble factor called "leukocytic" or "endogenous pyrogen" (*1*). This factor was later shown to have many diverse biological functions and was renamed interleukin-1 (IL-1) (*2*). Two other cytokines have since been shown to be endogenous pyrogens: cachectin, also known as tumor necrosis factor (TNF) (*3*), and interferon-α (IFN-α) (*4*).

We recently described a heparin-binding protein that macrophages secrete in response to endotoxin (*5*). This cytokine, which we refer to as "macrophage inflammatory protein–1" or MIP-1, is purified from macrophage-conditioned medium by sequential anion exchange, heparin Sepharose, and gel filtration. It migrates as an apparent equimolar doublet approximately 8 kD in size as analyzed by SDS–polyacrylamide gel electrophoresis (PAGE) yet forms multimers of varied molecular masses up to and exceeding 10^{6} daltons as assessed by gel filtration. Because of its tendency to aggregate, the MIP-1 used in this study could be isolated at >95% purity from the void volume of the gel filtration column as judged by SDS-PAGE and silver staining. This cytokine is composed of two related proteins, MIP-1α and MIP-1β, both of

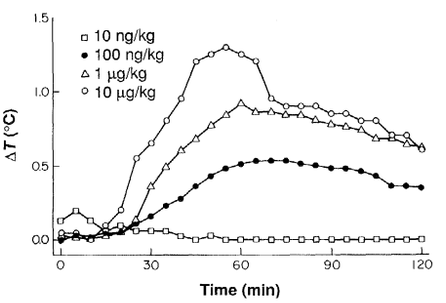

Fig. 1. Mean increases in temperature above normal (fevers) of white virgin female rabbits (2 to 3 kg) induced by MIP-1. Three rabbits were used for the two lowest doses; five and two rabbits were used for the doses of 1 and 10 μg/kg, respectively. The maximum SEM for any data point along the curve was 0.30°C. The animals were maintained and tested at 25°C. The animals were restrained, and basal temperatures were measured and recorded every 15 min by means of an indwelling rectal thermistor (Yellow Springs Instrument, Yellow Springs, Ohio) and digital meter (Markson Science, Phoenix, Arizona). After at least 3 hours of stabilization, with a minimum of 1.5 hours of recording a stable temperature, MIP-1 was injected intravenously into each rabbit. Temperatures were then recorded every 5 min for a minimum of 2 hours. The material used was purified as described (*5*). Polymyxin B (Sigma, 250 μg/ml) and antibody to cachectin/TNF were routinely added to the injected samples that were diluted up to 250 μl with nonpyrogenic physiological saline (0.9% NaCl).

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