Developmental Biology **212,** 440–454 (1999)
Article ID dbio.1999.9351, available online at http://www.idealibrary.com on IDEAL®

Member of the Neurotransmitter Transporter Family Required for Organisation of the Apical Cytocortex

Kevin Johnson,*,† Elisabeth Knust,† and Helen Skaer*,[1]

**Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, United Kingdom; and †Institut für Genetik, Heinrich-Heine Universität Düsseldorf, Universitätsstrasse 1, 40225 Düsseldorf, Germany*

We have identified a novel member of the vertebrate sodium- and chloride-dependent neurotransmitter symporter family from *Drosophila melanogaster*. This gene, named *bloated tubules (blot),* shows significant sequence similarity to a subgroup of vertebrate orphan transporters. *blot* transcripts are maternally supplied and during embryogenesis exhibit a complex and dynamic pattern in a subset of ectodermally derived epithelia, notably in the Malpighian tubules, and in the nervous system. Animals mutant for this gene are larval lethals, in which the Malpighian tubule cells are distended with an enlarged and disorganised apical surface. Embryos lacking the maternal component of *blot* expression die during early stages of development. They show an inability to form actin filaments in the apical cortex, resulting in impaired syncytial nuclear divisions, severe defects in the organisation of the cortical cytoskeleton, and a failure to cellularise. For the first time, a neurotransmitter transporter-like protein has been implicated in a function outside the nervous system. The isolation of *blot* thus provides the basis for an analysis of the relationship between the function of this putative transporter and epithelial morphogenesis.

***Key Words: Drosophila; bloated tubules (blot);* cellularisation; actin cytoskeleton; Malpighian tubules; neurotransmitter transporter.**

INTRODUCTION

The renal or Malpighian tubules of insects have proved to be an important model system for the analysis of both the development of epithelial tissues (Skaer, 1993, 1996) and the physiological activity of the mature transporting epithelium (Maddrell, 1981, 1991; O'Donnell *et al.*, 1996). Their function consists of the excretion of toxic waste products and the adjustment of ionic and osmotic balance of the body fluids (Wigglesworth, 1939).

In *Drosophila*, the embryonic development of the Malpighian tubules can be subdivided into distinct steps, which allows us to dissect the genetic and cell biological requirements for each of them. At the extended germ band stage, two pairs of primordia evaginate at the border between midgut and hindgut. Within each tubule primordium one cell is singled out by lateral inhibition from an equivalence group (Hoch *et al.*, 1994). This so-called tip cell controls the proliferation of its neighbours as the tubule grows (Skaer, 1989, 1992). Later, it expresses markers diagnostic of neural cells and forms close contacts with an A9 nerve branch (Hoch *et al.*, 1994). Further morphogenesis of the tubules includes an increase in polyteny and further growth by extensive cell rearrangement. The tubules persist through metamorphosis into adulthood.

The circumference of the tubule is occupied by two cells, the apical sides of which face the lumen and which are characterised by a highly organised cytoskeleton and well-elaborated microvilli (see Skaer, 1992, 1993, for reviews).

Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under Accession No. AJ243264.

[1] To whom correspondence should be addressed at current address: Developmental Genetics Programme, Krebs Institute, University of Sheffield, Firth Court, Western Bank, Sheffield S10 2TN, UK. Fax: 0114 222 2788. E-mail: h.b.skaer@sheffield.ac.uk.

0012-1606/99 $30.00

Secretory activity becomes obvious at late stages of embryogenesis by the deposition of uric acid in the tubule lumen. Taken together, the characteristics of this simple epithelium facilitate the analysis of problems applying to epithelial development in general, such as the control of cell proliferation and cell shape, the establishment of cell polarity, morphogenetic movements, and differentiation into a transporting epithelium.

This paper describes the isolation, molecular characterisation, and expression pattern of a novel gene, identified by P-element enhancer trapping to be expressed in the Malpighian tubules. We show that mutations in *bloated tubules (blot)* have a profound impact on the organisation of the apical membrane domain in the tubules. We also show an essential requirement for *blot* in the organisation of the cortical cytoskeleton during the syncytial blastoderm and cellularisation. Blot encodes a member of the 12-membrane-pass transporter superfamily exhibiting sequence similarities with a group of orphan neurotransmitter symporters. We present the pattern of *blot* expression which is the first putative neurotransmitter transporter expressed outside the CNS in *Drosophila.*

MATERIAL AND METHODS

Fly Stocks, Reversion Screens, and Germ-Line Clones

Fly culture and crosses were carried out according to standard procedures (Ashburner, 1989). The homozygous viable starter strain carrying the ry^{506} *P[lArB]A434* chromosome (*A434*) was generated by Bellen *et al.* (1989). The lethal P-element line *P{*ry^{+} *=PZ} l(3)01658* ry^{506}*/TM3,* ry^{RK} Sb^{1} *(l(3)1658)* was generated by Spradling *et al.* (1995). Deficiencies used include *Df(3L)st7 e/TM3 Sb e* (73A3; 74A3-B1) (Ashburner *et al.*, 1980) and *Df(3L)81K19 st tra/TM6B* (73A3; 74F1-F4) (James and Collier, 1992), *Df(3L)st-E23 st tra/TM3 Sb* (72E1-E5;74D1-D5) (Nöthiger *et al.*, 1989). Their breakpoints were confirmed on polytene chromosomes. In order to demonstrate that the lethal phenotype of *l(3)1658* is caused by the insertion of the PZ element, we mobilised the P element by hybrid dysgenesis using Δ2-3 as a source of transposase (Robertson *et al.*, 1988). Revertant *ry506* chromosomes, which complemented *l(3)1658* and *Df(3L)st-E23,* were analysed by Southern blot and showed restoration to the wild-type genomic arrangement in the vicinity of the original PZ insertion site (data not shown). Genomic deletions of adjacent sequences were induced by imprecise excision of the P-element insertion from the enhancer trap line *A434.* In order to enhance the stability of imprecise excision events, hybrid dysgenesis was initiated over the deficiency *Df(3L)st-E23,* thus preventing any possible gap repair by gene conversion (Engels *et al.*, 1990).

blot germ-line clones were generated using the FLP-DFS technique (Chou and Perrimon, 1996). For this, one of the alleles obtained by the reversion screen, $blot^{M55}$, and the lethal P-element-induced allele $blot^{1658}$ were recombined onto the $D\ FRT^{3L-2A}$ chromosome. Females carrying germ-line clones were mated to males heterozygous for the original mutant stock in order to assay for paternal rescue.

Molecular Techniques

Genomic DNA adjacent to the insertions was obtained by plasmid rescue of the PZ element (Mlodzik and Hiromi, 1992) and the P[lArB] element (Wilson *et al.*, 1989) using an *Xho*I digest of genomic DNA prepared from flies of the *A434* insertion line and an *Xba*I digest of genomic DNA prepared from the *l(3)1658* insertion line. A genomic 2.0-kb *Xho*I/*Hin*dIII fragment flanking the P element of the *A434* line was used to screen a *Drosophila* genomic λ phage library (provided by S. Russell) according to Sambrook *et al.* (1989). *In situ* hybridisations to polytene chromosomes were done according to Ashburner (1989). The transcriptional activity of the individual fragments of the genomic walk was analysed by whole-mount *in situ* hybridisations performed according to Tautz and Pfeifle (1989). A 5.8-kb *Xho*I insert fragment from genomic phage λ 3.3 was used to screen the λ cDNA library E6, made from poly(A)$^{+}$ RNA of 3- to 6-h embryos (Poole *et al.*, 1985). One of the cDNAs obtained, the 2.1-kb cDNA E1, was subsequently used to probe Northern blots containing poly(A)$^{+}$ RNA of different developmental stages and to screen the *Drosophila* 4- to 8-h embryonic cDNA library pNB40 (Brown and Kafatos, 1988). Two of the cDNAs, E1 (1–2036 bp) and NB7 (699–4017 bp) overlap and represent the full-length transcript of *blot*. Both cDNAs were sequenced on both strands (Sequenase 2.0 Kit; USB). The *blot* cDNA sequence was analysed using the Staden (R. Staden, Medical Research Council Laboratory of Molecular Biology, Cambridge, UK) and the Genetics Computer Group, Inc., software packages and the DNAstar software package (DNASTAR, Inc.). A FastA 3.08 alignment (Pearson and Lipman, 1988) set at default parameters was used to establish the alignment with proteins of the Swiss PROT "all" library.

Immunocytochemistry and β-Galactosidase Detection

Embryos were stained either as whole mounts or after dissection (Bate, 1990). Ovaries were dissected from 3- to 5-day-old females in Ringer (Roberts, 1986), the peritoneal sheath was slit, and the ovarioles were teased apart to allow access of reagents. Antibody staining and β-gal detection were carried out according to conventional protocols (Ashburner, 1989). We used X-Gal (Genetic Society of America) to assay for β-gal activity and rabbit anti-β-gal antibody (Cappel; used at 1:10,000) to reveal patterns of β-gal expression. Fixation and labelling of nuclei and the cytoskeleton were according to Theurkauf (1994). For the detection of F-actin, embryos were formaldehyde fixed (36%), hand devitellinised (von Dassow and Schubiger, 1995), and incubated with fluorescently labelled phalloidin (Molecular Probes) diluted 1:1000 in PBS/0.1% Triton X-100 (PBT). Nuclei were visualised using propidium iodide (Molecular Probes) at a concentration of 200 ng/ml in PBT or using TOTO-3 (Molecular Probes) diluted 1:3000 in PBT. The microtubule cytoskeleton was stained using a mouse anti-α-tubulin primary antibody (Sigma) diluted 1:800 in PBT/5% normal horse serum and a donkey anti-mouse IgG secondary antibody coupled to Cy3 (The Jackson Laboratory). Microscopical analysis was done on a Zeiss Axiophot2 supplied with Nomarski optics. Laser-scanning microscopy was performed using a Leica TCS NT confocal imaging system and pictures were mounted using Adobe PhotoShop 4.0 and Canvas 5.0.

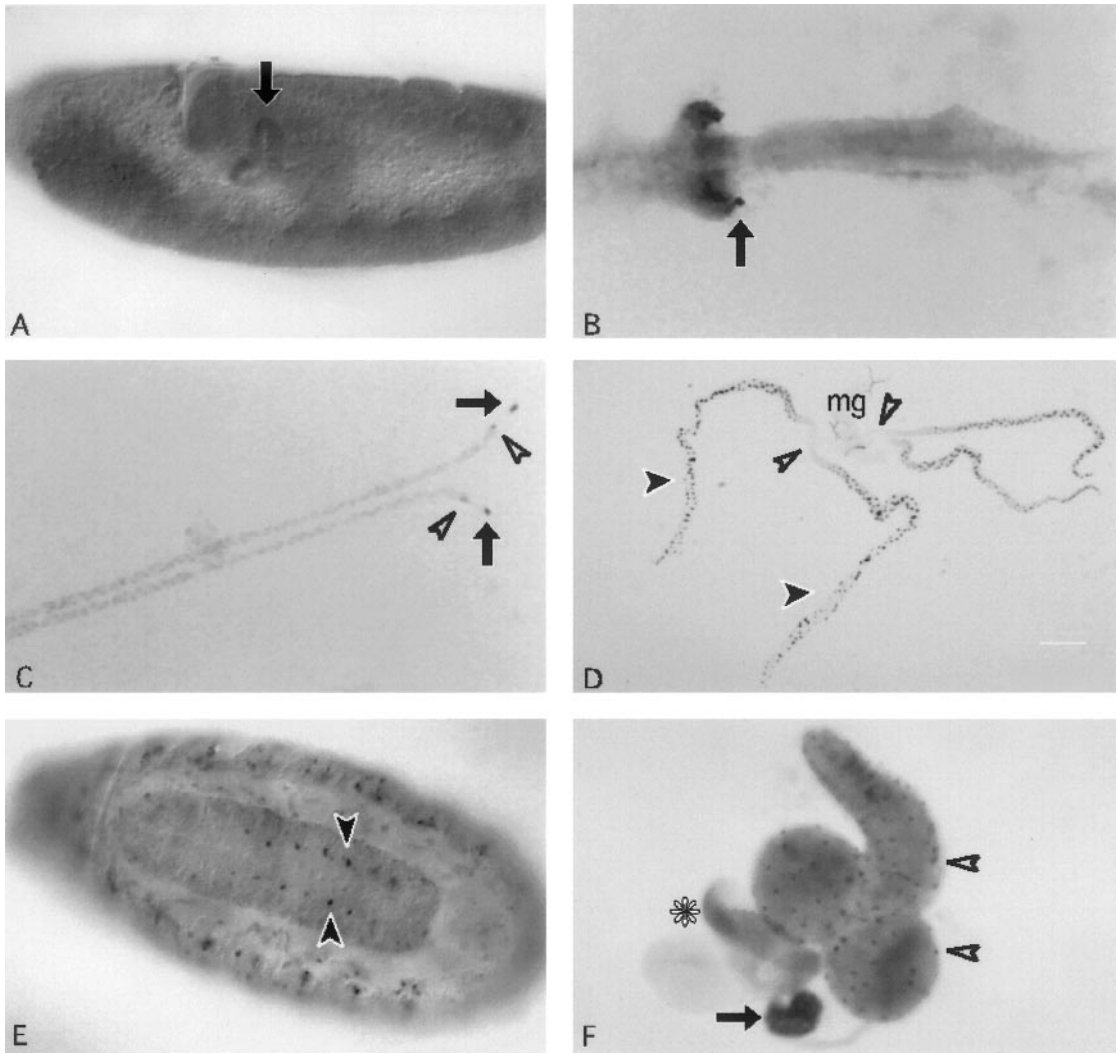


FIG. 1. β-Galactosidase expression in embryos and larvae of isolated enhancer trap lines. β-Galactosidase expression in lines *l(3)1658* (A, E) and *A434* (B, C, D, and F) revealed by immunocytochemical staining. (A) Staining in the Malpighian tubule primordia (arrow) of a stage 11 embryo. (B, C) Dissected preparations from stage 12 (B) and stage 15 (C) embryos to show the restriction of β-galactosidase expression from the tubule primordial cells into the tip cells (arrow) and their siblings (open arrowheads). (D) β-Galactosidase is present in all four Malpighian tubules in third-instar larvae (anterior tubules indicated by arrowheads). The ureters do not stain (open arrowheads). mg: midgut. (E) Stage 16 embryo shows stained cells in the ventral nerve cord (arrowheads). (F) Expression in the CNS and associated structures of a third-instar larva, including the ring gland (arrow) and eye-antennal disc (asterisk). Single cells on the surface of the brain lobes and ventral nerve cord express β-galactosidase (open arrowheads). (A) Lateral view, (E) ventral view. Stages according to Campos-Ortega and Hartenstein (1997).

RESULTS

Genetic and Phenotypic Analysis of blot

Our screen of enhancer trap lines with expression in the developing Malpighian tubules yielded two insertion lines, *l(3)1658* (Spradling *et al.*, 1995) and *A434* (Bellen *et al.*, 1989), which show very similar patterns of reporter gene expression. These lines are of particular interest because early *lacZ* expression throughout the tubule primordia, as they evert from the hindgut (Fig. 1A), is subsequently restricted to the tip cells and their siblings as they become apparent at the distal end of each Malpighian tubule (Figs. 1B and 1C). The *l(3)1658* insertion line is a late larval lethal (Spradling *et al.*, 1995). Homozygous animals are delayed in

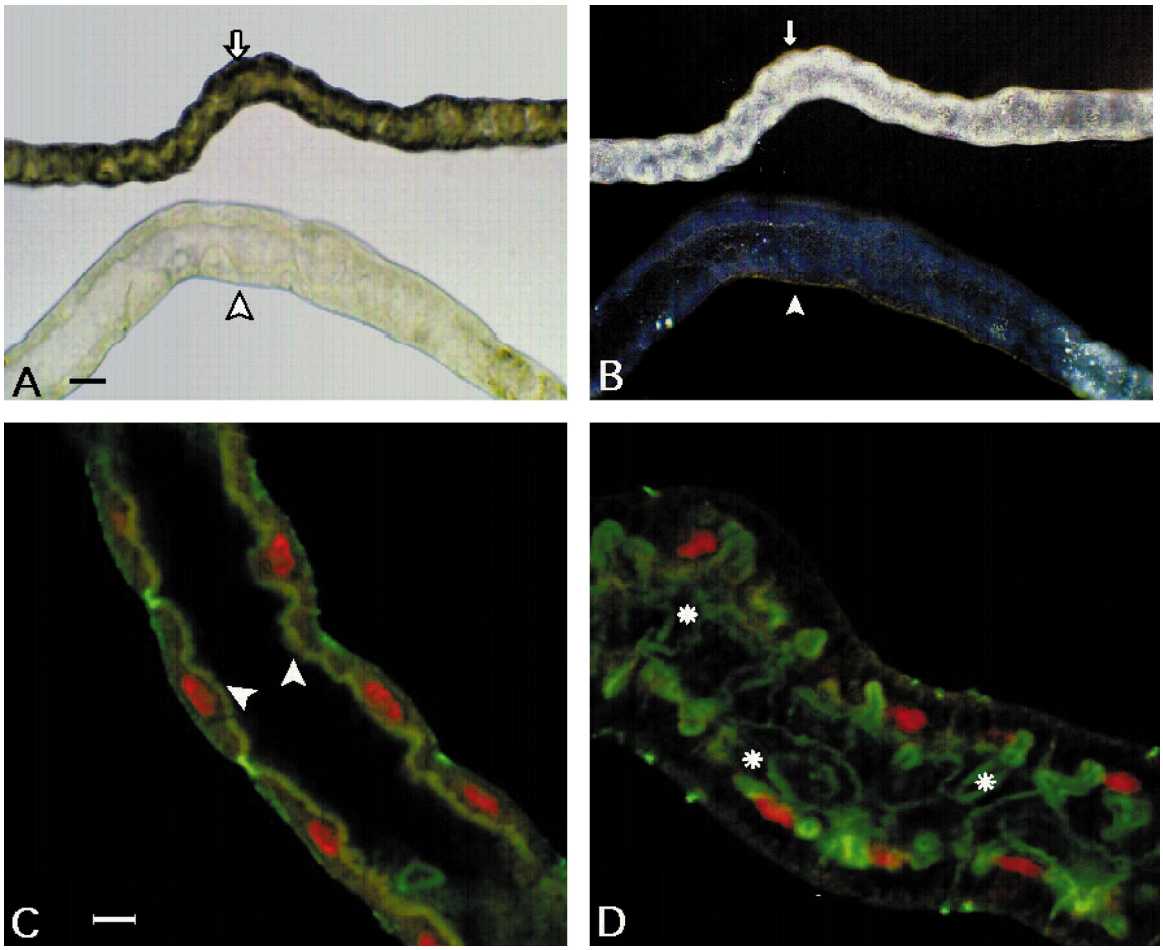
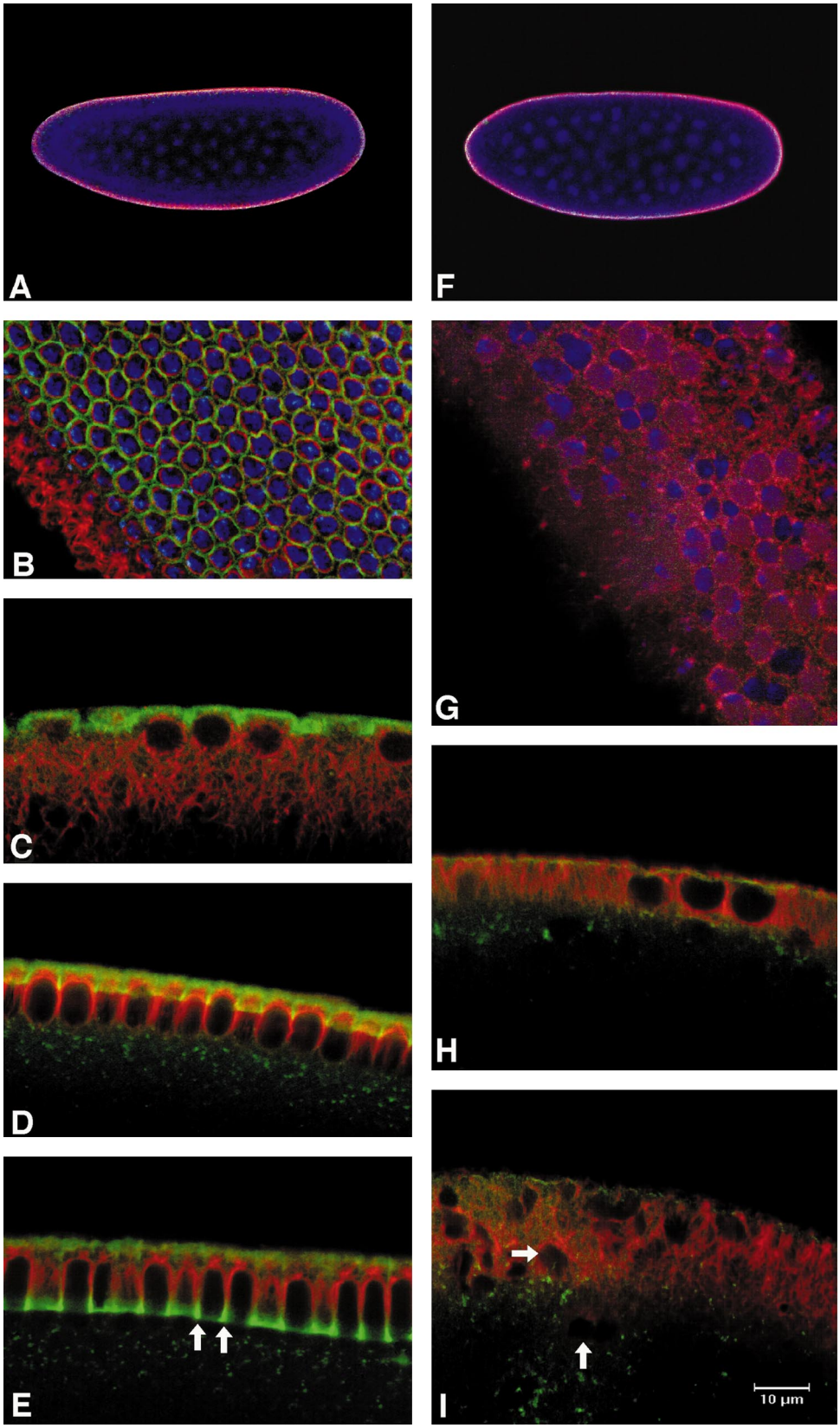


FIG. 2. Malpighian tubules of wild-type and *blot* mutant larvae. (A, B) Bright- and dark-field images of dissected wild-type (arrows) and $blot^{1658}$-mutant (arrowheads) tubules. Note the bloated appearance of the mutant tubules and the reduction in fluorescent intracellular concretions. (C, D) Confocal images of dissected, third-instar Malpighian tubules stained with phalloidin (F-actin, green) and propidium iodide (nucleic acids, red). (C) Detail of a wild-type main segment. The apical brush border (arrowhead) encircles the lumen of the tubule and shows concentrated F-actin staining in the microvilli. (D) Detail of a $blot^{1658}/blot^{1658}$ main segment. The cells appear swollen and extend apical projections (stars) outlined by phalloidin staining, into the lumen. Scale bars, 50 μm (A, B) and 20 μm (C, D).

their development but reach the mid/late third instar before development is arrested. The lethality could be reverted by excision of the P element, suggesting that the chromosome does not carry other zygotic lethal mutations.

Genetic analysis of the 74B region. *l(3)1658* was mapped to 74B on the left arm of the third chromosome (Spradling *et al.*, 1995; this work). It complements *Df(3L)st7*, placing *blot* proximal to 74A3-B1, the proximal breakpoint of this deficiency. Noncomplementation was found with *Df(3L)st-E23* and *Df(3L)81K19*. Animals hemizygous for *l(3)1658* over *Df(3L)st-E23* or *Df(3L)81K19* show premature arrest in growth during the second larval instar and subsequently die, indicating that the insertion of the PZ element in *l(3)1658* created a hypomorphic allele of *blot* (therefore designated $blot^{1658}$). Neither hemizygous nor homozygous $blot^{1658}$ larvae show any overt morphological abnormalities other than the Malpighian tubule phenotype described below and a slight thickening of the cuticular denticles.

In order to create stronger alleles of *blot*, the P element of the viable *A434* line was dysgenically mobilised over *Df(3L)st-E23* (see Material and Methods). Two chromosomes, named $blot^{M55}$ and $blot^{M51}$, which fail to complement *Df(3L)st-E23*, *Df(3L)81K19*, or the *l(3)1658* chromosome but complemented *Df(3L)st7*, were recovered. Animals hemizygous or homozygous for $blot^{M55}$ or $blot^{M51}$ show second-instar larval lethality and exhibit phenotypes similar to homo- or hemizygous $blot^{1658}$ animals (not



shown). Molecular analysis of these two lines shows that part of the *A434* element remains, suggesting that a small genomic deletion in the vicinity of the P element had been created (data not shown).

Phenotypic analysis of zygotic blot mutants. Wild-type larval Malpighian tubules consist of two pairs of slender tubes which have a yellowish appearance. Their cells are characterised by the inclusion of concretion bodies and a large number of fluorescent substances (Figs. 2A and 2B) (Wessing and Eichelberg, 1978). In *blot* mutant larvae, the overall organisation of the Malpighian tubules is normal. However, they differ from wild type in their bloated appearance (hence the name *bloated tubules*) and a dramatic reduction in the number of cellular concretion bodies (Figs. 2A and 2B).

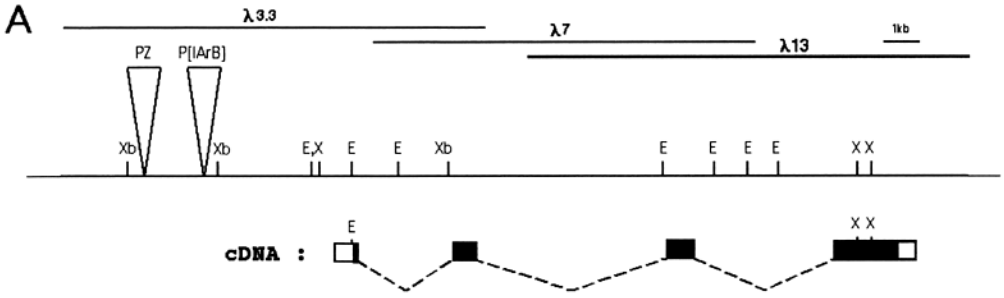
In tubule cells of wild-type larvae the cytoskeleton is strictly organised. Microtubules preferentially align along the basolateral side (data not shown). Actin filaments are highly concentrated in a submembranous region beneath the apical membrane, the cortex, and extend into the microvilli (Fig. 2C). The number of nuclei in the mutant tubules is similar to that of wild-type larvae (not shown) and the epithelium is still formed by a monolayer of cells, which exhibit apicobasal polarity. However, the apical side of the epithelial cells is highly abnormal; extensive folds protrude into the lumen almost obliterating it. Staining for F-actin shows that the folded membrane is still associated with an apical cytocortex, suggesting that microvilli are still present (Fig. 2D).

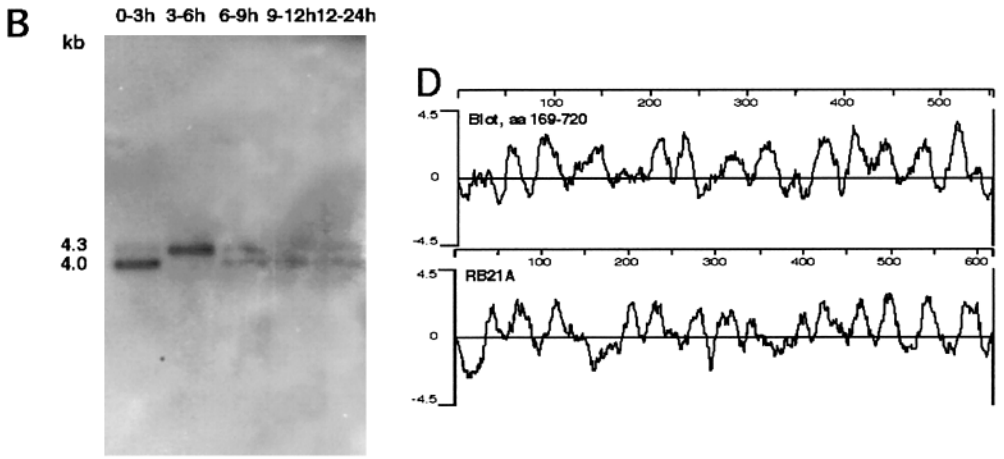
Phenotypic analysis of mutant embryos lacking maternal blot expression. Since *blot* is strongly expressed during oogenesis and its RNA is present in the freshly laid egg (see below), we analysed embryos lacking the maternal component of gene expression. Females with germ-line clones of either $blot^{M55}$ or $blot^{1658}$ show strongly reduced fertility. The embryonic phenotype for both alleles described below is fully penetrant and there is no evidence of paternal rescue.

Wild-type embryos show a dramatic reorganisation of the apical cortex, an actin-rich cortical layer beneath the egg's plasma membrane, on the arrival of nuclear centrosomes during nuclear cycle 10. As a result, evenly distributed actin filaments (Fig. 3A) are concentrated into interphase caps in the newly developing cytoplasmic domains. During the following syncytial mitosis, the actin caps are transformed into mitotic pseudo-cleavage furrows, which surround the spindles at metaphase during mitotic cycles 10–13 (Figs. 3B and 3C). During cellularisation, most of the actin filaments are recruited to the membrane furrow, with the highest concentration at the furrow canals (Figs. 3D and 3E, arrows) (see Warn and Robert-Nicoud, 1992; Foe *et al.*, 1993; Sullivan and Theurkauf, 1995, for reviews).

Embryos lacking maternal *blot* function show an apparently normal pattern of early nuclear division and nuclei migrate to the periphery at the preblastoderm stage (Fig. 3F). Small aggregates of F-actin, dispersed throughout the yolk, are present in numbers comparable to those of wild type (not shown). The earliest morphological defects are a delay in the formation and frequently a reduction in the number of pole cells formed (not shown). As the nuclei reach the periphery, few actin filaments are present and no organisation into F-actin caps or mitotic pseudo-cleavage furrows can be detected (Figs. 3G and 3H). Consequently, the structure of the apical cortex is severely impaired, the nuclei in the cortex lose synchrony of division, and the spacing between adjacent nuclei becomes irregular. Many nuclei display an abnormal shape, frequently fuse, and drop into the interior of the embryo (Fig. 3I). At the time when cellularisation should occur, the recruitment of F-actin normally associated with the developing membrane furrow does not occur and cellularisation completely fails (cf. Figs. 3D, 3E, and 3I). The embryo remains a polynucleate mass, which gradually degenerates, the yolk pushing out to displace the cortical cytoplasm, followed by shrinking of the entire mass and embryonic lethality.

FIG. 3. Cellularisation in wild-type embryos and embryos derived from *blot* germ-line clones. Optical sections of preblastoderm- and blastoderm-stage embryos stained with phalloidin (F-actin, green), anti-α-tubulin (microtubules, red), and TOTO-3 (nuclei, blue in A, B, F, G). (A–E) Wild-type and (F–I) mutant embryos derived from germ-line clones of $blot^{1658}$. (A) Wild-type preblastoderm embryo showing the even distribution of F-actin and microtubules beneath the plasma membrane before the nuclei-associated centrosomes reach the cortical cytoplasm. (B) Wild-type embryo during cellularisation showing the highly stereotyped pattern of microtubules aligned around the interphase nuclei, surrounded by a honeycomb network of F-actin. (C) Nuclear cycle 11. Nuclei show a regular arrangement in the cytocortex. The microtubule network extends several nuclear diameters into the egg. The interphase F-actin caps are in the process of being transformed into pseudocleavage furrows. (D) Slow phase of cellularisation. F-actin filaments are concentrated in caps above the nuclei and recruited to the newly formed cellularisation furrow. (E) Fast phase of cellularisation showing highly elongated nuclei and the strongest concentration of F-actin localised to the contractile rings of the furrow canals (arrows). (F–I) In embryos lacking the maternal component of *blot*, the early pattern of nuclear divisions and migration during the preblastoderm stage seems unaffected (F). However, at the syncytial blastoderm stage filamentous actin in the cortex is almost completely absent, although aggregates, present in the yolk, are still visible (G, H). F-actin caps, pseudo-cleavage furrows, and cellularisation furrows do not form. The early microtubule network extends only about one nuclear diameter into the egg (H). Nuclei lose synchrony, adopt an abnormal shape, and are irregularly arranged (G, I) with many of them dropping into the yolk (I, arrow). B and G, surface views; A, C–E, and F, H–I, sagittal views.





C

```
MSSSYKPRSS DEALAEQFSL ARAGSGTKPP ATPTYRQISG AMATLPRHDN  50
NHNHNDNSSR IRSDSTATSR TCVYIGGAPT GGGNGNEQIS VTLDRRQPQR 100
MSWLNMRRAK ANETDLPTVT PSPQTTRSCI STVSSVVDSG GGRTTATSGR 150
ISSSGIVTLS GSNNTLSEIQ GGYHDMDHDA VSKNFSISAS AHNITTASNA 200
KLLPAVEDES NKQTKCSVFR GLVLCLCLNL SYANVVRFPR ELDRYGSAYL 250
VPYVVLLFLV GLPMVLLEIS VGQFLGQGAA HTWRASPIFK GACMISRFAS 300
WLSAIWVSLQ AVLALAYIGM FASNDLPFRE CAGPVKLRLS GYLLTGTSGQ 350
ECLQLTFLTP FWRNPLYFGL LAAGLIGLWI VVMLCTHNAK ILRRSIPVFG 400
LVGLVLLCTL TGWEVRNSFS RHYFPELWGF DSNLLAESNI WFNALMQVLF 450
SVNCGFGALP MITGKFLYKG DAVRTSVVYL CFNLLINAIA VTLFMVQFDL 500
SSNGFQNMEE LKPLTAIYDR VLNGSREGDS HLLQHLVPSL IYALIILSAM 550
VSITVAVYTS TRLVPRRPNY VICLIGLVVA VISFAAPKFL IARVLDSRLV 600
GTMVVTALVF ELIAITWIYG AKNIYTDLEF SIGRPIFRVW MWLWVICPAI 650
LTGILVWWCA DDDQYDLLAE YLPRWAPILF VLAVIVIIAC VQIFRQVEYN 700
FFGMICEASK PAKEWGPADP LARHSWKQWR SVCQDTGRRD FTLRRRGTRD 750
YTHSIKKGQY SSATKYGVQN GGQTQTPMHQ QHWKSSTPGN SSPNYSGSMF 800
GDSAIEEDIS VDEFPGITQQ QYVPFQASDT KQSRYSQRIR QTAQPQTQTQ 850
MRPPRESVEK HREVVYIRRL SDGGTGTGHA TRIEISPSNE SITYGNGKSN 900
NNYSSAMSRN PLGRSTGCLA APASRRRPAC MSSGRPARWS MLTHTSCHLQ 950
RRPVERRITF AGGSSTSIRK SIPRSCEIRG GPGVGNEAFT FKKTCCIQNK 1000
RICICNPYCI YGTPNYKLRI TNHVHTESVS SSCEL·1035
```

Cloning and Sequence Analysis

A 2.0-kb *Hin*dIII/*Xho*I fragment adjacent to the *A434* insert was isolated and used to initiate a genomic walk of 30 kb from a phage library, as shown in Fig. 4A. Both P elements were found to have inserted into a genomic 2.7-kb *Xba*I fragment, which subsequent analysis indicated to be the 5′ upstream region of *blot*. Whole-mount *in situ* hybridisations using various probes which together covered the entire genomic walk revealed only a single pattern of transcription (described below).

The analysis of several partial cDNAs, isolated from phage and plasmid cDNA libraries, revealed a composite cDNA length of about 4 kb. Two transcripts of 4.3 and 4.0 kb were detected on a Northern blot containing RNA isolated from staged wild-type embryos, the shorter transcript appearing to be predominantly supplied maternally (Fig. 4B). Sequencing of the cDNA clones λE1 and NB7 revealed that they are partially overlapping and together represent a composite cDNA of 4017 nucleotides, which is thus likely to represent the full-length *blot* mRNA. This cDNA contains one long open reading frame (ORF), which is preceded by three in-frame translational stop codons. None of the in-frame initiation codons (AUG) conform precisely to the consensus sequence for *Drosophila* initiation codons (Cavener, 1987). The longest possible ORF predicts a protein of 1035 amino acids (Fig. 4C) with a calculated molecular mass of 114 kDa. A hydrophobicity plot reveals 12 domains of high hydrophobicity, each large enough to harbour a transmembrane domain (TMD) (Fig. 4D). The N-terminus does not contain a readily identifiable signal sequence, suggesting that the large hydrophilic N- and C-terminal regions are located in the cytoplasm. In this putative orientation there are three large extracellular domains (ECD) between TMD3 and TMD4, TMD5 and TMD6, and TMD7 and TMD8 with sizes of approximately 47, 31, and 36 amino acids (aa), respectively (Figs. 4C and 4D).

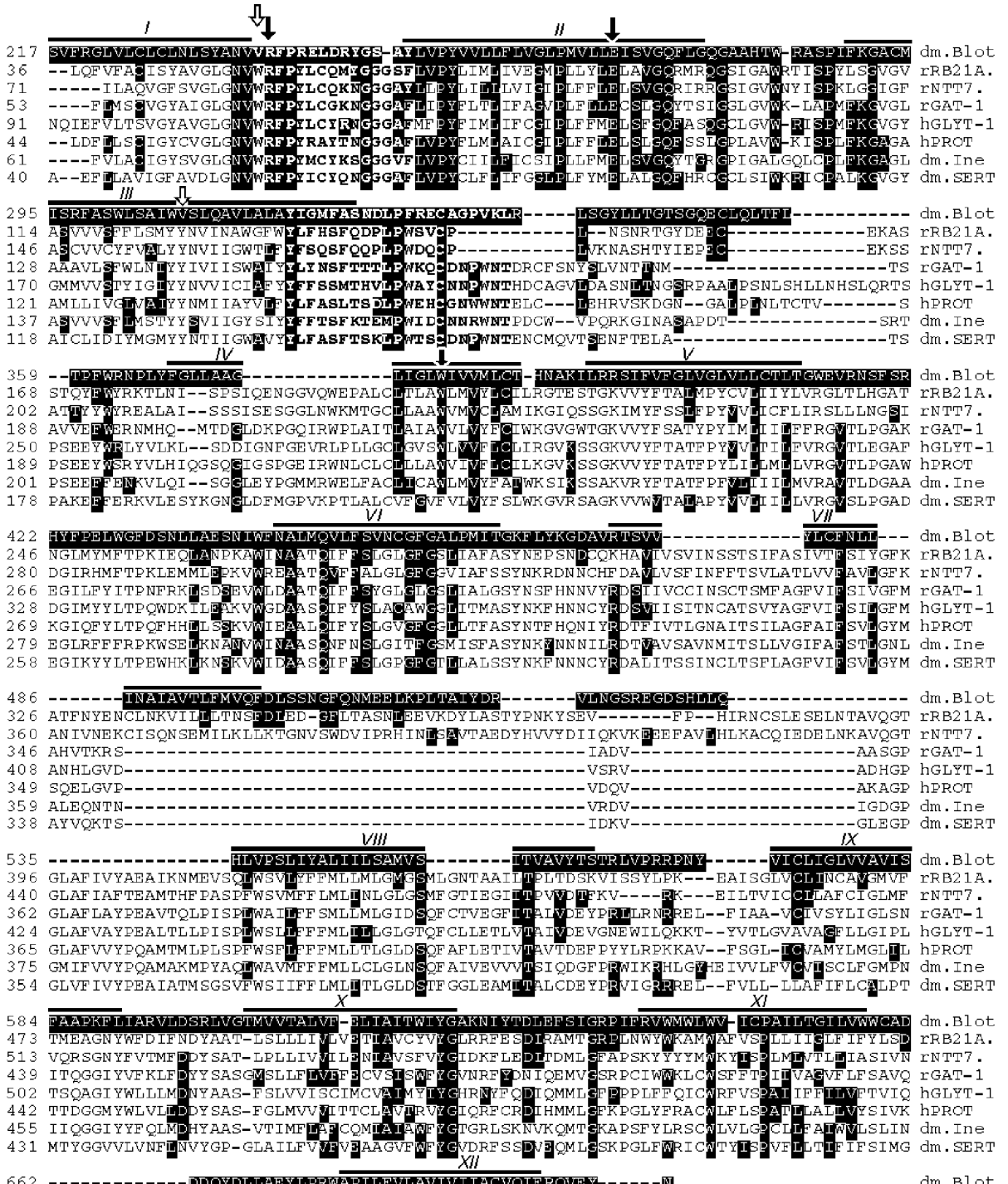
Comparison of the deduced amino acid sequence to peptide sequences in the databases reveals that the central, 12 transmembrane-spanning domains of Blot have significant similarities with a large family of sodium- and chloride-dependent neurotransmitter symporters (Fig. 5). Amongst the more strongly conserved regions are two signatures indicative of this family (highlighted in Fig. 5; Bairoch *et al.*, 1997; Bucher and Bairoch, 1994). The Blot sequence conforms to the Prosite signatures by 66 and 76%, respectively. The 100 most probable alignments of Blot to database sequences are exclusively to sodium- and chloride-dependent neurotransmitter symporter family members. There are no alignments to members of the second subfamily of potassium-dependent neurotransmitter antiporters (Kanai and Hediger, 1992) nor to other 12-transmembrane-domain transporters such as glucose transporters (reviewed in Wright *et al.*, 1993).

The highest probable match was identified between Blot and the rat orphan transporter Rb21A (Smith *et al.*, 1995). There is 28% identity in a stretch of 436 aa from Blot Leu-224 to Asp-661. Taking conserved amino acid exchanges into account, the degree of similarity reaches 64%. In hydrophobicity plots of Blot and RB21A the comparison reveals a striking conservation of both the number of putative TMDs and their arrangement and spacing (Fig. 4D).

Pattern of blot RNA Expression during Development

The spatiotemporal pattern of *blot* expression was analysed by *in situ* hybridisation in wild-type embryos and ovaries. There is expression of *blot* in all nurse cells during oogenesis (Fig. 7A), resulting in ubiquitous staining of early embryos (Fig. 6A). Patterned zygotic expression is initiated transiently in an anterior cap region which expands into a broad band of ectodermal *blot* transcription before cellularisation is complete (Fig. 6B). At gastrulation expression refines to six stripes and a posterior patch in the ectoderm (Fig. 6C). During late stages of germ-band extension, the most prominent element of the *blot* expression pattern is a ring of cells in the amnioproctodeum. This expression is refined to the Malpighian tubule primordia as they evert from the hindgut during stage 11 (Fig. 6D) and later becomes limited to the tip cells, which continue to express

FIG. 4. Molecular analysis of the *blot* locus at 74B. (A) Composite restriction map of 30 kb of genomic DNA in the region of the P-element insertions. Genomic λ phages are indicated. The transcription unit of *blot* is organised into at least four exons covering 17 kb of DNA. E, *Eco*RI; X, *Xho*I; Xb, *Xba*I. Solid lines represent genomic DNA; boxes represent the *blot* cDNA; the longest open reading frame is filled in black. PZ and P[lArB] indicate the insertion sites of the P element of the two enhancer trap lines used. (B) Northern blot analysis of poly(A)$^+$ RNA (10 μg/lane) isolated from staged wild-type embryos and hybridised with cDNA E1. Two transcripts of 4.3 and 4.0 kb, present at different levels of abundance during development, can be distinguished, the shorter of which is more strongly expressed before gastrulation, suggesting maternal origin, while the longer becomes more abundant as zygotic expression is initiated. (C) Amino acid sequence derived from the longest open reading frame of the full-length *blot* cDNA. The 12 putative transmembrane domains are indicated by numbered lines. There are three large putative extracellular domains, ECD I (aa 319–365), ECD II (aa 411–441), and ECD III (aa 499–534) (the precise borders are arbitrary). The potential consensus N-glycosylation site (N523) in the third large extracellular domain is circled. (D) Hydrophobicity plots of the Blot protein (aa 169–720) and the full-length rat orphan transporter RB21A. Note the high structural conservation in the region of the 12 TM domains in terms of the spacing and hydrophobicity profile. The plots are based on the Kyte and Doolittle algorithm (1982), averaging 12 residues at a time.



blot throughout embryogenesis (Fig. 6E). *blot* RNA is also expressed in the posteriormost row of cells in each segment, in segmentally reiterated cells in the CNS, in head sensory structures such as the antennomaxillary complex (Fig. 6F), and in the tracheal system in a subset of the putative tracheal tip cells (not shown) and in the anterior and posterior spiracles (Fig. 6F).

In the wild-type larva, expression of *blot* is reinitiated in all cells of the Malpighian tubules (Figs. 6G and 6H). In contrast, tubules derived from homozygous $blot^{1658}$ animals show no *blot* expression (Figs. 6I and 6K), suggesting that the phenotype observed is due to the absence of *blot* function. We also tested whether the expression of *blot* is abolished in ovaries and embryos derived from $blot^{1658}$ germ-line clones. While wild-type cysts of stage 9–11 show strong *blot* expression (Fig. 7A) and the transcript is found ubiquitously in early embryos (Fig. 7B), there are no *blot* transcripts detectable in $blot^{1658}$ germ-line clone ovaries and embryos (Figs. 7C and 7D) until the paternally introduced $blot^{+}$ allele is zygotically activated (not shown). *In situ* hybridisations of a *lacZ* probe to $blot^{1658}$ germ-line clone ovaries and embryos reflect the wild-type pattern of *blot* expression (Figs. 7E and 7F), indicating that the activity of the trapped enhancer is unaffected.

The activation of *bicoid* transcription during oogenesis and the localisation of its RNA in $blot^{1658}$ germ-line clone embryos are also indistinguishable from wild type, suggesting that the anterior–posterior axis formation remains unperturbed in embryos lacking the maternal component of *blot* (Figs. 7G and 7H).

DISCUSSION

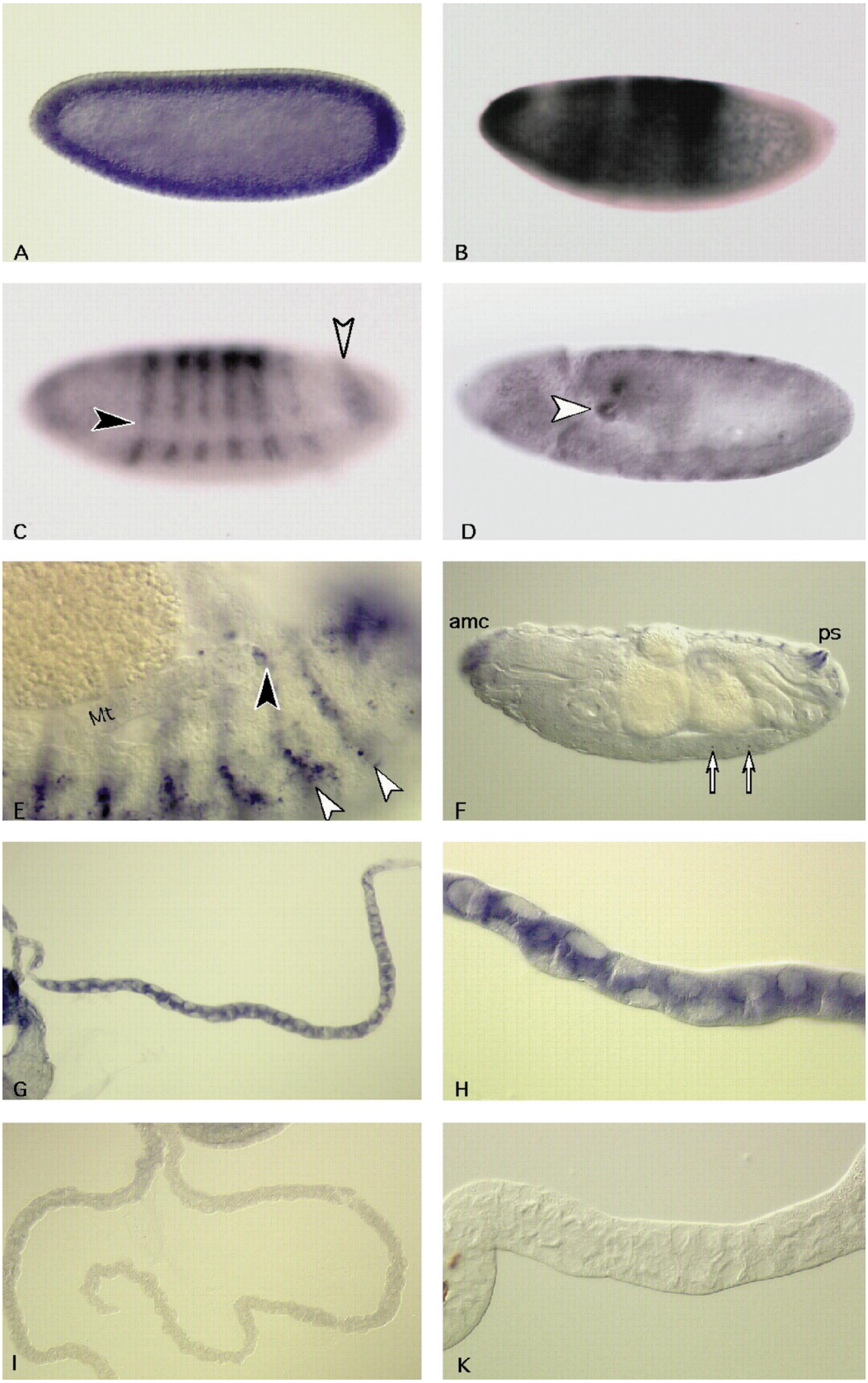
In this paper we describe the isolation and characterisation of a novel *Drosophila melanogaster* gene, *bloated tubules*. The product of this gene exhibits significant similarity with members of the sodium- and chloride-dependent neurotransmitter transporter family, which play important roles in intercellular signalling and vectorial transport. The effects of neurotransmitter transporters are primarily modulatory, such as terminating the process of synaptic transmission by clearing the synaptic cleft of neurotransmitters and thereby additionally retrieving signalling molecules for recycling (Iverson and Kelly, 1975; Uhl and Johnson, 1994). In vertebrates, members of the neurotransmitter transporter family are expressed predominantly in the nervous system but their gene products are also found in epithelial tissues (Guimbal and Kilimann, 1993; Rasola *et al.*, 1995). However, nothing is known about their function in these epithelia.

Analysis of the deduced amino acid sequence of Blot reveals that it has the highest sequence and structural similarities to a subfamily of neurotransmitter transporter proteins for which no substrate has been identified. These orphan transporters include rRB21A, rNTT7, rNTT4, and clone VTA 1732 (Smith *et al.*, 1995; Uhl *et al.*, 1992). Hydrophobicity plots reveal that these proteins have more than one large potential extracellular domain, which may be glycosylated, thus distinguishing them from the other members of the family which show only one large, potentially glycosylated, extracellular domain (e.g., rGAT-1; Bennett and Kanner, 1997).

blot is expressed primarily in epithelial tissues of ectodermal origin and in the nervous system of the embryo and larva. Substantial levels of transcript are also found in the developing oocyte and the freshly laid egg. Lack or reduction of *blot* function during oogenesis results in early arrest of embryonic development. The cuticular phenotype of enhancer trap line *l(3)1658* was previously analysed in a screen for P-element-induced maternal-effect mutations (Perrimon *et al.*, 1996). *l(3)1658* was included in a class of mutations in which germ-line clones produced normal eggs but embryos that fail to produce cuticle.

Ledent *et al.* (1998) identified *l(3)1658* as a P-element insertion reflecting the expression of a bHLH protein target of *poxn* (*tap;* Gautier *et al.*, 1997; Bush *et al.*, 1996). These authors generated a lethal excision ("a large deletion"; Ledent *et al.*, 1998), tap^{74}, which resulted in a reduction of *tap* expression but no discernible morphological phenotype.

FIG. 5. Comparison of the Blot protein with members of the Na^{+}/Cl^{-}-dependent neurotransmitter symporter family. Amino acid sequence comparison of the central regions comprising the 12 transmembrane domains. Each transmembrane region of Blot is indicated by a bar and a Roman numeral. Blot shows closest similarity to a subgroup of orphan symporters (rRB21A and rNTT7), which show two large ECDs, one between TMD III and IV and the other between TMD VII and VIII. The other Na^{+}- and Cl^{-}-dependent neurotransmitter symporters show only one large extracellular domain between transmembrane helices III and IV. Two signatures characteristic of this symporter family are highlighted in bold (Prosite Database). The Blot sequence conforms 66 and 76% to the consensus motifs. The rGAT-1 transporter contains 5 amino acids which were shown to be critical for transport function (W68, R69, E101, Y140, and W222) (Kleinberger-Doron and Kanner, 1994; Keshet *et al.*, 1995; Bismuth *et al.*, 1997). They are indicated by arrows. W68 and Y140 were indicated to be essential for the recognition and/or transport of the substrate γ-aminobutyric acid itself rather than the transport of sodium or chloride ions. The fact that these two residues are not conserved in Blot (open arrows) may indicate a profound structural difference in the substrate transported by Blot. Accession numbers are rRB21A (Q64093), rNTT7 (Q08469), rGAT-1 (P23978), hGLYT-1 (P48067), hPROT (Q99884), dm Ine (Q94917), and dm SERT (U02296). The alignment was performed using the Clustal algorithm in conjunction with a PAM 250 matrix (DNAstar).



tap^{74} does not complement *l(3)1685* or *blot*^{M55} (Ledent *et al.*, 1998; J. Bourne and H.S., unpublished). Comparison of restriction maps for the two genomic regions reveals no overlap and whole-mount *in situ* hybridisations with fragments of the *blot* genomic walk do not stain *tap*-expressing cells (Bush *et al.*, 1996; K. Johnson, unpublished). These data indicate that *tap* and *blot* have distinctive genomic locations. Comparison of the expression patterns of *tap* and *blot* shows that *LacZ* expression in l(3)1658 more closely reflects the expression of *blot;* in particular, *tap* is not expressed in the Malpighian tubules, in the tracheal system, or during oogenesis in the adult (H.S., unpublished). We suggest that the lethality of both *l(3)1685* and *tap*^{74} results from the loss of *blot* function since neither line complements *blot*^{M55} and the Malpighian tubules of *tap*^{74} larvae appear swollen, as we have described for *l(3)1685* (H.S., unpublished).

We have identified *l(3)1658* to be an allele of *blot* and have named it *blot*^{1658}. The phenotypic analysis of embryos derived from *blot*^{1658} germ-line clones described here confirms the importance of the maternal contribution of *blot* (Perrimon *et al.*, 1996); embryos from germ-line clones display a dramatic reduction in the concentration of cortical actin filaments. The resulting absence of pseudo-cleavage furrows is likely to be the cause of the nuclear defects observed. This phenotype is reminiscent of that produced by injection of cytochalasin B into syncytial blastoderm wild-type embryos. This treatment disrupts the monolayered arrangement of cortical nuclei by blocking microfilament organisation. In contrast, blocking microtubule organisation by injection of low concentrations of colcemid prevents the exclusion of yolk from the cleared cytoplasm but has no influence on the monolayered peripheral nuclei (Edgar *et al.*, 1987).

Individual aspects of this phenotype have been described in embryos of the maternal-effect lethal mutations *sponge*, *daughterless-abo-like*, *nuclear fallout*, *grapes*, and *scrambled* (reviewed in Foe *et al.*, 1993; Sullivan and Theurkauf, 1995) and the zygotic genes *nullo* and *serendipity-α* (reviewed in Schejter and Wieschaus, 1993a). Embryos lacking maternal *sponge* gene product fail to assemble actin into the interphase caps and the pseudo-cleavage furrows. Consequently, an irregular spacing of nuclei and a fusion of mitotic spindles can be observed (Postner *et al.*, 1992). In contrast, embryos derived from germ-line clones of *daughterless-abo-like, nuclear fallout,* or *scrambled* show no effect on the actin cap, but exhibit disruption of the pseudo-cleavage furrows (Sullivan *et al.*, 1993). In the case of *daughterless-abo-like* embryos, this phenotype seems to be due to a failure to separate the centrosomes correctly (Sullivan *et al.*, 1990), whereas *nuclear fallout* embryos show a defect in the recruitment of actin from the caps to the pseudo-cleavage furrows (Rothwell *et al.*, 1998). The zygotic genes *nullo*, *bottleneck*, and *α-serendipity* are required for the organisation of the hexagonal actin network during membrane formation. Mutations in any of these genes lead to the formation of multinucleate cells (Postner and Wieschaus, 1994; Schejter and Wieschaus, 1993b; Schweisguth *et al.*, 1990). The fact that embryos lacking *blot* function show defects in several different steps before and during cellularisation suggests either a recurring requirement for its gene product or a requirement as a prerequisite for the organisation of the F-actin cytoskeleton in early embryonic development.

The unifying feature of the early embryonic phenotype of germ-line clones and the larval Malpighian tubule phenotype is a dramatic change in the organisation of the apical cytocortex, although the effects observed are to some extent opposite. While in embryos derived from germ-line clones the F-actin organisation is completely abolished, epithelial cells of the Malpighian tubules apparently exhibit an increase in F-actin. Whether this is the cause or the consequence of the expanded apical surface remains to be elucidated. The phenotypes observed raise the question of how the loss of or reduction in activity of a putative transporter produces the defects observed. We have not yet identified a substrate transported by Blot. One could imagine that the normal intracellular supply of this substrate, by transport across the plasma membrane, is required for the synthesis, polymerisation, or organisation of the cytoskeleton. Alternatively, the morphological changes observed could result, as a secondary effect, from physiological changes produced by alterations in transport. Although data from genetic and cell biological analysis have given some insight into the

FIG. 6. Expression of *blot* transcripts. *In situ* hybridisation of digoxygenin-labelled cDNA fragments to wild-type embryos (A–F) and third-instar Malpighian tubules of wild-type (G, H) and *blot*^{1658} (I, K) larvae. (A) Embryo at nuclear cycle 11 shows weak but even distribution of *blot* transcripts. (B) Stage 5 embryo showing *blot* transcripts in an anterior cap and in three broad stripes in the prospective segmented ectodermal region. (C) Early stage 6 embryo, showing five strong and one weak ectodermal stripes of *blot* expression, which are interrupted in the ventrolateral region (arrowhead). Posterior-lateral patches of staining mark the rim of the amnioproctodeal invagination (open arrowhead). (D) Stage 11 embryo, showing expression in the primordia of the Malpighian tubules (open arrowhead). (E) Detail of a stage 13 embryo showing *blot* expression in the tip cell of an anterior Malpighian tubule (Mt) (arrowhead). Open arrowheads indicate stripes of cells in the epidermis expressing *blot* transcripts. (F) Stage 16 embryo, showing expression in single, segmentally reiterated cells along the ventral nerve cord (open arrows), the antennomaxillary complex (amc), and the posterior spiracles (ps). Anterior is to the left and dorsal is up. (G, H) Malpighian tubule of a wild-type third-instar larvae showing *blot* expression in all tubule cells. (I, K) There is no expression of *blot* in Malpighian tubules derived from homozygous third-instar *blot*^{1658} larvae.

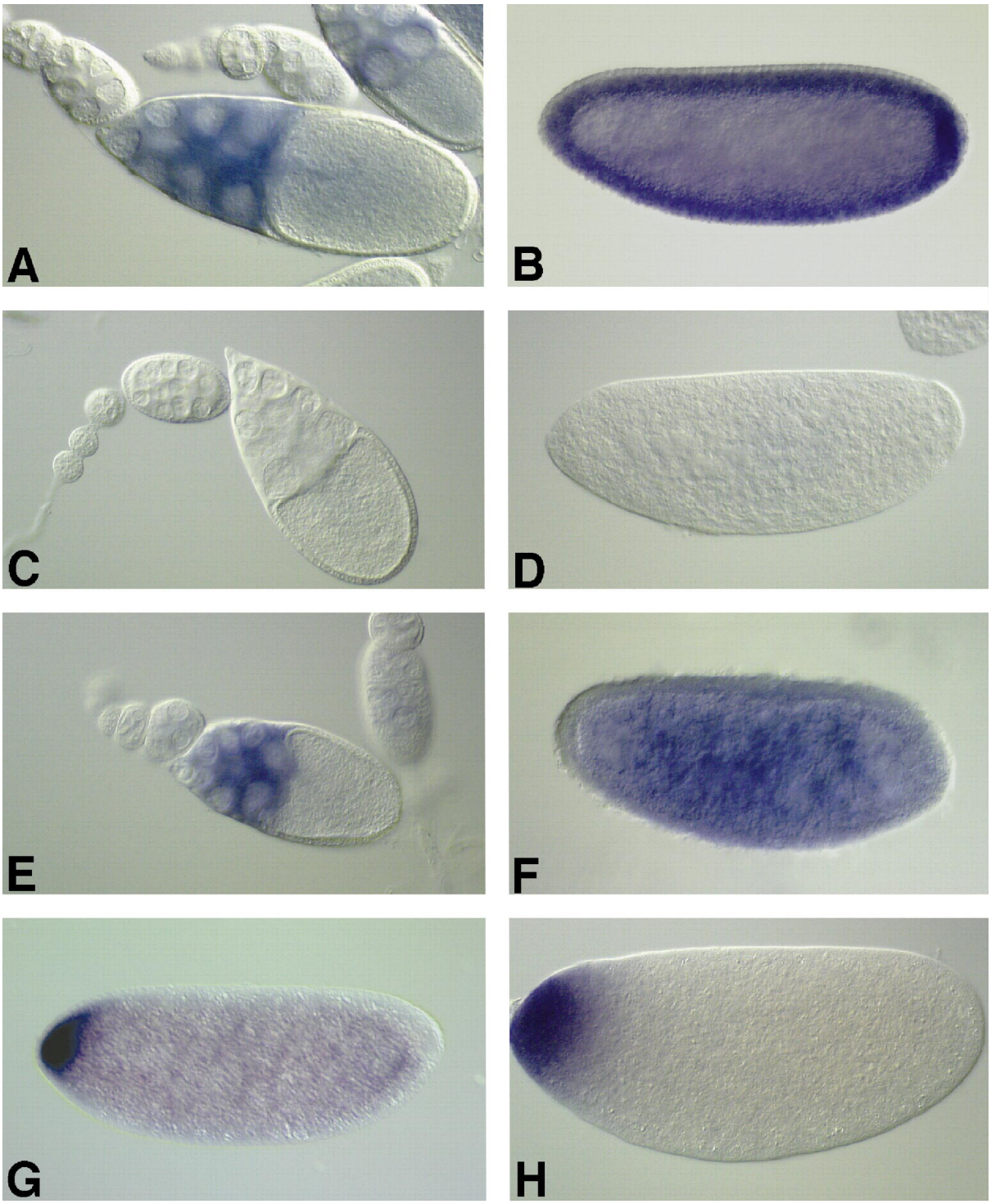


FIG. 7. Maternal expression and early zygotic localisation of *blot*, *lacZ*, and *bicoid* transcripts in wild-type and $blot^{1658}$ germ-line clones. (A, B) Wild-type expression of *blot*. There is expression of *blot* in all nurse cells, which is particularly strong during stages 9–11. Subsequently, *blot* transcripts are evenly distributed in early embryos. (C, D) $blot^{1658}$ germ-line clone ovaries and embryos, processed in parallel, show no expression of *blot*, whereas the transcription of the *lacZ* reporter gene (E, F) in mutant ovaries and embryos remains unaffected. (G, H) Neither expression nor the subsequent localisation of *bicoid* RNA is perturbed in $blot^{1658}$ germ-line clone embryos (H) compared to wild type (G).

molecular components of the apical cytoskeleton, comparatively little is known about the signals and factors that trigger its co-ordinated organisation and modification. Our finding, that a molecule with characteristics of a transporter is required at two different stages of *Drosophila* epithelial development to regulate this important subcellular domain, is an opportunity to study this mechanism in more detail.

ACKNOWLEDGMENTS

We thank Micheal Akam, Nick Brown, and Steve Russell for donating libraries. We are grateful to Arno Müller and Andreas Wodarz for critically reading the manuscript. K.J. specifically thanks Alexandra Gampel and Alfonso Martinez-Arias for many useful discussions. This work was supported by Wellcome grants to H.S., DFG grants to E.K., and a Leslie Wilson Scholarship of Magdalene College, Cambridge (UK), as well as grants from the Zoology Balfour Fund and the Cambridge Philosophical Society to K.J.

REFERENCES

Ashburner, M. (1989). "*Drosophila:* A Laboratory Manual." Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Ashburner, M., Faithfull, J., Littlewood, T., Richards, G., Smith, S., Velissarion, V., and Woodruff, R. (1980). Report of new mutants. *Drosophila Inf. Serv.* **55,** 193–195.

Bairoch, A., Bucher, P., and Hofmann, K. (1997). The PROSITE database, its status in 1997. *Nucleic Acids Res.* **25,** 217–221.

Bate, C. M. (1990). The embryonic development of larval muscles in *Drosophila. Development* **113,** 471–485.

Bellen, H. J., O'Kane, C. J., Wilson, C., Grossniklaus, U., Pearson, R. K., and Gehring, W. J. (1989). P-element-mediated enhancer detection: A versatile method to study development in *Drosophila. Genes Dev.* **3,** 1288–1300.

Bennett, E. R., and Kanner, B. I. (1997). The membrane topology of GAT-1, a (Na^+ + Cl^-)-coupled gamma-aminobutyric acid transporter from rat brain. *J. Biol. Chem.* **272,** 1203–1210.

Bismuth, Y., Kavanaugh, M. P., and Kanner, B. I (1997). Tyrosine 140 of the γ-aminobutyric acid transporter GAT-1 plays a critical role in neurotransmitter recognition. *J. Biol. Chem.* **272,** 16096–16102.

Brown, N. H., and Kafatos, F. H. (1988). Functional interactions during early neurogenesis of *Drosophila melanogaster. J. Mol. Biol.* **203,** 425–437.

Bucher, P., and Bairoch, A. (1994). A generalized profile syntax for biomolecular sequence motifs and its function in automatic sequence interpretation. *In* "Proceedings 2nd International Conference on Intelligent Systems for Molecular Biology" (R. Altman, D. Brutlag, P. Karp, R. Lathrop, and D. Searls, Eds.), pp. 53–61. AAAI Press, Menlo Park, CA.

Bush, A., Hiromi, Y., and Cole, M. (1996). *biparous:* A novel bHLH gene expressed in neuronal and glial precursors in *Drosophila. Dev. Biol.* **180,** 759–772.

Campos-Ortega, J. A., and Hartenstein, V. (1997). "The Embryonic Development of *Drosophila melanogaster.*" Springer-Verlag, Heidelberg.

Cavener, D. R. (1987). Comparison of the consensus sequence flanking translational start sites in *Drosophila* and vertebrates. *Nucleic Acids Res.* **15,** 1353–1361.

Chou, T., and Perrimon, N. (1996). The autosomal FLP-DFS technique for generating germline mosaics in *Drosophila melanogaster. Genetics* **144,** 1673–1679.

von Dassow, G., and Schubiger, G. (1994). How an actin network might cause fountain streaming and nuclear migration in the syncytial *Drosophila* embryo. *J. Cell Biol.* **127,** 1637–1653.

Edgar, B. A., Odell, G. M., and Schubiger, G. (1987). Cytoarchitecture and the patterning of *fushi tarazu* expression in *Drosophila* blastoderm. *Genes Dev.* **1,** 1126–1132.

Engels, W. R., Johnson-Schiltz, D. M., Eggleston, W. B., and Sved, J. (1990). High frequency P element loss in *Drosophila* is homolog dependent. *Cell* **62,** 515–525.

Foe, V. E., Odell, G. M., and Edgar, B. A. (1993). Mitosis and morphogenesis in the *Drosophila* embryo: Point and counterpoint. *In* "The Development of *Drosophila melanogaster*" (M. Bate and A. Martinez Arias, Eds.), pp. 149–300. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Gautier, P., Ledent, V., Massaer, M., Dambly-Chaudiere, C., and Ghysen, A. (1997). *tap,* a *Drosophila* bHLH gene expressed in chemosensory organs. *Gene* **191,** 15–21.

Guimbal, C., and Kilimann, M. W. (1993). A sodium-dependent creatin transporter in rabbit brain, muscle, heart and kidney. cDNA cloning and functional expression. *J. Biol. Chem.* **268,** 8418–8421.

Hoch, M., Broadie, K., Jäckle, H., and Skaer, H. le B. (1994). Sequential fates in a single cell are established by the neurogenic cascade in the Malpighian tubules of *Drosophila. Development* **120,** 3439–3450.

Iverson, L. L., and Kelly, J. S. (1975). Uptake and metabolism of gamma-aminobutyric acid by neurones and glial cells. *Biochem. Pharmacol.* **24,** 933–938.

James, J. M., and Collier, G. E. (1992). Early gene interaction during prepupal expression of arginine kinase. *Dev. Genet.* **13,** 302–305.

Kanai, Y., and Hediger, M. A. (1992). Primary structure and functional characterization of a high-affinity glutamate transporter. *Nature* **360,** 467–471.

Keshet, G. I., Bendahan, A., Su, H., Mager, S., Lester, H. A., and Kanner, B. I. (1995). Glutamate-101 is critical for the function of the sodium and chloride-coupled GABA transporter GAT-1. *FEBS Lett.* **371,** 39–42.

Kleinberger-Doron, N., and Kanner, B. I. (1994). Identification of tryptophan residues critical for the function and targeting of the γ-aminobutyric acid transporter (subtype A). *J. Biol. Chem.* **269,** 3063–3067.

Kyte, J., and Doolittle, R. F. (1982). A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* **157,** 105–132.

Ledent, V., Gaillard, F., Gautier, P., Ghysen, A., and Dambly-Chaudiere, C. (1998). Expression and function of *tap* in the gustatory and olfactory organs of *Drosophila. Int. J. Dev. Biol.* **42,** 163–170.

Maddrell, S. H. P. (1981). The functional design of the excretory system. *J. Exp. Biol.* **90,** 1–15.

Maddrell, S. H. P. (1991). The fastest fluid secreting cell known: The upper Malpighian tubule cell of *Rhodnius. BioEssays* **13,** 357–362

Mlodzik, M., and Hiromi, Y. (1992). Enhancer trap method in *Drosophila:* Its application to neurobiology. *Methods Neurosci.* **9,** 397–413.

Nöthiger, R., Jonglez, M., Leuthold, M., Meier-Gerschwiler, P., and Weber, T. (1989). Sex determination in the germ line of *Drosoph-*

ila depends on genetic signals and inductive somatic factors. *Development* **107,** 505–518.

O'Donnell, M. J., Dow, J. A. T., Huesmann, G. R., Tublitz, N. J., and Maddrell, S. H. P. (1996). Separate control of anion and cation transport in Malpighian tubules of *Drosophila melanogaster*. *J. Exp. Biol.* **199,** 1163–1175.

Pearson, W. R., and Lipman, D. J. (1988). Imported tools for biological sequence comparison. *Proc. Natl. Acad. Sci. USA* **85,** 2444–2448.

Perrimon, N., Lanjuin, A., Arnold, C., and Noll, E. (1996). Zygotic lethal mutations with maternal effect phenotypes in *Drosophila melanogaster*. II. Loci on the second and third chromosomes identified by *P*-element-induced mutations. *Genetics* **144,** 1681–1692.

Poole, S. J., Kauvar, L. M., Drees, B., and Kornberg, T. (1985). The engrailed locus of *Drosophila:* Structural analysis of an embryonic transcript. *Cell* **40,** 37–43.

Postner, M. A., and Wieschaus, E. F. (1994). The *nullo* protein is a component of the actin-myosin network that mediates cellularization in *Drosophila melanogaster* embryos. *J. Cell Sci.* **107,** 1863–1873.

Postner, M. A., Miller, K. G., and Wieschaus, E. (1992). Maternal-effect mutations of the *sponge* locus affect cytoskeletal rearrangements in *Drosophila melanogaster* embryos. *J. Cell Biol.* **119,** 11205–11218.

Rasola, A., Galietta, L. J., Barone, V., Romeo, G., and Bagnasco, S. (1995). Molecular cloning and functional characterisation of a GABA/betain transporter from human kidney. *FEBS Lett.* **373,** 229–233.

Roberts, D. B. (1986) "*Drosophila:* A Practical Approach." IRL Press, Oxford.

Robertson, H. M., Preston, C. R., Phillis, R. W., Johnson, D. M., Benz, W. K., and Engels, W. R. (1988). A stable source of P-element transposase in *Drosophila melanogaster*. *Genetics* **118,** 461–470.

Rothwell, W. F., Fogarty, P., Field, C. M., and Sullivan, W. (1998). Nuclear-fallout, a *Drosophila* protein that cycles from the cytoplasm to the centrosomes, regulates cortical microfilament organization. *Development* **125,** 1295–1303.

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). "Molecular Cloning: A Laboratory Manual." Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Schejter, E. D., and Wieschaus, E. (1993a). Functional elements of the cytoskeleton in the early *Drosophila* embryo. *Annu. Rev. Cell Biol.* **9,** 67–99.

Schejter, E. D., and Wieschaus, E. (1993b). Bottleneck acts as a regulator of the microfilament network governing cellularization of the *Drosophila* embryo. *Cell* **75,** 373–385.

Schweisguth, F., Lepesant, J. A., and Vincent, A. (1990). The *serendipity alpha* gene encodes a membrane associated protein required for the cellularisation of the *Drosophila* embryo. *Genes Dev.* **4,** 922–931.

Skaer, H. le B. (1989). Cell division in the development of the Malpighian tubules of *Drosophila melanogaster* is regulated by single, specialised cells. *Nature* **342,** 566–569.

Skaer, H. le B. (1992). Cell proliferation and rearrangement in the development of the Malpighian tubules of the Hemipteran, *Rhodnius prolixus*. *Dev. Biol.* **150,** 372–380.

Skaer, H. le B. (1993). Development of the alimentary canal. *In* "The Development of *Drosophila melanogaster*" (M. Bate and A. Martinez Arias, Eds.), pp. 941–1012. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Skaer, H. le B. (1996). Cell proliferation and development of the Malpighian tubules in *Drosophila melanogaster*. *Exp. Nephrol.* **4,** 119–126.

Smith, K. E., Fried, S. G., Durkin, M. M., Gustafson, E. L., Borden, L. A., Branchek, T. A., and Weinshank, R. L. (1995). Molecular cloning of an orphan transporter. A new member of the neurotransmitter transporter family. *FEBS Lett.* **357,** 86–92.

Spradling, A. C., Stern, D., Kiss, I., Roote, J., Laverty, T., and Rubin, G. M. (1995). Gene disruptions using P transposable elements: An integral component of the *Drosophila* genome project. *Proc. Natl. Acad. Sci. USA* **92,** 10824–10830.

Sullivan, W., and Theurkauf, W. E. (1995). The cytoskeleton and morphogenesis of the early *Drosophila* embryo. *Curr. Opin. Cell Biol.* **7,** 18–22.

Sullivan, W., Minden, J. M., and Alberts, B. M. (1990). *daughterless-abo-like*, a *Drosophila* maternal-effect mutation that exhibits abnormal centrosome separation during late blastoderm divisions. *Development* **110,** 311–323.

Sullivan, W., Fogarty, P., and Theurkauf, W. (1993). Mutations affecting the cytoskeletal organization of syncytial *Drosophila* embryos. *Development* **118,** 1245–1254.

Tautz, D., and Pfeifle, C. (1989). A non-radioactive in situ hybridisation method for the localisation of specific RNAs in *Drosophila* embryos reveals translational control of the segmentation gene *hunchback*. *Chromosoma* **98,** 81–85.

Theurkauf, W. E. (1994). Immunofluorescence analysis of the cytoskeleton during oogenesis and early embryogenesis. *In* "Methods in Cell Biology" (L. S. B. Goldstein and E. A. Fyrberg, Eds.), Vol. 44. Academic Press, San Diego.

Uhl, G. R., and Johnson, P. S. (1994). Neurotransmitter transporters: Three important gene families for neuronal function. *J. Exp. Biol.* **196,** 229–236.

Uhl, G. R., Kitayama, S., Gregor, P., Nanthakumar, E., Persico, A. M., and Shimada, S. (1992). Neurotransmitter transporter family cDNAs in a rat midbrain library: 'Orphan transporters' suggest sizable structural variations. *Brain Res. Mol. Brain Res.* **16,** 353–359.

Warn, R. M., and Robert-Nicoud, M. (1992). The formation and fate of the blastoderm epithelium of the *Drosophila* embryo. *In* "Epithelial Organization and Development" (T. P. Fleming, Ed.), pp. 136–161. Chapman & Hall, London.

Wessing, A., and Eichelberg, D. (1978). Malpighian tubules, rectal papillae and excretion. *In* "The Genetics and Biology of *Drosophila.*" (M. Ashburner and T. R. F. Wright, Eds.), Vol. 2c, pp. 1–42. Academic Press, London.

Wigglesworth, V. B. (1939). "The Principles of Insect Physiology." Menthuen, London.

Wilson, C., Pearson, R. K., Bellen, H. J., O'Kane, C. J., Grossniklaus, U., and Gehring, W. J. (1989). P-element enhancer detection: An efficient method for isolating and characterizing developmentally regulated genes in *Drosophila. Genes Dev.* **3,** 1301–1313.

Wright, E. M., Loo, D. D. F., Panayotova-Heiermann, M., Lostao, M. P., Hirayama, B. H., Mackenzie, B., Boorer, K., and Zampighi, G. (1993). Active sugar transport in eukaryotes. *J. Exp. Biol.* **196,** 197–212.

Received for publication March 18, 1999
Accepted May 18, 1999