bicoid and the terminal system activate tailless expression in the early Drosophila embryo

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Summary

In the early Drosophila embryo, the maternal terminal genes are required for formation of the acron at the anterior and the telson at the posterior. We show here that the terminal system, a signal transduction pathway active at the poles of the embryo, is required to activate transcription of the key zygotic gene tailless (tl) in two symmetrical domains. Consistent with the characterization of the tl protein as a putative transcription factor (a member of the steroid receptor superfamily) that represses segmentation genes and activates terminal-specific genes, we observe a correlation between the presence of the posterior cap of tl expression and differentiation of a telson.

While the maternal patterning systems of the Drosophila embryo function for the most part independently, the maternal anterior system, in which the bicoid (bcd) protein functions as a graded morphogen, is required together with the terminal system to establish the acron. This dual requirement is evident at the molecular level in the control of tl expression. We find that bcd and the terminal system are required together to activate the anterior-dorsal stripe of tl expression that is correlated with formation of the acron. In the absence of bcd, the anterior cap of tl expression established by the terminal system persists and an ectopic telson forms at the anterior, while in the absence of terminal system activity only an abnormal anterior stripe forms. This is the first described example of how, by jointly controlling expression of the same gene, two systems of positional information function together to set unique positional values.

Key words: maternal morphogens, Drosophila, pattern formation, gap genes, tailless, terminal domains.

Introduction

Four maternal systems of genetic information are involved in setting up the pattern of the Drosophila embryo. Three establish positions along the anterior-posterior axis and one establishes positions along the dorsal-ventral axis (see review by Nüsslein-Volhard, 1991). The activity of these systems results in the transcriptional activation of zygotic genes in an ordered array that subdivides the embryo into different regions. In particular, the anterior system controls the gnathal and thoracic regions, while the terminal system controls the two termini, i.e., the acron (defined here as the brain and parts of the head skeleton) and the telson (defined here as the dorsal portion of the eighth abdominal segment and all structures posterior to it) (Nüsslein-Volhard et al., 1987).

The pattern deletions observed in these maternal effect mutants are essentially non-overlapping, leading to the view that the anterior and terminal systems are largely independent of each other (Nüsslein-Volhard et al., 1987). An exception to this generalization is provided by an additional feature of the bcd phenotype: in embryos from bcd females, the acron is replaced by a telson (Frohnhofer and Nüsslein-Volhard, 1986). Therefore, while the maternal terminal system suffices to establish a telson at either pole and bcd the gnathal and thoracic regions, the combined activity of both the maternal terminal system and the bcd morphogen are required to establish the acron (Frohnhofer and Nüsslein-Volhard, 1986).

The anterior domain of the early Drosophila embryo can be viewed as a field in which positional information is provided by a gradient of the bcd protein morphogen. The bcd protein is a homeodomain transcription factor distributed in a monotonic gradient with its highest concentration at the anterior pole; different concentrations of the bcd protein along the anterior-posterior axis lead to expression of different zygotic genes (Driever and Nüsslein-Volhard, 1988a and b; Tautz, 1988; Driever and Nüsslein-Volhard, 1989; Struhl et al., 1989; Driever et al., 1989; Gaul and Jackie, 1989;

The maternal terminal system also appears to establish a system of graded positional information (Casanova and Struhl, 1989). The terminal genes specify the components of a signal transduction pathway that is thought to function as follows. A uniformly distributed receptor tyrosine kinase, encoded by the torso (tor) gene, is activated at the poles of the embryo by a locally released ligand controlled by the torsolike (Isl) gene (Sprenger et al., 1989; Casanova and Struhl, 1989; Stevens et al., 1990). The tor receptor then activates the serine/threonine kinase encoded by the \( t(1) \) polehole (\( t(1) \)ph) gene, the Drosophila homolog of the vertebrate proto-oncogene c-raf-1 (Mark et al., 1987; Nishida et al., 1988; Ambrosio et al., 1989a and b). Ultimately, the \( \text{tor}/D-\text{raf} \) pathway activates one or more as yet unidentified transcription factors that control the expression of zygotically expressed, terminal-specific genes.

The requirement for both the terminal system and \( \text{bed} \) in the anterior terminal domain indicates that the combined activity of these two maternal systems is necessary to establish the specific program of gene expression required for the development of the acron. While shared zygotic targets have been identified, in these cases \( \text{bed} \) and the terminal system have been found to act independently of one another and as antagonists, the former activating and the latter repressing expression of the same gene (Finkelstein and Perrimon, 1990; Tautz, 1988; Driever and Nusslein-Volhard, 1989; Struhl et al., 1989). The regulation of the zygotic terminal gene \( \text{tailless} \) (\( \text{til} \)) exemplifies a different mode of interaction between these two maternal systems: \( \text{bed} \) and the terminal system function together to activate and to repress \( \text{til} \) expression in specific domains in the anterior of the Drosophila embryo.

The requirement for \( \text{til} \) gene function in the terminal regions is shown by the lack of acron derivatives at the anterior and the absence of the telson at the posterior of \( \text{til} \) mutant embryos (Strecker et al., 1988). While initially activated in two symmetrical caps at both poles, expression of the \( \text{til} \) gene becomes restricted to a dorsolateral stripe at the anterior of blastoderm stage embryos (Pignoni et al., 1990). We show here that the early \( \text{til} \) expression in two terminal caps is mainly under the control of the maternal terminal system, while the anterior \( \text{til} \) stripe is generated in response to complex interactions between the anterior, terminal and dorsal-ventral systems. Our analysis of the regulation of \( \text{til} \) expression by the anterior and terminal systems provides the first evidence that two positional information systems are required together to both activate and repress transcription of a specific gene.

Materials and methods

Localization of \( \text{til} \) transcripts

Whole-mount in situ hybridization was carried out with single-stranded DNA probes labeled by unidirectional PCR (Nipam Patel, personal communication), or with double-stranded DNA probes labeled by random priming with digoxigenin-dATP (Genius kit, Boehringer Mannheim), as described previously (Pignoni et al., 1990). Embryonic stages are those of Campos-Ortega and Hartenstein (1985); nuclear cycles were determined as in Foe and Alberts (1983).

Data analysis

Embryos were examined and photographed using a Zeiss Axioshot microscope with differential interference contrast optics. Measurements were made from photographs in which all views were lateral. Measurements were made midlaterally for the early anterior and posterior caps in syncytial blastoderm embryos, and for the posterior cap in cellularizing embryos; the position of the resolved dorsolateral stripe in cellularizing embryos was determined on the dorsal side. Ten or more embryos of the appropriate stage were measured in each case (with the exception of cellular blastoderm stage \( \text{exc} \) var embryos, of which five were measured). All measurements were converted to percentage EL. Sample mean and standard deviations (between 1 and 2%) were calculated; differences of less than 2% EL were considered insignificant. The wild-type standard was taken to be the pattern of \( \text{til} \) expression in Oregon-R embryos stained separately or mixed with mutant embryos.

Generation of \( \text{til} \) antiserum

A construct expressing a \( \beta \)-gal-\( \text{til} \) fusion protein was made by inserting the 1150 \( \text{PstI}-\text{HindIII} \) fragment (this encodes amino acids 38-421 of the \( \text{til} \) protein) from the cDNA N4 (Pignoni et al., 1990) into the vector pTRBO (Burglin and DeRobertis, 1987). A construct expressing a glutathione-S-transferase-\( \text{til} \) fusion protein was made by adding \( \text{EcoRI} \) linkers to a 1200 bp \( \text{TagI} \) fragment (this encodes amino acids 25-425 of the \( \text{til} \) protein) from the cDNA N4, digesting with \( \text{EcoRI} \) and \( \text{HindIII} \), and inserting this piece into a pGEX vector (Smith and Johnson, 1988) that had been modified to contain a \( \text{HindIII} \) site. The \( \beta \)-gal-\( \text{til} \) fusion protein was induced with IPTG and purified by SDS-PAGE; a homogenized gel slice containing the fusion protein was used to immunize rats. Initial injections were with 100 \( \mu \)g of fusion protein in Freund’s adjuvant (complete for the first injections, incomplete for subsequent boosts). The final boost was with 500 \( \mu \)g of the glutathione-\( \text{til} \) fusion protein that was isolated as inclusion bodies according to the method of Hoey (1989), and sonicated prior to injection.

Antibody staining

Embryos were fixed and stained as described by Hartenstein and Campos-Ortega (1986), with the following modifications. The anti-\( \text{til} \) antiserum, biotin-SP-AffiniPure anti-rat antibodies and streptavidin-HRP (the latter two from Jackson ImmunoResearch) were diluted 1:10 and preabsorbed to 3-20 hour wild-type embryos, then used at final dilutions of 1:400, 1:2000 and 1:2000, respectively. The staining reaction was carried out in the presence of 

\[ \text{NiCl}_2 \]

using the Vector Laboratories DAB substrate kit for HRP. After they were stained, embryos were washed with PBST (PBS + 0.3% Triton X-100), dehydrated in ethanol and acetone, and mounted in Polystain (Polysciences). Approximately one-quarter of the embryos collected from a \( \text{Df(3R)til}^{\text{PSX}} \) stock (\( \text{Df(3R)til}^{\text{PSX}} \) deletes the entire \( \text{til} \) gene, Pignoni et al., 1990) are not stained by the anti-\( \text{til} \) antiserum, consistent with the notion that this antiserum stains only the protein encoded by the \( \text{til} \) locus in early embryos.
Fly stocks

The strongest available mutant alleles were selected, based on previous phenotypic analyses of the homozygous and hemizygous combinations (L. Ambrosio personal communication; Klingler, 1989, Sprenger et al., 1989). One terminal mutant allele is available for each of the $fs(l)N$ and $fs(l)ph$ loci: $fs(l)N^{211}$ and $fs(l)ph^{R01}$ are hypomorphic, as they are the only alleles that do not result in the more severe collapsed egg phenotype (Degelmann et al., 1986 and 1990; Perrimon et al., 1986). Germline clones of $l(1)ph^{A75}$ were generated by using the dominant female sterile technique (Perrimon, 1984, Perrimon et al., 1984). Other alleles used were: $tor^{XRI}$, a 9.5 kb deletion of the locus (Sprenger et al., 1989), and $tor^{PM51}$, a strong allele (Schupbach and Wieschaus, 1986); $tsr^{R01}$ and $tsl^{146}$, both strong alleles, and $tsl^{MK}$, the weakest $tsl$ allele (Klingler, 1989), $trk^{10D}$, a strong allele (Schupbach and Wieschaus, 1986); $l(1)ph^{E475}$ and $l(1)ph^{1-25}$, both strong alleles (Perrimon et al., 1985; Ambrosio et al., 1989a); $bcd^{14}$, a small deletion of the locus (Berleth et al., 1988), $exu^{A}$, a strong allele (Schupbach and Wieschaus, 1986); and $dl^{1}$, a strong allele (Roth et al., 1989). Females carrying four copies ($bcd^{4+}$) and six copies ($bcd^{6+}$) of the wild-type $bcd$ gene were obtained from the 5.8/FM7 stock (Driever and Nusslein-Volhard, 1988b). Double mutant chromosomes used were $tor^{wk}trk^{RA}$, both strong alleles (Schupbach and Wieschaus, 1986); $bcd^{14}tsl^{146}$, both strong alleles (Lehmann and Frohnhofer, 1989); and $exu^{Dl}var^{OD}$ (Schupbach and Wieschaus, 1986). The $tor^{XRI}$, $tor^{R01}$, $tsl^{146}$, $tsl^{MK}$, and $trk^{10D}$ stocks were provided by R. Tearle and C. Nusslein-Volhard; the $l(1)ph^{E475}$ stock by L. Ambrosio; the $tor^{wk}trk^{RA}$ stock by J. Casanova, the $bcd^{14}tsl^{146}$ stock by R. Lehmann; the $fs(l)N^{211}$, $fs(l)ph^{PM51}$, $bcd^{14}$, and $tor^{PM51}$ stocks by T. Strecker; the multiple-bcd" stock by N. McGunnis; the $exu^{Dl}$ and $exu^{Dl}var^{OD}$ stocks by T. Schupbach; the $dl^{1}$ and $ovo^{Dl}$ stocks by K. V Anderson. The stock P75.2-3, carrying extra copies of the wild-type $ill$ gene, was obtained from an original transformat type line that had two inserts, one on the second and one on the third chromosome (Baldarelli, 1990).

Results

Patterns of $ill$ RNA and protein distribution in the early embryo

We have increased the sensitivity of our detection of $ill$ transcripts and have extended our analysis of $ill$ localization to the protein level. $ill$ mRNA was detected by in situ hybridization to whole embryos using a full-length cDNA clone (Pignoni et al., 1990), while $ill$ protein was detected with a rat polyclonal anti-$ill$ antibody (see Materials and methods).

Transcripts of the $ill$ gene are first detected during nuclear cycle (NC) 9 as two dots of stain in nuclei in the terminal regions of the syncytial blastoderm embryo (Fig. 1A, and data not shown). Staining is stronger in the most terminal nuclei, which appear labeled throughout, and progressively less intense in subterminal nuclei, appearing as two dark dots and then two very faint dots of stain (Fig. 1A). During NC10, 11 and 12, staining increases first over the nuclei (Fig. 1B) and then in the surrounding cytoplasm, forming two solid caps of staining at both termini (Fig. 1C).

After the formation of the terminal caps, the RNA expression pattern resolves into smaller domains at both anterior and posterior termini (Fig. 1D-F). By the beginning of cellularization, the posterior cap has retracted from approximately 20 to 16% egg length (EL), while expression at the anterior pole has undergone a series of dramatic changes. Soon after anterior cap formation, staining is lost, first from the extreme anterior tip progressing toward the posterior (Fig. 1D), and then from the ventral midline progressing laterally (Fig. 1E); at the same time, expression on the dorsal side extends posteriorly by a few per cent EL. As a result, by the end of the syncytial blastoderm stage, the anterior $ill$ domain has become a horseshoe-shaped stripe located between 75 and 88% EL and extending about two-thirds of the way towards the ventral midline (Fig. 1F). Although activation appears to occur simultaneously at both poles, staining in the anterior domain (cap or stripe) is generally weaker than staining in the posterior domain.

The $ill$ protein appears in the same pattern as the RNA. Two caps of $ill$ protein are seen in late syncytial blastoderm stage embryos (Fig. 1G), while the resolved pattern is visible in cellular blastoderm stage embryos (Fig. 1H). As was seen for the RNA staining, the intensity of antibody staining in the posterior cap is greater than that at the anterior. The pattern of $ill$ expression detected in cellular blastoderm stage embryos corresponds to the domains deleted in $ill$ mutants as projected onto the blastoderm fate map (Pignoni et al., 1990).

In what follows, the domains of $ill$ expression in syncytial blastoderm stage embryos are referred to as anterior and posterior caps (Fig. 1C) while the anterior $ill$ domain in cellular blastoderm stage embryos is referred to as the $ill$ stripe (Fig. 1F). The repression of $ill$ extending from the anterior tip towards the posterior will be referred to as anterior repression (Fig. 1D), while the repression extending from the ventral midline laterally will be referred to as ventral repression (Fig. 1E).

Activation of $ill$ by the maternal terminal system

Several lines of evidence suggest that the initial activation of $ill$ transcription at both poles of the embryo is controlled by the maternal terminal system. Phenotypic analysis of loss-of-function mutants shows that the terminal regions deleted in $ill$ embryos are large subdomains of those deleted in $tor$ or $trunk$ ($trk$) embryos (embryos from homozygous maternal effect mutant mothers will be referred to by the maternal genotype in what follows) (Strecker et al., 1988). Gain-of-function mutations in the $tor$ gene give an opposite phenotype in which the terminal domains are expanded; this expansion depends on the ectopic expression of $ill$ that occurs in these mutant embryos (Klingler et al., 1988; Strecker et al., 1989; Steingrimsson et al., 1991).

To examine the role of the maternal terminal pathway in activation of $ill$ transcription, we analyzed $ill$ expression in the strongest alleles available of each of the six described maternal terminal genes: $tor$, $trk$, $tsl$, $l(1)ph$, $fs(l)Nasrat$, and $fs(l)polehole$. (Fig. 1H)
Fig. 1. Activation and resolution of \textit{til} expression pattern in wild-type embryos. Spatial distribution of \textit{til} RNA (A-F) or protein (G,H) was detected by in situ hybridization or antibody staining, respectively, as described in Materials and methods. All views are lateral, with anterior on the left and dorsal up. The embryos shown are wild-type except for the embryo in B which carries extra copies of the wild-type \textit{til} gene. (A) Syncytial blastoderm stage embryo, NC 11. Note staining in a pair of dots in many nuclei. That each dot corresponds to the accumulation of transcripts from one copy of the \textit{til} gene is suggested by the appearance of additional dots in the nuclei of embryos carrying extra copies of \textit{til} on the second chromosome (data not shown). Similar nuclear dots corresponding to transcripts of a single gene copy have been described for the gene \textit{string} (O’Farrell et al., 1989). (B) Syncytial blastoderm stage embryo, NC 12. (C-E, G) Syncytial blastoderm stage embryos. (F, H) Cellular blastoderm stage embryos. (Schupbach and Wieschaus, 1986; Stevens et al., 1990; Ambrosso et al., 1989a; Perrimon et al., 1985, 1986; Degelmann et al., 1986, 1990). Essentially the same pattern was obtained when the spatial distribution of either \textit{til} RNA or protein was examined: at the posterior, little or no \textit{til} expression can be detected in either syncytial or cellular blastoderm embryos; at the anterior, the early cap does not appear while an anterior stripe, although abnormal, appears by the late syncytial blastoderm (Fig. 2A-D and data not shown). These results are seen even in null \textit{tor}^{XR1} embryos and in \textit{tor trk} embryos, mutant in two components of the terminal pathway (Fig. 2A,B and E). The maternal terminal system, therefore, is necessary to activate \textit{til} expression in the terminal caps; at the anterior, however, an abnormal \textit{til} stripe appears in the absence of a functional terminal system.

Since the \textit{bcd} morphogen is active in the anterior, and is required to make the distinction between anterior and posterior termini (Frohnhöfer and Nüsslein-Volhard, 1986), it seemed likely that \textit{bcd} might be involved in activating \textit{til} at the anterior. Consistent with this hypothesis, \textit{bcd tsl} embryos, which lack both anterior and terminal gene functions, show no \textit{til} expression at
Maternal control of tailless expression

Fig. 2. Control of \textit{tll} expression by maternal terminal genes

Localization of \textit{tll} RNA (A, C-F) and protein (B) in cellular blastoderm stage embryos, views as described in Fig. 1. (A, B) \textit{tor} embryo; (C) \textit{tsl} embryo; (D) \textit{l(1)ph} embryo; (E) \textit{trk} \textit{tor} embryo; (F) \textit{bcd tsl} embryo. The bracket above the embryo in A indicates the position of the \textit{tll} stripe in wild-type embryos. In situ hybridization to embryos mutant for \textit{fs(l)N}, \textit{fs(l)ph}, and \textit{trk} gave the same results as shown in panels A, C and D. Antibody staining of \textit{tsl}, \textit{l(1)ph} and \textit{bcd tsl} embryos gave results consistent with the in situ hybridization to RNA shown in panels C, D and F. In some of the embryos from mothers homozygous mutant for most terminal alleles, there was a very low level of staining in the posterior domain (data not shown). This suggests either that most of the terminal alleles tested are hypomorphs, or that the terminal pathway retains a low residual level of activity in the absence of any one of these gene products. A low level of posterior \textit{tll} expression was usually associated with ectopic expression of \textit{tll} in the trunk region and/or the pole cells. This might be related to the presence of systems for the repression of \textit{tll} in these regions of the embryo (see Fig. 3 legend).

either pole (Fig. 2F). We conclude that \textit{bcd} is responsible for the activation of \textit{tll} transcription at the anterior pole in terminal mutant embryos.

We can therefore use \textit{bcd} embryos to examine the activation of \textit{tll} transcription by the terminal system alone. In \textit{bcd} embryos, \textit{tll} transcription is activated at both poles in two terminal caps (Fig. 3A,B). Two features of these early caps differ from those seen in wild-type embryos. First, the level of staining of the caps is approximately equal, in contrast to the wild-type situation in which staining is more intense at the posterior. The presence of \textit{bcd} protein, therefore, appears to have a negative effect on the anterior cap of \textit{tll} expression. Second, the caps are less symmetrical and, in some embryos, considerably larger than those seen in wild-type embryos, extending up to 45% EL from the posterior pole and up to 72% EL from the anterior pole (compare Fig. 1C with Fig. 3A). By the beginning of cellularization, these caps have generally retracted towards the termini, extending from 0 to 17% EL in the posterior and from 87 to 100% EL in the anterior (see Fig. 3C for the RNA and 3D for the protein). The anterior cap does not resolve into the stripe seen in wild-type embryos, but mimics the behavior of a normal posterior cap of \textit{tll} expression (Fig. 3E), disappearing from the ectopic anterior amnioproctodeal invagination during germ band extension (Fig. 3F). The presence of caps at both poles of \textit{bcd} embryos and the absence of caps in terminal mutant embryos indicate that the maternal terminal system activates \textit{tll} expression in these domains.

Activation by \textit{bcd}

When activation is only by \textit{bcd} (in \textit{tor} embryos for example) \textit{tll} RNA is first detected during NC 12 in the dorsal nuclei at the anterior tip of the embryo (Fig. 4A); this domain of expression then spreads posteriorly and ventrally (Fig. 4B). By the beginning of cellularization, the most anterior expression is lost, and a broad dorsolateral stripe is seen between 80 and 96% EL (Figs
Fig. 3. Activation of *til* by the maternal terminal system in the absence of *bcd*. Localization of *til* RNA (A-C, E-F) and protein (D) in *bcd* embryos, staging and views as described in Fig. 1. (A, B) Syncytial blastoderm stage; (C, D) cellular blastoderm stage; (E) gastrulation, (F) germ band extension. The two caps of *til* expression behave similarly, from their initial appearance through germ band extension. The region of *til* activation in *bcd* embryos is sometimes considerably larger than expected from studies of *til* expression in wild-type embryos (compare A with Fig. 1C), indicating that the maternal terminal system is potentially active over a larger domain than initially postulated. In cellular blastoderm stage embryos, the posterior cap is slightly expanded when the level of *bcd* protein is lowered (C and Fig. 5A) and is slightly contracted when the level of *bcd* protein is raised (Fig. 5B, 5C, 6C); the anterior cap responds to the *bcd* gradient in an opposite fashion (Fig. 6C, and data not shown). These variations could be the result of changes in gap gene expression; since *til* expression is not significantly altered in embryos mutant for any one of the known gap genes, however (FP unpublished), a combinatorial effect of multiple gap genes would have to be invoked.

2A and 4C). This domain is expanded and shifted anteriorly compared to the *til* stripe seen in wild-type embryos (compare Figs 1F and 2A). That this stripe is activated by *bcd* in a concentration-dependent fashion can be demonstrated by altering the *bcd* concentration gradient (Driever and Nüsslein-Volhard, 1988b). In embryos from *tor* mutant females bearing 4 wild-type copies of the *bcd* gene (*tor* *bcd* 4+), the anterior domain extends more posteriorly than in *tor* embryos containing the normal dosage of *bcd*, i.e. from 70 to 94% EL (Fig. 4D).

**tor and *bcd* together control the anterior *til* stripe**

*Activation*

To investigate the relationship between the *bcd*-dependent *til* domain seen in terminal mutant embryos (Fig. 2A-E) and the *til* stripe seen in wild-type embryos (Fig. 1F), we tested the effect of altering either the *bcd* gradient or the activity of the terminal system on anterior *til* expression.

Evidence that the position of the *til* stripe in wild-type embryos is under the control of the *bcd* morphogen comes from experiments in which the *bcd* gradient is altered by changing the number of *bcd* copies. The *til* stripe, which lies between 88 and 75% EL in wild-type embryos, is narrower and shifted anteriorly in *bcd* 1+ embryos, while it is shifted posteriorly in *bcd* 4+ embryos and becomes broader in *bcd* 6+ embryos (Fig. 5E; compare Fig. 1F with Fig. 5A-C for the RNA pattern, and Fig. 1H with Fig. 5D for the protein pattern). These results suggest that *bcd* plays a critical role in the formation of the *til* stripe. Since the shape and position of this domain is abnormal whenever terminal system activity is absent (Figs 2A-E and 4D), the maternal terminal pathway must also be involved in the correct establishment of the stripe domain. Further manipulation of the terminal and anterior activities was carried out to elucidate the roles played by these two systems in forming the *til* stripe.

The domain in which the terminal system is active can
Maternal control of tailless expression

be reduced by using hypomorphic alleles of the \( \text{ts} \) gene, which is believed to control the ligand for the \( \text{tor} \) receptor (Stevens et al., 1990). Embryos mutant for the weak allele \( \text{ts}^\text{MK} \), although showing no cuticular defects at the anterior (i.e. acron development is normal), show posterior phenotypes ranging from an almost complete telson to a lack of all structures posterior to A7 (Kl{"u}nger, 1989).

In \( \text{ts}^\text{MK} \) embryos, the initial activation of \( \text{til} \) in two symmetrical domains is not seen. By cellular blastoderm stage, a smaller than normal posterior cap (extending up to 7\% EL from the posterior pole) can be seen in approximately one third of the embryos (Fig. 6B); the \( \text{til} \) stripe, on the other hand, narrower by a few per cent EL but otherwise normal in shape and location, is present in all \( \text{ts}^\text{MK} \) embryos (Fig. 6A,B). These results indicate that when the activity of the terminal pathway is reduced to a point where activation of \( \text{til} \) in terminal caps is no longer detected (Fig. 6A) an essentially normal \( \text{til} \) stripe can still be formed. Since all \( \text{ts}^\text{MK} \) embryos develop normal heads, the stripe seen in these embryos apparently provides sufficient \( \text{til} \) activity for normal acron development. Thus the requirement for the terminal system in establishing the stripe in the correct position (Fig. 2A-E) and in forming the acron (Sch{"u}pbach and Wieschaus, 1986; N{"u}sslein-Volhard et al., 1987) can be satisfied by a relatively low level of terminal system activity (i.e., that present in \( \text{ts}^\text{MK} \) embryos).

A shallower \( \text{bed} \) gradient, i.e. lower than normal at the anterior tip but higher than normal at more posterior positions, is obtained in \( \text{exuperantia vasa} \) (\( \text{exu vas} \)) embryos (Driever and N{"u}sslein-Volhard, 1988b; Struhl et al., 1989). In these embryos, \( \text{til} \) expression is initially activated in two, smaller than normal, terminal caps (Fig. 6C). By the beginning of cellularization, the anterior cap has retracted to a smaller domain while a thinner than normal \( \text{til} \) stripe appears between 86 and 92\% EL (Fig. 6D). This stripe, in contrast to that seen in strong terminal class mutants, behaves like a wild-type stripe in that it separates into two lateral anterior domains late in the cellular blastoderm stage (data not shown). The anterior cap, on the other hand, behaves like the posterior cap in that it disappears early during gastrulation (Fig. 6E). The lower level of \( \text{bed} \) protein at the anterior of \( \text{exu vas} \) embryos presumably results in the anterior shift of the \( \text{til} \) stripe. The position of this stripe is consistent with the expression of another \( \text{bed} \)-regulated gene \( \text{empty spiracles} \) (\( \text{ems} \)); \( \text{ems} \) is expressed just posteriorly to the \( \text{til} \) stripe in \( \text{bed}^1, 2^+ \) and \( 4^+ \) embryos, and appears at about 84\% EL in \( \text{exu vas} \) embryos (Dalton et al., 1989; Figs 1F, 5A,B, and 6D). The absence of the \( \text{til} \) stripe in \( \text{exu vas} \) embryos (Fig. 6F) is consistent with the lack of \( \text{til} \) expression posterior to 80\% EL in strong terminal mutant embryos and indicates that the level of \( \text{bed} \) protein at the anterior of \( \text{exu vas} \) embryos is not sufficient to activate \( \text{til} \) in the absence of terminal gene activity.

The results obtained with \( \text{ts}^\text{MK} \) and \( \text{exu vas} \) embryos indicate that the activation of the \( \text{til} \) stripe requires the activities of both \( \text{bed} \) and the terminal system. In the absence of the maternal terminal system, higher levels of \( \text{bed} \) are required to activate \( \text{til} \) than in wild-type. One apparent exception to this is that the \( \text{til} \) stripe extends as far posteriorly in \( \text{bed}^4+ \) embryos as it does in \( \text{bed}^4+ \) embryos (70\% EL; compare Figs 4D and 5B). Since terminal system activity does not extend much further than 20\% EL from the poles (Casanova and Struhl, 1989; Figs 1C and 5B, C, see Repression below), activation of \( \text{til} \) by \( \text{bed} \) around 70\% EL in \( \text{bed}^4+ \) embryos is probably independent of terminal gene function.

Repression

The resolution of the \( \text{til} \) domain from a cap into a stripe at the anterior, but not at the posterior, and the lack of
Fig. 5. The \textit{til} stripe depends on the \textit{bcd} concentration gradient. Localization of \textit{til} RNA (A-C) and protein (D), staging and views as described in Fig. 1; significance of brackets as in Fig. 2. (A) Cellular blastoderm stage \textit{bcd} 1+ embryo; (B) cellular blastoderm stage \textit{bcd} 4+ embryo; (C, D) cellular blastoderm stage \textit{bcd} 6+ embryos. With increasing copies of \textit{bcd}, the \textit{til} stripe moves more posteriorly; (E) depicts this shift graphically for the anterior (○) and posterior (■) boundaries of the \textit{til} stripe in \textit{bcd} 1+, 2+, 4+ and 6+ embryos. The positions of equivalent \textit{bcd} immunostaining intensities (△) are shown for comparison (from Dreher and Nüsslein-Volhard, 1988).

this resolution in \textit{bcd} embryos, indicate that anterior repression requires \textit{bcd} function. Since the domain in which \textit{til} is repressed at the anterior expands as the number of wild-type copies of the \textit{bcd} gene is increased from 1 to 4 (Figs 5A, 1F and 5B), the extent of anterior repression is dependent on the concentration of \textit{bcd} protein.

As shown by the anteriorward expansion of the \textit{til} stripe in all terminal system mutants (Fig. 2A-E), anterior repression also requires terminal gene activity. The requirement for the terminal system in anterior repression can be observed in a \textit{bcd} 2+ background (compare the wild-type embryo in Fig. 1F with the \textit{tor} embryo in Fig. 2A) as well as in a \textit{bcd} 4+ background (compare the \textit{bcd} 4+ embryo in Fig. 5B with the \textit{tor bcd} 4+ embryo in Fig. 4D). That the anterior repression domain extends as far posteriorly as 78% EL but not beyond when the number of \textit{bcd} gene copies is increased from 4 to 6 (Fig. 5B,C) can also be explained by the dependence of anterior repression on terminal gene activity.

\textbf{Role of \textit{dl} in ventral repression}

The retreat of the \textit{til} stripe from the ventral side of the embryo (Fig. 1E,F) suggests a role for an additional patterning system in regulating \textit{til} transcription. Positional values along the dorsal-ventral axis are initially established by the maternal dorsal group genes. These genes encode the components of a signal transduction pathway that controls the graded nuclear localization of a transcription factor, the \textit{dorsal (dl)} protein (Steward 1987, 1989; Rushlow et al., 1989; Roth et al., 1989; reviewed by Nüsslein-Volhard, 1991).

Examination of \textit{til} expression in \textit{dl} embryos reveals that the \textit{dl} gene product is required for ventral repression. While in a wild-type cellular blastoderm stage embryo the \textit{til} stripe extends about 240° around the circumference of the embryo (Figs 7A and 1F), in \textit{dl} mutant embryos the stripe extends completely around the circumference of the embryo (Fig. 7B,C). Repression by \textit{dl} appears to require \textit{bcd} function, since ventral repression does not occur either at the posterior of wild-type embryos or at the anterior of \textit{bcd} mutant embryos.

\textbf{Discussion}

The main conclusion to be derived from the results
Maternal control of tailless expression

Fig. 6. Formation of the \textit{tll} stripe: activation by \textit{bcd} and the terminal system. Localization of \textit{tll} RNA, staging and views as described in Fig. 1, brackets as in Fig. 2. (A, B) Cellular blastoderm stage \textit{ts} \textsuperscript{MK} embryos. While the posterior cap is absent from about two-thirds of the embryos and present but reduced in the remaining third, the anterior \textit{tll} stripe is present (although thinner by a few per cent EL) in all embryos. (C) Syncytial blastoderm stage \textit{exu} \textit{vas} embryo; (D) cellular blastoderm stage \textit{exu} \textit{vas} embryo; (E) beginning of gastrulation, \textit{exu} \textit{vas} embryos; (F) cellular blastoderm stage \textit{exu} \textit{vas tor} embryo. Mutant \textit{exu} embryos gave results similar to those seen for the \textit{exu} \textit{vas} embryos (data not shown), although separation of the anterior expression domain into a cap and a stripe was less pronounced in the \textit{exu} than in the \textit{exu} \textit{vas} embryos. This slight difference can probably be attributed to a difference in the \textit{bcd} protein gradient at the anterior of the embryos from these two stocks, since mutations in the posterior system alone (\textit{oskar} and \textit{nanos}) do not affect either the \textit{bcd} protein gradient (Driever and Nusslein-Volhard, 1988b) or the anterior \textit{tll} pattern (FP, unpublished).

The presented here is that, while the terminal system, as predicted, activates the \textit{tll} gene in symmetrical domains at the embryonic poles, both the \textit{bcd} protein and the terminal system act together to establish the anterior \textit{tll} stripe. The postulated interaction between the anterior and terminal activities in establishing the acron is thus shown to occur at the level of regulation of a single gene, \textit{tll}: both maternal activities are required together for both activation and repression of this gene. A summary of the genetic control of \textit{tll} expression by these two activities, as well as by the dorsal-ventral system, is provided in Fig. 8.

Control of \textit{tll} expression by three maternal systems

The terminal system activates \textit{tll} in two polar caps

The initial pattern of \textit{tll} activation depends mainly on the terminal system (Figs 1A-C and 8A) and is consistent with the proposed model for the functioning of the terminal pathway (see Introduction). The activation of the zygotic genes \textit{huckebein (hkb)} and \textit{tll} in caps of different sizes, and the observation that the posteriormost region of the embryo is most sensitive to loss of terminal gene activity, suggest that there is a gradient of terminal system activity, highest at the poles and decreasing toward the center of the embryo (Pignoni et al., 1990; Weigel et al., 1990; Casanova and Struhl, 1989). Our data on the pattern of \textit{tll} expression in \textit{exu} \textit{vas} embryos also support this hypothesis. The presence of both a cap and a stripe in \textit{exu} \textit{vas} embryos (Fig 6D) can be explained if the lower level of \textit{bcd} protein present at the anterior of these embryos is sufficient to repress the activation of \textit{tll} transcription around 90\% EL, but not sufficient to override the \textit{tll} activation caused by a higher activity of the terminal system at the very tip. The pattern of \textit{tll} RNA staining at the posterior of wild-type embryos provides the most direct evidence for graded terminal pathway activity. As longer staining periods are used to reveal lower levels of \textit{tll} RNA by in situ hybridization, the size of the domain where \textit{tll} RNA is detected increases by several per cent EL (data not shown). Moreover, the intensity of \textit{tll} RNA staining in the nuclei of very early embryos
Fig. 7. \(dll\) is required for ventral repression. Localization of \(dll\) RNA and staging as described in Fig. 1. (A, B) Transverse optical sections through the anterior domain; (C) lateral view. (A) Cellular blastoderm stage wild-type embryo: the anterior dorsal stripe extends about 240° around the circumference of the embryo. (B) Cellular blastoderm stage \(dll\) embryo: the anterior dorsal stripe extends completely around the circumference of the embryo. (C) Cellular blastoderm stage \(dll\) embryo.

decreases from terminal to subterminal positions (Fig. 1A). The maternal terminal genes can therefore be viewed as establishing a coordinate system of positional information (Wolpert, 1989).

\(bcd\) and the terminal system act together to turn on the \(tll\) stripe

By the cellular blastoderm stage, the anterior \(tll\) cap has been replaced by a dorsal stripe. While repression of the early cap plays a role in the appearance of the stripe (see below), transcription in the stripe appears to be largely independent of that in the anterior cap. Thus in strong terminal mutant and in \(tsplMK\) embryos, the early caps of \(tll\) expression are not seen, but the stripe does appear by late syncytial blastoderm (Figs 2A-E and 6A,B). Also, the posterior border of the stripe extends beyond the border of the anterior cap. Finally, the dependence of the position of the stripe on the \(bcd\) gradient (Figs 1F, 4D, 5A-D) shows that \(tll\) transcription in this domain is activated by specific concentrations of the \(bcd\) protein (as indicated in Fig. 8B). The anterior shift of the \(tll\) stripe in strong terminal mutant embryos (Fig. 2A-E) shows that the terminal system is necessary for establishing the stripe in the correct position. The appearance of a relatively normal stripe in \(tsplMK\) embryos (where terminal activity is reduced) suggests that a low level of terminal system activity is sufficient for activation of the stripe in the correct position.

\(bcd\) and the terminal system act together to repress \(tll\) at the anterior

The anterior repression domain (indicated in Fig. 8B) is defined as the region of repression seen at the anterior

Fig. 8. Regulation of \(tll\) expression by the terminal, anterior and dorsal-ventral maternal systems. Summary of the genetic control of \(tll\) expression. The wild-type domains of \(tll\) expression are shown in the embryo cartoons. The activating and repressing activities of the different genes are not meant to imply direct protein-DNA interactions, but to indicate the pathway in which the gene is active. (A) Syncytial blastoderm stage pattern. The more intensely staining posterior cap of \(tll\) expression is due to activation by the terminal system, tor (thick arrow); the more weakly staining anterior cap results from activation by the terminal system, tor (thick arrow) reduced to thin arrow in the presence of \(bcd\). (B) Cellular blastoderm stage pattern. The formation of the \(tll\) stripe requires the wild-type activity of all three maternal systems. The lines above the embryo represent, non-quantitatively, the gradient of anterior system activity (\(bcd\)) and the postulated gradient of activated terminal system (tor). Activation of the \(tll\) stripe is primarily by intermediate levels of \(bcd\) activity (solid arrow leading from \(bcd\) gradient), but also requires terminal gene activity (dashed line leading from activated tor gradient). Anterior repression \([-;\square]\) requires a high level of both \(bcd\) and terminal system activity (solid line leading from both \(bcd\) and activated tor gradients). Ventral repression \([-;\bullet]\) depends on the gene \(dll\); the requirement for \(bcd\) may reflect a role of \(dll\) in repressing \(bcd\)-dependent activation of \(tll\), \(dll\) (\(bcd\)).

of \(dll\) mutant embryos (Fig. 7C). Anterior repression by \(bcd\) displays a stricter requirement for terminal gene function than does \(bcd\)-dependent activation, since even the highest levels of \(bcd\) protein present in wild-type embryos seem to be insufficient to repress \(tll\) in the absence of terminal gene function. Thus in tor \(dll\) embryos, which have a normal complement of \(bcd\)
Maternal control of tailless expression

protein but lack the dorsal and terminal systems, there is a complete lack of anterior repression, i.e., the anterior domain appears as a cap (data not shown). The failure of the anterior repression domain to extend beyond 80% EL, as the number of wild-type bcd copies is increased from 4 to 6 (Fig. 5B,C), is also consistent with the notion that terminal activity is required for anterior repression.

dl represses the til stripe
That dl acts to repress the til stripe ventrally (as indicated in Fig. 8B) is shown by the extension of this stripe to the ventral midline in dl embryos (Fig. 7). Since ventral repression is absent at the posterior of wild-type embryos and at the anterior of bcd embryos, repression on the anterior ventral side depends on both dl and bcd. In exu vas embryos, however, ventral repression occurs around 90% EL (where the stripe forms) but not at the anterior tip (where a small cap persists) even though somewhat higher levels of bcd are present at the pole (Fig. 6D; Struhl et al., 1989). This could be explained if dl represses the bcd-dependent activation of til (the stripe), but not the terminal system-dependent activation of til (the cap). This hypothesis also provides an explanation for the observation that, although nuclear localization of the dl protein is detected as early as NC 10 (Roth et al., 1989; Rushlow et al., 1989; Steward, 1989), ventral repression is not seen in wild-type embryos until late syncytial blastoderm (around NC 13), after the onset of anterior repression and after the time when activation by bcd is seen in terminal mutant embryos.

Maternal factors controlling til: direct or indirect effects?
The early (detectable by NC 9) activation of til transcription at both poles by the terminal pathway argues for a direct activation of til by one or more maternally provided transcriptional activators. The later activation and repression involving bcd, on the other hand, might be achieved indirectly through zygotic genes which in turn regulate til transcription. Mutations in genes such as orthodenticle (otd), hunchback (hb) and hkb (Finkelstein and Perrimon, 1990; Driever and Nüsslein-Volhard, 1989; Struhl et al., 1989; Weigel et al., 1990), however, do not significantly affect the anterior expression of til (FP, unpublished). Thus, if there are zygotic targets of bcd that regulate anterior til expression, these have not yet been identified.

Alternatively, the bcd protein might activate and repress til transcription directly. As its concentration increases to a threshold level, the bcd protein might activate transcription of til by binding to high affinity binding sites in the til promoter. The similarity between the spreading from the anterior tip of newly synthesized bcd protein and of til RNA expression in terminal mutant embryos (Fig. 4A-C; Driever and Nüsslein-Volhard, 1988a) supports such a direct activation model. As the bcd protein concentration continues to increase at the anterior pole, binding to low affinity binding sites might repress til transcription. Models in which the bcd protein both activates and represses have also been proposed for the control of the gap genes Kruppel and giant (Gaul and Jäckle, 1989; Hülskamp et al., 1990; Eldon and Pirrotta, 1991; Kraut and Levine, 1991). While the bcd protein has been shown to directly activate transcription of the hb gene (Driever and Nüsslein-Volhard, 1989; Struhl et al., 1989), a repressor function has not been demonstrated directly.

Since the dl protein can act as a repressor of transcription (Ip et al., 1991) and the til stripe appears normal in embryos mutant for the ventral zygotic genes twist and snail (Thiße et al., 1987; Boulay et al., 1987; FP, unpublished), ventral repression of til by dl is probably direct.

Relationship of altered til expression patterns to mutant phenotypes
The absence of the posterior til cap from terminal mutant embryos, and the requirement for both the terminal system and the til gene to form the telson, demonstrate that the posterior cap of til expression is required to establish the telson. Consistent with this conclusion, the persistence of the anterior cap of til expression into the cellular blastoderm stage, as is seen in bcd and exu vas embryos, is correlated with the formation of ectopic posterior structures at the anterior. Thus an ectopic posterior midgut invagination is seen in bcd and exu vas embryos (Fig. 3E,F; Schüpbach and Wieschaus, 1986) and an ectopic telson differentiates in bcd embryos (Frohnhöfer and Nüsslein-Volhard, 1986).

The early anterior cap of til expression seen in wild-type embryos, however, does not appear to be necessary for proper acron development. Thus tslMK embryos, even thought they lack this early cap, develop a normal acron. The anterior til stripe, however, does appear to be required, as it is always present when acron development is normal. That an anterior stripe alone is not sufficient is shown by the fact that the thin stripe present in exu vas embryos and the broader stripe seen in strong terminal mutant embryos do not result in normal acron formation. Since til tsl double mutant embryos do not differ in either cuticular or nervous system morphology from tsl mutants (Strecker et al., 1988; data not shown), the til protein present at the anterior of terminal mutant embryos (Fig. 2B) is unable to provide all functions necessary for acron development. Presumably other genes controlled by bcd and the terminal system are also required for this process.

Two systems of positional information interact to control one gene
The terminal system and the bcd protein gradient are each independently capable of defining a coordinate system of positional information. Thus the terminal pathway in the posterior and the bcd protein in the prospective gnathal and thoracic regions activate different sets of target genes (Pignoni et al., 1990; Driever and Nüsslein-Volhard, 1989; Struhl et al., 1989; Gaul and Jäckle, 1989).

That bcd and the terminal system are required
Degelmann, A., Hardy, P. A. and Mahowald, A. P. (1990) Genetic expression of a particular gene. Thus both terminal activity and bcd are necessary to activate transcription in the anterior stripe of ill, as well as to establish anterior repression of ill. Our discovery of these interactions provides an explanation for the dependence of positional values at the anterior of the Drosophila embryo on both maternal systems. Other zygotic genes expressed at the anterior, such as hkb (Weigel et al., 1990) and other yet unidentified loci, might be controlled by both systems in a similar fashion.

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