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Genetic and molecular characterization of tube, a *Drosophila* gene maternally required for embryonic dorsoventral polarity

(pattern formation/signal transduction/Toll/pelle)

ANTHEA LETSOU, SHERRY ALEXANDER, KIM ORTH, AND STEVEN A. WASSERMAN

Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX 75235

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ABSTRACT **Loss of maternal function of the tube gene disrupts a signaling pathway required for pattern formation in *Drosophila*, causing cells throughout the embryo to adopt the fate normally reserved for those at the dorsal surface. Here we demonstrate that tube mutations also have a zygotic effect on pupal morphology and that this phenotype is shared by mutations in Toll and pelle, two genes with apparent intracellular roles in determining dorsoventral polarity. We then describe the isolation of a functionally full-length tube cDNA identified in a phenotypic rescue assay. The tube mRNA is expressed maximally early in embryogenesis and again late in larval development, corresponding to required periods of tube activity as defined by distinct maternal and zygotic loss-of-function phenotypes in tube mutants. Sequence analysis of the cDNA indicates that the tube protein contains five copies of an eight-residue motif and shares no significant sequence similarity with known proteins. These results suggest that tube represents a class of protein active in signal transduction at two stages of development.**

Signals specifying the dorsoventral polarity of the *Drosophila melanogaster* embryo are generated, transmitted, and interpreted through the action of 12 maternal-effect genes (1, 2). Of these, tube, dorsal, and 9 other loci share a common loss-of-function maternal-effect lethal phenotype; all embryonic cells adopt the fate normally reserved for those at the dorsal surface of the embryo (1, 3). Fertilized eggs laid by females lacking function for any of these 11 genes, collectively designated the dorsal group, produce embryos that gastrulate symmetrically and arrest 24 hr after egg laying as hollow tubes of dorsal cuticle (4). Loss of function of a 12th gene, cactus, results in an opposite, or ventralized, phenotype (5).

Genetic and molecular studies support a model in which the dorsal group loci and cactus act to produce a gradient of nuclear import of dorsal protein (6–8); this concentration gradient then defines position along the dorsoventral axis through the regional activation of downstream genes. In dorsal-positive embryos loss of function at tube or any of the other dorsal group loci disrupts signal transduction, preventing nuclear translocation of the dorsal protein (6). Similarities in sequence and function among the dorsal protein, the enhancer binding protein NFκB, and the proteins encoded by the vertebrate oncogene v-*rel* and its cellular homolog c-*rel* suggest that dorsoventral axis formation in *Drosophila* occurs by a conserved mechanism for regulated nuclear import (6–11).

A critical question that remains unanswered is how information regulating nuclear import is transduced in the cell. In *Drosophila* it appears that plasma membrane-bound Toll protein transmits information directing localized import of dorsal protein (6–8, 12); the gene products required for transduction of the Toll signal to dorsal have not been identified. To address the relationship of tube to dorsal, Toll, and other dorsal group loci, we have characterized the tube locus on a genetic and molecular level.*

METHODS

***Drosophila* Strains.** Balancers, marker mutations, and dorsal group mutations, including the tube alleles tub^1 (tub^{118}) and tub^2 (tub^{238}), have been described (13–15). The tub^4 allele was isolated in a screen for mutations failing to complement the maternal defect of the tub^2 mutation (S.A.W. and K. V. Anderson, unpublished data). The *P*-element insertion *l(3)ry147* was a gift of C. Berg and D. McKearin (Carnegie Institution of Washington, Baltimore).

Embryo Injection and Scoring of Maternal Phenotype. Dechorionated embryos were injected prior to pole-cell formation and embryonic phenotypes were scored as described (16, 17). Embryos exhibiting formation of dorsal folds, cephalic and ventral furrows, posterior and anterior midgut invaginations, pole-cell migration, and germ band extension were scored as strongly rescued at gastrulation. Such embryos differentiate dorsolaterally derived filzkörper and often ventrolaterally derived ventral denticles. Embryos exhibiting a few, but not all, of the asymmetric features of wild-type gastrulation were scored as weakly rescued.

Scoring of Zygotic Phenotype. Pupal axial ratios (length/width) were determined from photographs taken 12–45 hr after pupariation: length was measured from the base of the anterior spiracles to the base of the posterior spiracles; width measurements reflect the diameter of the pupa at its widest point. Pupae were sorted according to differences of 0.1 in axial ratio. The genotypes of adults that emerged from each class were determined on the basis of visible markers.

Mapping of the tube Locus. To construct compound third chromosomes (18), tub^2, *ru h th st* p^p/*TM8* females were exposed to 2000 R γ-irradiation (1 R = 0.258 mC/kg) and mated with *C(3L)P2, ri; C(3R)VK1,* e^s males. F_1 flies carrying new attached 3L or 3R chromosomes were identified as *ru h th st* e^s or *ri* p^p progeny, respectively. F_1 females were tested directly for the tube maternal-effect phenotype; F_1 males were mated to *C(3L)P2, ri; C(3R)VK1,* e^s females to generate F_2 females for testing. To further map the tube locus, flies segmentally aneuploid for 82A-C in proximal 3R were generated by crossing stocks carrying the T(Y;3)A154 and T(Y;3)J17 translocations (19). *Df(3R)2-2* (81F4-5; 82F10-11) was isolated in a γ-ray screen for deficiencies in proximal 3R. *Df(3R)Z-1* (82A5-6; 82E4) was provided by S. DiNardo (Rockefeller Univ., New York), and *Df(3R)XM3* (82A3-6; 82B) and *Df(3R)A321R1* (81F; 82A4-6) were provided by D. Weigel and B. Wild (Universität München, Munich).

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*The sequence reported in this paper has been deposited in the GenBank data base (accession no. M59501).

Nucleic Acids. To construct the *l(3)ry147* genomic library, DNA was isolated from adult flies (14), digested with *Bam*HI, and subcloned into the bacteriophage λ vector λ DASH (20). Overlapping cosmid clones from a library generously supplied by J. Tamkun (Univ. of Colorado, Boulder) were isolated using standard techniques (21). For transcription *in vitro*, pNB448 DNA was linearized with *Not* I at a site 3′ of the cDNA insert (22) and SP6 transcripts were generated *in vitro* essentially as described (23). Subclones in the bacteriophage M13 vectors mp18 and mp19 were sequenced by the dideoxynucleotide chain-termination method (24) using T7 and *Thermus aquaticus* DNA polymerases on an Applied Biosystems model 370A DNA sequencer. Both strands of the cDNA and all cloning sites were sequenced.

RESULTS

Maternal Effect tube Phenotype. Wild-type embryos undergo asymmetric gastrulation (Fig. 1*A*), characterized by the formation of dorsal folds, ventral and cephalic furrows, and anterior and posterior midgut invaginations (25). Wild-type larval cuticles are similarly distinctive (Fig. 1*B*) and feature structures derived from ventrolateral, dorsolateral, and dorsal positions in the embryonic fate map: ventral denticle belts, filzkörper, and dorsal hairs, respectively (25). In contrast, embryos derived from tub^1/tub^2 females gastrulate symmetrically (13), as evidenced by the circumferential presence of dorsal folds (Fig. 1*C*), and secrete a twisted and hollow tube of dorsal cuticle (Fig. 1*D*). This recessive maternal-effect lethal phenotype is common to all dorsal group mutants (4).

At 25°C females heteroallelic for a tube allele, tub^4, and a deletion that fails to complement tube, *Df(3R)XM3*, produce embryos that develop filzkörper, whereas embryos from tub^4/tub^1 or tub^4/tub^4 females develop some ventral denticles in addition to filzkörper. Thus the tube alleles tub^1 and tub^4 exhibit partial loss-of-function maternal phenotypes and we conclude that the strongly dorsalized maternal phenotype of embryos derived from tub^1/tub^2 females does not require a null genotype. Temperature-shift experiments further reveal that the weak maternal phenotype of tub^4/tub^1 is cold sensitive. For example, at 25°C all embryos from tub^4/tub^1 females develop ventral denticle belts in addition to filzkörper, whereas at 18°C 80% of these embryos develop only filzkörper.

Zygotic tube Phenotype. Although the tube gene was identified as a maternal-effect locus (13), we found that tube mutations also have a zygotic effect, altering pupal morphology. Pupae homozygous for strongly dorsalizing tube alleles were noticeably shorter than wild-type pupae. This squat phenotype, unlike that of the dominant Tubby locus (26), could not be scored in crawling larvae. The phenotype was, however, readily distinguishable at the white prepupal stage.

The squat tube phenotype was quantitated by measuring pupal dimensions; the axial ratio (length/width) was significantly reduced in *tub/tub* pupae. For example, in a cross between tube heterozygotes, *tub/+* pupae had an axial ratio of 3.0, whereas the axial ratio of *tub/tub* pupae was only 2.5 (Table 1). In addition, we noted an allele-dependent gradation of zygotic phenotype that correlated with the strength of the maternal-effect phenotype; in particular, pupae heteroallelic for the partial loss-of-function tub^4/tub^1 combination exhibited wild-type pupal morphology.

When other dorsal group mutants were tested, pupae carrying recessive dorsalizing alleles of pelle or Toll were also found to have significantly reduced axial ratios. A zygotic phenotype had not been described previously for pelle; Toll null allele combinations had been shown to produce zygotic semilethality (27). The axial ratios of pupae carrying strongly dorsalizing mutations in any of four dorsal group genes that appear to act extracellularly (12, 28, 29), nudel (*ndl*), snake (*snk*), gastrulation defective (*gd*), and pipe (*pip*), were indistinguishable from wild-type values (see Table 1). In contrast, cactus mutations, which have a ventralizing rather than dorsalizing maternal effect, yielded significantly increased axial ratios, in the range of 3.2–3.4 (S.A., unpublished data).

Genetic and Physical Mapping of the tube Locus. The tube gene had been mapped by recombination to the central region of the third chromosome (13, 15). To determine on which side of the centromere the locus lies, we generated six compound third chromosomes consisting of two attached copies of either the left (3L) or right (3R) arm of a tube-bearing chromosome. The tube maternal effect segregated only with the newly generated four compound 3R chromosomes, thereby placing the tube locus to the right of the centromere. Synthetic deficiencies constructed from segmental aneuploid stocks (19) and stable deficiencies generated in proximal 3R were tested for their ability to complement tube. At the cytological level, the tube-containing interval was defined by *Df(3R)XM3* and further delimited by the distal breakpoint of *Df(3R)A321R1* and the proximal breakpoint of *Df(3R)Z-1*; the tube gene thus lies in the 82A4-6 polytene interval (Fig. 2).

Chromosomal Walk to the tube Locus. The *P*-element insertion *l(3)ry147* at the 82A-B border was used to initiate a chromosomal walk to the tube gene. Overlapping cosmid clones were isolated that extend 170 kilobases (kb) proximally from the insertion site. Fragments from throughout this

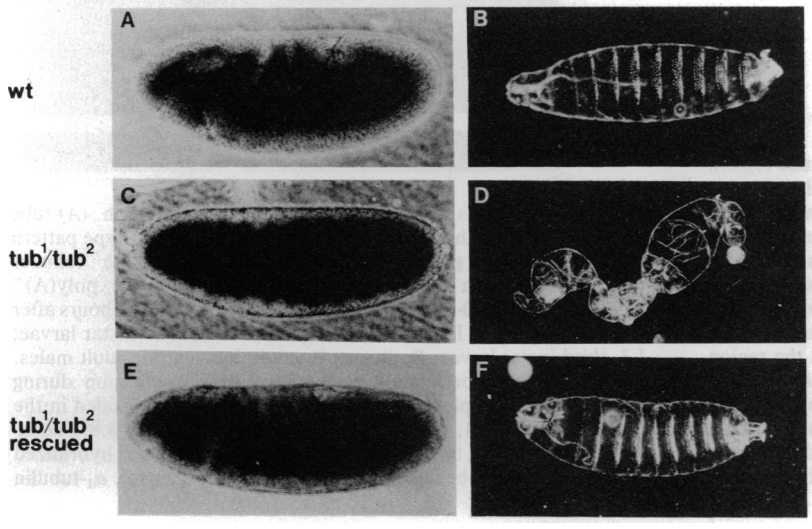

FIG. 1. Maternal effect phenotype of wild-type, tube, and rescued embryos. Embryos are oriented anterior left and dorsal up. Gastrulating wild-type embryos exhibit an asymmetric pattern of folds, furrows, and invaginations (*A*); wild-type larvae are characterized by a detailed array of cuticle elements (*B*). Embryos of maternal genotype tub^1/tub^2 are strongly dorsalized in the pattern of cell movements at gastrulation (*C*) and in the array of cuticle elements displayed by unhatched embryos (*D*). Microinjection of tub^1/tub^2 embryos with RNA at 10 μg/ml produced by transcription *in vitro* of the pNB448 cDNA results in a wild-type gastrulation pattern (*E*) and the reappearance of dorsolateral cuticle elements. Microinjection of tub^1/tub^2 embryos with pNB448 at 200 μg/ml transcripts also results in a completely wild-type gastrulation pattern and yields hatching larvae (*F*).

Table 1. Zygotic phenotype of dorsal group genes

Genotype of pupae	Axial ratio	Number of pupae
st e/st e	2.9 ± 0.1	60
tub/tub	2.5 ± 0.1	77
tub/+	3.0 ± 0.1	154
pll/pll	2.4 ± 0.1	59
pll/+	2.9 ± 0.1	196
Tl/Tl	2.5 ± 0.1	80
Tl/+	2.9 ± 0.1	90
gd/gd	2.9 ± 0.1	54
gd/+	3.0 ± 0.1	170
ndl/ndl	2.9 ± 0.1	62
ndl/+	2.9 ± 0.1	92
pip/pip	2.9 ± 0.1	94
pip/+	3.0 ± 0.1	69
snk/snk	2.9 ± 0.1	64
snk/+	3.0 ± 0.2	112

Pupae were measured for a *st e* stock and for the dorsalizing genotypes *tub2/Df(3R)XM3,pll^{2}/pll^{7},Tl^{r444}/Tl^{r444}, gd^{2}/gd^{2}, ndl^{046}/ndl^{169}, pip^{386}/pip^{664}*, and *snk^{073}/snk^{229}*. The (+) balancers were *FM3* and *TM3* or *TM8*. For pairs of alleles results were pooled for the two balanced classes, except that for pipe only the *pip^{386}/+* class was recovered and scored. Axial ratio data are expressed as mean ± SD.

walk were then used as probes in Southern blot hybridizations to identify the proximal breakpoint of *Df(3R)Z-1* and the distal breakpoint of *Df(3R)A321R1*. At the molecular level, the deficiencies delimit a 17-kb region that contains the tube gene (Fig. 2).

Identification of tube cDNA by a Microinjection Assay of Phenotypic Rescue. To enable us to identify the tube transcript within the 17-kb interval defined by deficiency breakpoints, we first employed a microinjection assay to measure the activity of the gene at various stages of development. Embryos from females lacking function of tube or any of five other dorsal group loci can be rescued phenotypically by transplantation of cytoplasm or poly(A)$^+$ RNA from wild-type embryos (13, 30). Although microinjection of poly(A)$^+$ RNA isolated from several stages of the *Drosophila* life cycle weakly rescued injected embryos from *tub^{1}/tub^{2}* females, reversing to some degree their dorsalized phenotype, only RNA isolated from 0- to 3-hr embryos, third-instar larvae, and adult females completely restored the normal gastrulation pattern (Fig. 3*A*).

Microinjection of 0- to 3-hr embryonic poly(A)$^+$ RNA fractionated on a 15–30% linear sucrose gradient indicated that the active tube RNA was ≈2 kb long (S.A.W. and K. V. Anderson, unpublished data). RNA blot hybridization was employed to screen for developmentally regulated transcripts encoded within the 17-kb region containing the tube gene. A direct correspondence was observed between the level of a 2.1-kb transcript detected by genomic probes f310 and f307 and the tube gene activity profile (Fig. 3). The 2.1-kb transcript was induced ≈20-fold in 0- to 3-hr embryos and 5-fold in third-instar larvae relative to late stage embryos (5–18 hr). This transcript was also abundant in adult females, as expected for a maternal message. *In situ* hybridization experiments with a strand-specific RNA probe revealed that the transcript was uniformly distributed in mature oocytes and precellular embryos (K.O., unpublished data).

By using the genomic fragment f310 as a probe, we isolated a cDNA, pNB448, from a 0- to 4-hr embryonic library (22). RNA transcripts from the cloned cDNA were generated *in*

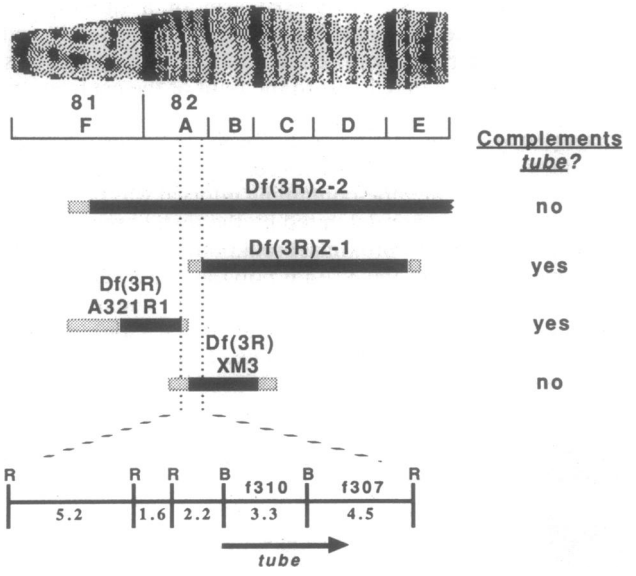

FIG. 2. Cytologic, genetic, and molecular map of the tube region. The polytene chromosome banding pattern near tube is illustrated at the top (proximal left and distal right). Deficiencies within the region are depicted by solid bars; deficiency breakpoint limits are depicted by stippled bars. Whether each deficiency complements or fails to complement tube is indicated to the right. A restriction endonuclease map with lengths in kilobases is shown below for a 17-kb genomic region that extends across the distal and proximal breakpoints of *Df(3R)A321R1* and *Df(3R)Z-1*, respectively, and defines the region that contains the tube locus. *Eco*RI (R) sites, as well as *Bam*HI (B) sites within the 10-kb *Eco*RI fragment, are indicated. Also shown are the identities of the genomic fragments, f310 and f307, that hybridize to the 2.1-kb tube mRNA. The approximate position and the direction of transcription of the tube transcript are indicated by the bold arrow.

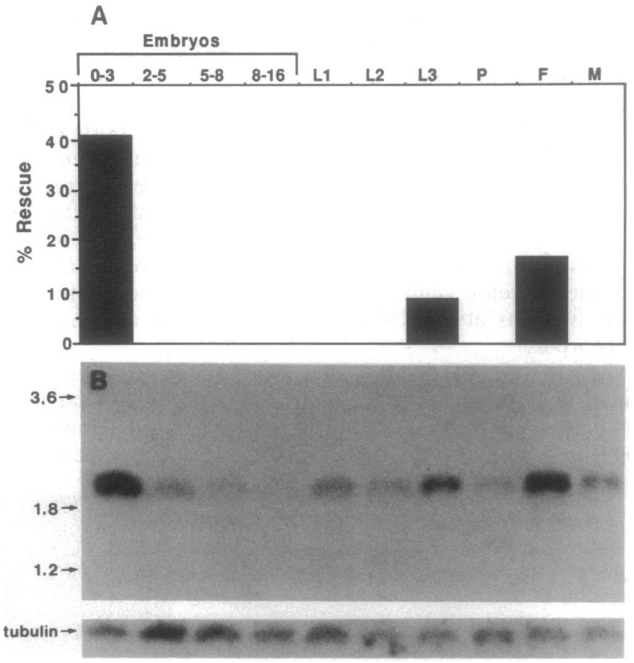

FIG. 3. Analysis of tube activity and gene expression. (*A*) tube activity above the threshold required to produce a wild-type pattern of gastrulation is graphed for embryos from *tub^{1}/tub^{2}* females microinjected with developmentally staged wild-type poly(A)$^+$ RNAs at 2 mg/ml. 0-3, 2-5, 5-8, and 8-16 represent ages in hours after egg laying at 25°C; L1, first-instar larvae; L2, second-instar larvae; L3, third-instar larvae; P, pupae; F, adult females; M, adult males. (*B*) RNA blot hybridization analysis of gene expression during development. Samples (8 μg) of poly(A)$^+$ RNA were loaded in the order depicted in *A*, fractionated by electrophoresis on 0.8% denaturing agarose gels, transferred to nylon membranes, and hybridized to radiolabeled probes specific for tube or for a control, α_1-tubulin (31).

Table 2. Rescue of tube-negative embryos by microinjected SP6 transcripts from pNB448 cDNA

Template	Injected RNA, μg/ml	Number of embryos injected	% embryos strongly rescued at gastrulation	% embryos with muscle contractions	% embryos hatched as larvae
Total library	1000	50	0	0	0
pNB448	1000	50	100	94	48
	200	14	100	64	21
	10	36	94	0	0

Embryos from tub^1/tub^2 females were injected prior to pole-cell formation with transcripts generated *in vitro* from linearized plasmids.

vitro using an SP6 promoter located in the cloning vector and were subsequently assayed for tube activity by microinjection (Table 2). Restoration of wild-type gastrulation and cuticle patterns was observed with injection of pNB448 transcripts at concentrations as low as 10 μg/ml (Fig. 1*E*). Moreover, at RNA concentrations of 200 μg/ml or greater, embryos deficient for maternal tube function could be rescued to hatching (Fig. 1*F*), a degree of rescue never achieved with cytoplasm or total poly(A)$^+$ RNA. These experiments confirmed both that the 2.1-kb transcript hybridizing to the pNB448 cDNA encodes the tube gene product and that this cDNA encodes a protein sufficient to rescue mutant embryos completely.

Sequence Analysis of the tube cDNA. When the nucleotide sequence of the pNB448 cDNA was determined, a single long open reading frame encoding a 50-kDa protein was identified (Fig. 4). The C-terminal half of this protein is notable for the presence of an eight-amino acid motif at five positions (see Fig. 4). The consensus eight-residue tube repeat is defined by six conserved positions: Asn-1, Pro-3, Leu-8, Thr- or Ser-6, and hydrophobic residues at positions 2 and 5. There is no apparent amino acid preference for positions 4 and 7. Both the second and third repeats can be extended by the sequence Ile-Xaa-Asn-Ser-Gly-Asp. If this extension is taken into consideration, a sixth degenerate repeat contained in residues 420–433 can be identified (6/11 matches).

The tube repeat is distinctive. The frequency (1.2×10^{-6}) at which matches to the tube repeat consensus were observed in the NBRF data base (32) was not significantly different from that expected for the random occurrence of the consensus in proteins of average amino acid composition; in no protein was the consensus sequence repeated.

No portion of the tube protein exhibited significant sequence similarity to proteins in the data base. The tube acidic region comprising residues 388–401 resembles a calcium-binding sequence, including the invariant positions Asp-1, Gly-6, and Glu-12 of known helix–loop–helix and related calcium-binding pockets (33); the conserved position 8 hydrophobic residue is, however, absent.

DISCUSSION

Although tube was originally identified as having a maternal-effect lethal phenotype, here we have demonstrated a clear zygotic phenotype: pupae homozygous for strong alleles of tube are squat. Thus the tube gene functions at least twice in *Drosophila* development. Maternal tube activity is required for determining embryonic dorsoventral polarity; zygotic tube functions at the end of larval development. Peaks in tube

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                                                                    ACACTGAAATACT  -180
      GCGAGCAGCGAGCAGTAGCTGTGGGCATTGGCAAAAACATAGAGAGAGAAGATAGATGCAAACATGGTTTTATAGCGGAAATCTCATACA   -90
      ATCGCACTGGACATCTGGGAACTTGAACCATCGACCTGCCACAGGCAAAGGCTTTCCCGTAAGGTTATCCTGGGTAGCTAAGGGAACACC    -1

      ATGGCGTATGGCTGGAACGGATGCGGAATGGGCGTGCAGGTGAACGGAAGCAACGGCGCCATAGGTTTATCCTCGAAGTATTCTCGCAAC    90
    1 M  A  Y  G  W  N  G  C  G  M  G  V  Q  V  N  G  S  N  G  A  I  G  L  S  S  K  Y  S  R  N

      ACGGAGCTGCGACGCGTTGAGGACAATGACATTTACCGGCTTGCCAAAATACTAGATGAGAACTCATGCTGGCGCAAACTGATGTCGATC   180
   31 T  E  L  R  R  V  E  D  N  D  I  Y  R  L  A  K  I  L  D  E  N  S  C  W  R  K  L  M  S  I

      ATACCCAAGGGCATGGATGTGCAGGCCTGCAGCGGAGCCGGATGCTTGAATTTTCCGGCGGAAATCAAAAAGGGATTTAAGTACACTGCG   270
   61 I  P  K  G  M  D  V  Q  A  C  S  G  A  G  C  L  N  F  P  A  E  I  K  K  G  F  K  Y  T  A

      CAGGACGTGTTCCAGATTGACGAGGCTGCAAACCGACTACCGCCGGACCAAAGCAAGTCGCAGATGATGATCGACGAGTGGAAGACCTCT   360
   91 Q  D  V  F  Q  I  D  E  A  A  N  R  L  P  P  D  Q  S  K  S  Q  M  M  I  D  E  W  K  T  S

      GGCAAGCTCAACGAGCGGCCCACGGTTGGGGTATTGCTCCAACTTCTGGTGCAGGCAGAGCTCTTCAGTGCGGCAGACTTTGTGGCACTA   450
  121 G  K  L  N  E  R  P  T  V  G  V  L  L  Q  L  L  V  Q  A  E  L  F  S  A  A  D  F  V  A  L

      GACTTCCTAAATGAGTCCACCCCTGCCCGGCCTGTTGACGGTCCCGGTGCGCTCATAAGCCTTGAGCTGCTGGAAGAGGAAATGGAAGTG   540
  151 D  F  L  N  E  S  T  P  A  R  P  V  D  G  P  G  A  L  I  S  L  E  L  L  E  E  E  M  E  V

      GACAACGAGGGGCTGAGCCTCAAATACCAGTCAAGCACTGCCACTCTTGGGGCAGACGCTCAAGGTAGCGTTGGCCTGAACCTGGACAAC   630
  181 D  N  E  G  L  S  L  K  Y  Q  S  S  T  A  T  L  G  A  D  A  Q  G  S  V  G  L  N  L  D  N

      TTTGAAAAAGACATTGTGAGGCGGGACAAGAGTGTGCCCCAGCCGTCAGGAAACACCCCTCCCATAGCGCCACCTCGACGACAGCAAAGG   720
  211 F  E  K  D  I  V  R  R  D  K  S  V  P  Q  P  S  G  N  T  P  P  I  A  P  P  R  R  Q  Q  R

      AGTACTACTAACTCCAACTTTGCAACATTGACAGGCACCGGAACTACGTCGACGACCATCCCGAATGTGCCCAATCTGACAATTCTCAAT   810
  241 S  T  T  N  S  N  F  A  T  L  T  G  T  G  T  T  S  T  T  I  P  N  V  P  N  L  T  I  L  N

      CCCAGCGAACAAATTCAGGAGCCAGTGCTGCAGCCACGACCGATGAACATCCCCGATCTGTCTATACTCATTTCGAACTCTGGTGATTTG   900
  271 P  S  E  Q  I  Q  E  P  V  L  Q  P  R  P  M  N  I  P  D  L  S  I  L  I  S  N  S  G  D  L

      CGGGCAACTGTGTCGGACAACCCAAGCAACAGAACTAGCTCAACGGATCCGCCTAACATTCCGCGAATCACTCTTTTAATTGATAATTCT   990
  301 R  A  T  V  S  D  N  P  S  N  R  T  S  S  T  D  P  P  N  I  P  R  I  T  L  L  I  D  N  S

      GGAGATGTAAACTCTCGACCAAATCACGCTCCAGCGAAAGCTTCAACGGCTACCACACCAACGGCGAGCTCAAACAACTTGCCAATGATA  1080
  331 G  D  V  N  S  R  P  N  H  A  P  A  K  A  S  T  A  T  T  P  T  A  S  S  N  N  L  P  M  I

      AGCGCCTTGAATATAAGTAAAGGCAGCAAGGAGACCTTGCGTCCTGAGAGCAGAAGCAGCTCCAGCAGCTTGAGCAAAGATGATGACGAT  1170
  361 S  A  L  N  I  S  K  G  S  K  E  T  L  R  P  E  S  R  S  S  S  S  S  L  S  K  D  D  D  D

      GATAACGATGGTGAGGAGGATGGAGAGGAAGAGTACCCAGATGCCTTCTTGCCAAATCTGAGTAACTCAGAGCAGCAGAGCTCAAACAAT  1260
  391 D  N  D  G  E  E  D  G  E  E  E  Y  P  D  A  F  L  P  N  L  S  N  S  E  Q  Q  S  S  N  N

      GACTCCAGCCTGACAACGGTTACTGGCACCAGTGGTGATAACAGCTTTGAGCTAACCAACGACTCCAGCTCCACCTCAAACGACGATTAC  1350
  421 D  S  S  L  T  T  V  T  G  T  S  G  D  N  S  F  E  L  T  N  D  S  S  S  T  S  N  D  D  Y

      GCTTGCAACATTCCAGACTTGAGTGAGCTGCAGCAATAGATAAAAGCACCGACCATCGGCGGAGATGACATTCCGTTCGATAACTTATGC  1440
  451 A  C  N  I  P  D  L  S  E  L  Q  Q  end

      AACCCAGATGTATTCATTTGGCAGCTGGGTGTGGACCTTCTTATGTTATGCATGAGGTCCAACTAGAATCTAAGATTCAGTGCACGATTT  1530
      GTTCATATCACGTCTGTAATCGTGACAGTACATAACAAGTACGGCAATATCAAATATTTCCGTTTCAAGAAAGCCATAACCCAATACCAA  1620
      AATTTAACAAATTTTGTATACTTACGAAATACTCCTTCATTTTCTAGTATAAATTTCCTTAAACGCATAATGGGTTTCTGTGACAGTTTT  1710
      GTCTTGGGACAAACTGCGATCTTATGACATTTTTCGTTATTAATTGATTGTTGCCTAAAATATTTCACAAGTACGATATAATGGCTAAAT  1800
      ACAAATAAAATATTTTTGAAAAAAAAAAAA
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FIG. 4. Nucleotide sequence of the tube cDNA in pNB448 and the deduced amino acid sequence. Nucleotide position is indicated in the right margin; amino acid position is indicated in the left margin. The five tube repeats are underlined.

gene expression that correspond to the times at which maternal and zygotic gene activities are required were observed in 0- to 3-hr embryos and in third-instar larvae.

Our data indicate that the maternal and zygotic tube gene products must encode similar, if not identical, activities. (*i*) The tube cDNA pNB448 hybridizes to a single 2.1-kb RNA, with no discernible size difference between maternal and zygotic transcripts. (*ii*) Microinjection of zygotic poly(A)$^+$ RNA isolated from third-instar larvae can rescue embryos maternally deficient for tube. (*iii*) tube allele strengths are coincident with respect to the maternal-effect and zygotic phenotypes. Similar results have been obtained for the Toll locus (12, 27).

When embryos maternally deficient for tube were injected with transcripts generated *in vitro* from pNB448, hatching larvae were produced, confirming that the pNB448 insert is a tube cDNA. The same approach has been used previously to identify an easter cDNA (16). In injecting embryos of tub^1/tub^2 maternal origin with increasingly dilute SP6 transcripts, we observed a phenotypic gradation similar to that seen among the various tube alleles. Thus, for tube, as for several other dorsal group loci (4), activity, whether titrated genetically or biochemically, is proportional to the degree of shift in the fate map.

The deduced tube polypeptide sequence is consistent with the hypothesis that the tube protein is intracellular. Like dorsal (9), but unlike the three other sequenced dorsal group genes (12, 16, 29), tube encodes neither a transmembrane domain nor a signal sequence directing protein secretion. Injection assays suggest that pelle also acts in the intracellular compartment of the embryo, since cytoplasmic pelle rescuing activity, like that of dorsal (34), becomes ventrally localized in 2- to 3-hr embryos (30).

If positional signals are transmitted from Toll to dorsal through localized pelle activity, the tube protein may facilitate pelle activation or promote association of pelle with other components of the signaling pathway. The hypothesis that tube and pelle act coordinately is supported by the observation that both genes mutate to cold sensitivity, whereas cold-sensitive phenotypes have not been observed for other dorsal group mutants (4). If a modified form of the tube protein itself encodes positional information for dorsal protein import, the activation must be transient or labile, since microinjection experiments have not revealed any localization of tube rescuing activity (13).

The fact that tube, pelle, and Toll share a zygotic, as well as maternal, phenotype indicates that their function is not limited to the syncitial environment of the early *Drosophila* embryo and suggests that the products of these genes act in concert during both embryonic and post-embryonic development. Given that the transcription factor NFκB has extensive sequence similarity with dorsal and that the nuclear translocation of both proteins is regulated by signals communicated through a transmembrane receptor, it is likely that proteins similar to tube, pelle, and Toll will be found to participate in vertebrate signaling pathways.

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