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Phil. Trans. R. Soc. Lond. B 2000 **355**, 931-937
doi: 10.1098/rstb.2000.0628

THE ROYAL SOCIETY

doi 10.1098/rstb.2000.0628

Coordinating cell fate and morphogenesis in *Drosophila* renal tubules

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Using the renal tubules of *Drosophila* as an example, we explore how cell specification leads to the morphogenetic movements that underlie the generation of tissue architecture. Taking two stages of development, we show first that the tubule cells are allocated by signalling between the endodermal and ectodermal compartments of the posterior gut. Activation of the Wnt pathway patterns the ectodermal anlage, resulting in the expression of tubule genes in a subset of cells and their eversion from the hindgut to form the tubule primordia. We argue that early gene expression directs these morphogenetic movements but not the complete programme of tubule differentiation. In the second example we show that the allocation of the mitogenic tip cell lineage in each tubule is required not only for the normal pattern of cell division but also for the stereotyped three-dimensional arrangement of the mature tubules. Analysis of mutants in which the tip cell lineage is misspecified reveals that both daughters of the tip cell progenitor are required for the tubules to navigate through the body cavity, so that the distal tips locate in their characteristic positions. We show that the regulator of Rac, Myoblast city, is essential for this second morphogenetic process.

Keywords: Malpighian tubules; cell fate; morphogenesis; Wnt signalling; terminal genes; *myoblast city*

1. INTRODUCTION

Underlying the development of a tissue or organ is a series of cellular events that includes changes in cell adhesion and shape, the acquisition of cell fate, cell migration, cell proliferation and cell differentiation. During development these processes are integrated by regulatory networks that coordinate them to produce a coherent, functional tissue.

Analysis of the complex set of cell interactions that together coordinate tissue development is hampered in many systems because several cell activities occur at the same time. In the renal or Malpighian tubules of *Drosophila* the cellular events that underlie tissue development occur, by and large, in sequence (figure 1*a–d*). The allocation of tubule cells within the embryonic hindgut is followed by changes in cell shape and adhesion to produce four evaginated buds; the tubule primordia (figure 1*a*,*b*). Cell proliferation in the primordia produces the mature number of tubule cells and occupies only a short period of embryogenesis. This is followed by a dramatic convergent-extension rearrangement of cells, which transforms the short cylindrical structures (figure 1*c*) into the elongated tubules that course through the body cavity; each tubule having a precise three-dimensional arrangement (figure 1*d*). Finally, cell growth, resulting from regulated endoreplication, and patterned cell differentiation results in the onset of physiological function, which can be monitored by the appearance of uric acid, the nitrogenous waste product, in the tubule lumen (figure 1*f*). Thus the development of the Malpighian tubules, in which specific cell activities occur during particular periods of embryogenesis, provides an opportunity to tease apart the mechanisms that operate during organogenesis to integrate the generation and specification of cells, tissue morphogenesis and patterned cell differentiation.

In this paper we discuss the relationship between cell specification and morphogenetic movements at two stages in tubule development, as a first step towards understanding how these processes are coordinated during organogenesis.

2. THE ALLOCATION OF MALPIGHIAN TUBULE CELLS AND THE FORMATION OF EVERTED PRIMORDIA

(a) *Allocation results from signalling between gut compartments*

The gut in *Drosophila* develops from the two ends of the embryo, from primordia that grow out and meet to form a continuous tube. The anterior and posterior primordia consist of terminal ectodermal cells, the fore- and hindgut, and endodermal cells, which come together to form the midgut (figure 2). Specific structures form at the junctions between the ectoderm and endoderm; the gut tube folds back on itself to form the three-layered proventriculus in the anterior, while in the posterior four buds evert and grow to form the renal or Malpighian tubules (figure 2*d*). The Malpighian tubule cells become distinguishable in the hindgut even before they start to evert, by the expression of the zinc-finger transcription factor

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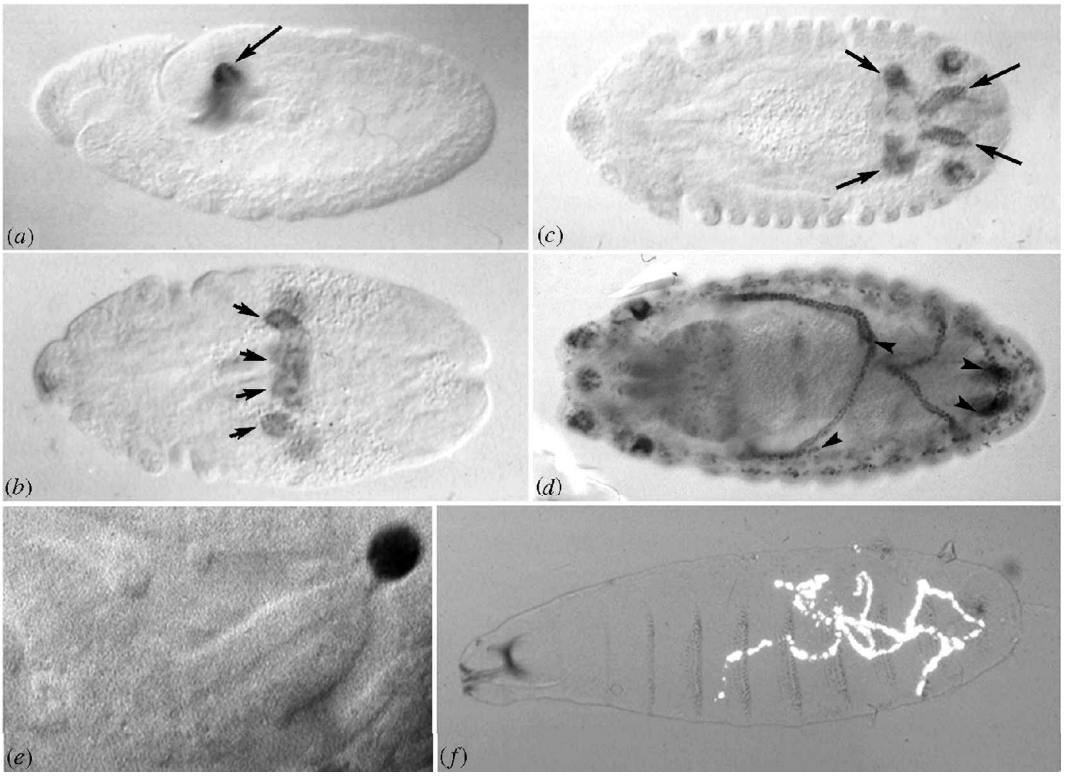

Figure 1. Malpighian tubule development. (*a–d*) Embryos stained with an antibody to Cut. (*a*) Tubule cells are allocated (arrow) and evert from the embryonic hindgut during the extended germ-band stage (stage 9/10). (*b*) The four tubule primordia (arrows) grow by cell proliferation until (*c*) the end of germ-band retraction, when they are short tubular structures (arrows). (*d*) The tubules elongate by cell rearrangement and take a stereotyped course through the body cavity. The characteristic sites of the tip cells at the distal ends are indicated by arrowheads. (*e*) The tip cell is distinctive in its position, morphology and gene expression (stained here for Krüppel). (*f*) Cell differentiation and the onset of physiological activity are marked by the secretion of uric acid, which precipitates in the tubule lumen. Living embryo viewed in polarized light. In all figures anterior is to the left. (*a*, *f*) Lateral view; (*b–d*) dorsal view.

Krüppel (Kr) and its target, a homeodomain-containing protein, encoded by *cut* (figure 1*a*).

Fate mapping by Hartenstein *et al*. (1985) established that the posterior gut develops from two neighbouring anlagen; the endodermal posterior midgut anlage and the ectodermal proctodeum that later gives rise to the hindgut and Malpighian tubules. Thus the tubules arise from an anlage that is shared with the hindgut at the blastoderm stage. They also showed that the future tubule cells lie close to the boundary separating the two anlagen. This suggests a model in which signalling between two clonally restricted groups of cells (compartments) initiates patterning within them, leading to the specification of new cell identities. This is a familiar paradigm in development, illustrated by the patterning of segments in the *Drosophila* embryo and of the adult wing and leg (reviewed in Lawrence 1992).

A simple way to test whether the tubule cells are specified in the shared ectodermal anlage by signalling from the endoderm is to remove or misspecify the endoderm. We did this by removing the activity of two genes, *huckebein* and *serpent*, that are known to be expressed in the midgut (Bronner & Jäckle 1991; Abel *et al*. 1993). Embryos mutant for both genes lack any morphological sign of the midgut, and do not express midgut markers. Strikingly, Malpighian tubules fail to be specified in the hindgut; there are no *cut*-expressing cells (figure 3*a*), no tubules evert from the hindgut tube and in late embryos no uric acid appears (Ainsworth 1999). The posterior midgut therefore appears to be required for the segregation of Malpighian tubule cells from within the neighbouring compartment, suggesting that signalling from the endoderm patterns the ectodermal anlage and specifies the tubule cells.

Perturbation of the pathway underlying this signalling would be expected to alter tubule cell allocation. We have found that embryos lacking the serine threonine kinase, encoded by *zeste-white3* (*zw3*, a homologue of vertebrate glycogen synthase kinase) or with a reduced activity of *armadillo* (*arm*, a homologue of vertebrate β-catenin) show this phenotype; loss of *zw3* results in too many tubule cells being specified (approximately double the normal number; cf. figure 3*d*,*f*), whereas embryos with reduced *arm* have very few tubule cells (approximately half the normal number; cf. figure 3*d*, *e*). As the complete loss of *arm* is cell lethal, we have not been able to test the hypothesis that in the absence of its product, tubule cell specification fails completely. Both *zw3* and *arm* act in the *wingless* signalling pathway; *zw3* as a negative and *arm* as a positive regulator. Wingless is required for normal tubule development but the mutant phenotype reveals that it plays a role in tubule cell eversion and in the

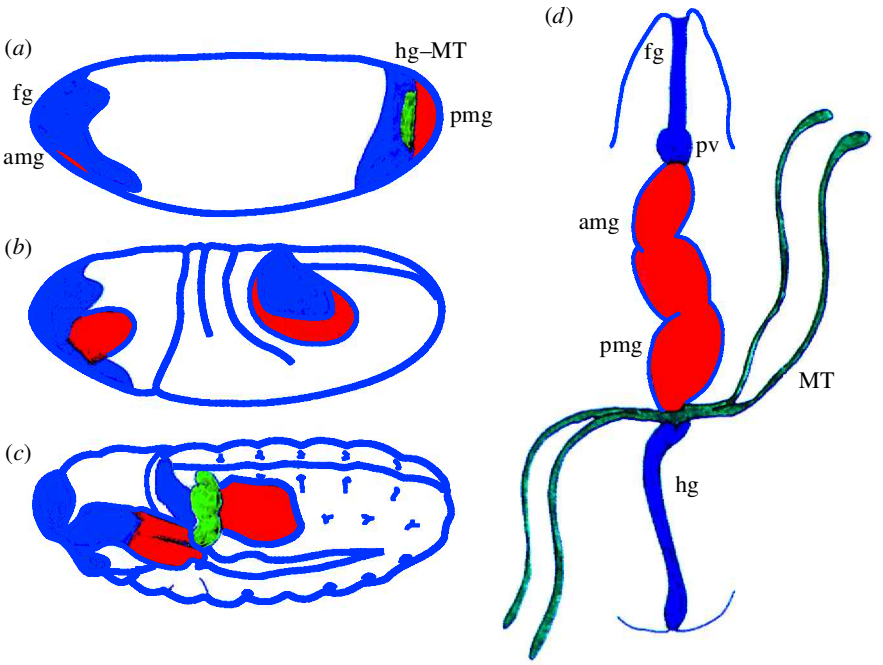

Figure 2. *Drosophila* gut development. Diagrams (after Hartenstein 1993) showing (*a*) the blastoderm fate map for the gut, (*b*) the invaginating primordia during gastrulation, (*c*) the segregation and eversion of the Malpighian tubules in the posterior gut, and (*d*) the mature embryonic gut. fg, foregut; pv, proventriculus; amg, anterior midgut; pmg, posterior midgut; MT, Malpighian tubules; hg, hindgut. Endoderm is shown in red, ectodermal fore- and hindgut in blue and the position of cells that will become Malpighian tubules in green in (*a*) and after they are specified in (*c*) and (*d*).

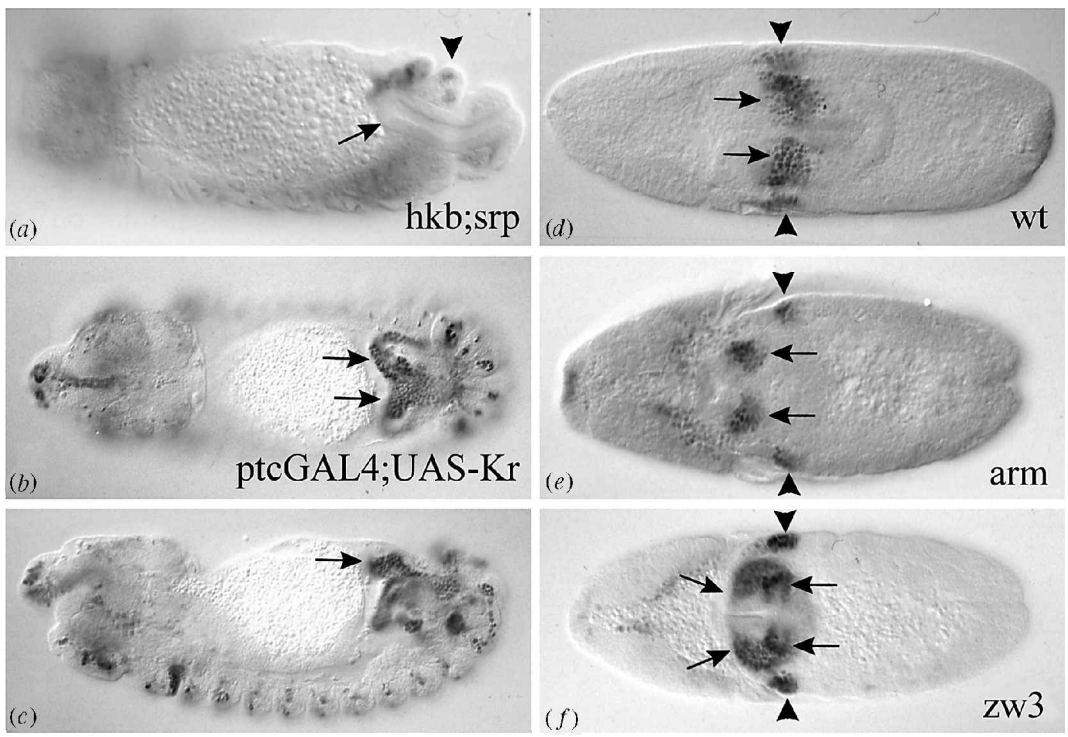

Figure 3. Tubule cell allocation. Embryos stained with an antibody to Cut. (*a*) In *huckebein*^{2};serpent^{9L} mutant embryos the midgut is lost and no *cut*-expressing cells appear in the hindgut or evert from it. Site of normal tubules arrowed. (*b*, *c*) When *Krüppel* is expressed ectopically in the hindgut, extra cells express *cut* and evert from the hindgut (arrows). (*d*) *cut*-expressing tubule cells in the hindgut (arrows) before they have evaginated in a wild-type embryo at stage 9. (*e*) Stage 9 embryo with reduced *armadillo* function. Fewer *cut*-expressing tubule cells are specified in the hindgut (arrows). *arm*^{XM19} mutant embryo derived from *arm*^{XM19} germ-line clone. (*f*) Stage 9 embryo lacking *zw3*. Extra *cut*-expressing tubule cells are specified in the hindgut. *zw3*^{M11} mutant embryo derived from *zw3*^{M11} germ-line clone. (*a*, *d–f*) Arrowheads indicate *cut* staining in the posterior tracheal spiracles. In all figures anterior is to the left. (*a*, *c*) Lateral view; (*b*, *d–f*) dorsal view.

regulation of tubule cell proliferation rather than in the allocation of tubule cell fate (Skaer & Martinez Arias 1993; Wan *et al*. 2000).

Wingless is a member of the widely conserved family of Wnt secreted glycoproteins (Cadigan & Nusse 1997), represented in *Drosophila* by at least four members. Thus it is possible that another *Drosophila* Wnt acts as the ligand to specify the tubule cells. Of the *Drosophila* Wnts, only *DWnt2* is expressed in the posterior gut primordium at the appropriate time. However, flies homozygous for a

null allele of *DWnt2* are viable (Kozopas *et al.* 1998), suggesting either that *Dwnt2* is not active in specifying tubule cells or that a maternal contribution of *Dwnt2* provides the ligand. Further work is required to assess the role of *Dwnt2* and if necessary, to search for other members of the Wnt family, as the full sequence of the fly genome becomes available.

(b) *Early tubule-specific gene expression drives eversion from the hindgut*

The allocation of Malpighian tubule cells in the hindgut is marked by the restriction of *Kr* expression to a ring of cells in the hindgut and soon after this by the expression of its target *cut* (Gaul *et al.* 1987; Gaul & Jäckle 1987). This is followed by the changes in cell shape that mark the onset of tubule eversion. It has been suggested that the expression of *Kr* alone is sufficient to propel cells into Malpighian tubule differentiation; Krüppel acts to switch potential hindgut cells into the tubule cell fate (Harbecke & Janning 1989). Indeed, in embryos mutant for *Kr* no tubules bud out from the hindgut (Gloor 1950). However, uric acid is still deposited in the lumen of the enlarged hindgut and cells in the mutant hindgut express a tubule cell marker (Skaer 1993). Thus in *Kr* mutant embryos, cells that remain in the hindgut differentiate some tubule-specific characteristics. Liu & Jack (1992) showed that these cells also express hindgut markers, suggesting that they are partially transformed towards a hindgut fate. However, it is clear that *Kr* alone is not sufficient to dictate complete tubule cell differentiation. In the absence of Kr, it is tubule eversion that fails and in mutants where *Kr* expression is reduced, but not lost, the tubules evert but their morphogenesis later in embryogenesis is defective (Harbecke & Janning 1989; Liu & Jack 1992).

Interestingly the tubules of embryos mutant for *cut* also fail to emerge normally from the hindgut. Instead of everting as four epithelial buds, the tubule cells form a multilayered blister on the surface of the hindgut and tubules never form (Liu *et al.* 1991), though uric acid is deposited (Skaer 1993) and several tubule markers are expressed (Liu & Jack 1992).

Thus both *Kr* and its target *cut*, genes the expression of which defines tubule cells in the embryonic hindgut, are required for the first morphogenetic movements that these cells make. In the embryonic mesoderm, the coexpression of two transcription factors Twist and Snail defines those cells that change their shape and move into the interior of the embryo during gastrulation (see Leptin 1999). In a similar way it might be that the coexpression of *Kr* and *cut* defines the cells in the embryonic hindgut that change shape and evert from the hindgut to become tubule cells.

We tested this hypothesis by driving the expression of *Kr* in an expanded domain in the hindgut using the heterologous transcriptional activation (Gal4-UAS) system (Brand & Perrimon 1994). As a result, the number of cells in the hindgut that expressed *cut* also increased and more cells everted from the hindgut to form enlarged tubule primordia (figure 3*b*,*c*). This confirms that the coexpression of *Kr* and *cut* is sufficient to drive the changes in cell adhesion and shape that underlie tubule eversion. Interestingly, in the anterior gut, *Kr* but not *cut* is transiently expressed at the midgut–foregut boundary and the *Kr*-expressing cells do not evert from the foregut but later contribute to the proventriculus (C. Ainsworth, unpublished data). As both Kr and Cut are transcription factors, it is possible that in the hindgut they act together to regulate the expression of cell adhesion molecules or cytoskeletal regulators that directly bring about the morphogenetic movements associated with tubule eversion.

3. CELL SPECIFICATION IN THE TUBULE PRIMORDIA INFLUENCES TUBULE MORPHOGENESIS

(a) *Allocation of the tip cell lineage involves multiple signalling pathways*

As the tubule primordia evert, a subset of about six cells in each bud starts to express basic helix-loop-helix transcription factors of the proneural gene family (Hoch *et al.* 1994). The mechanisms that pattern expression to these cell clusters are not known. However, we have shown recently that the activity of the *wingless* signalling pathway is a prerequisite for the normal activation of proneural gene expression; in *wingless* mutants proneural gene expression is weak and patchy in the tubule primordia (Wan *et al.* 2000). Once proneural gene expression is established, repressive cell interactions within the cluster result in its refinement into a single cell in each tubule. This process of lateral inhibition requires the activity of the neurogenic genes, which encode the ligand, Delta, the receptor, Notch, and elements of the signal transduction machinery. Lateral inhibition establishes a single progenitor cell (the tip mother cell), which divides once to produce two daughters, one of which becomes the tip cell (Hoch *et al.* 1994). This morphologically distinctive cell is found at the distal end of the tubules (figure 1*e*) and has a mitogenic function. Removal of the tip cell, either genetically, for example in deficiencies for the proneural genes (Hoch *et al.* 1994), or mechanically, by cell ablation (Skaer 1989), results in tubules in which cell division fails, so that the mature number of cells is only half that in wild-type embryos.

Immediately after division of the progenitor cell, both daughters express proneural genes but a second set of inhibitory interactions between these two cells, again mediated by the neurogenic signalling pathway, restricts proneural gene expression to just one cell. Persistence of proneural gene expression establishes the tip cell fate, while the sibling cell loses this potential, shows no distinctive morphology but remains as a neighbour of the tip cell. Although both daughters initially express the neurogenic ligand Delta, their tip cell potential is not equal because the determinant Numb is asymmetrically segregated into one daughter as the tip mother cell divides (Wan *et al.* 2000). Numb blocks signalling through Notch (Guo *et al.* 1996; Zhong *et al.* 1996; Spana & Doe 1996) so that the cell inheriting Numb protein is assured tip cell fate. In mutants for *numb*, this bias is removed so that in a percentage of embryos Notch signalling is activated in both daughters. As a result tip cells are not specified but two sibling cells differentiate (inset in figure 4*b*; Wan *et al.* 2000). Conversely, when *numb* is overexpressed using the Gal4-UAS system, signalling through Notch is blocked in both cells and two tip cells differentiate at the expense of the siblings cell (inset in figure 4*c*; Wan *et al.* 2000).

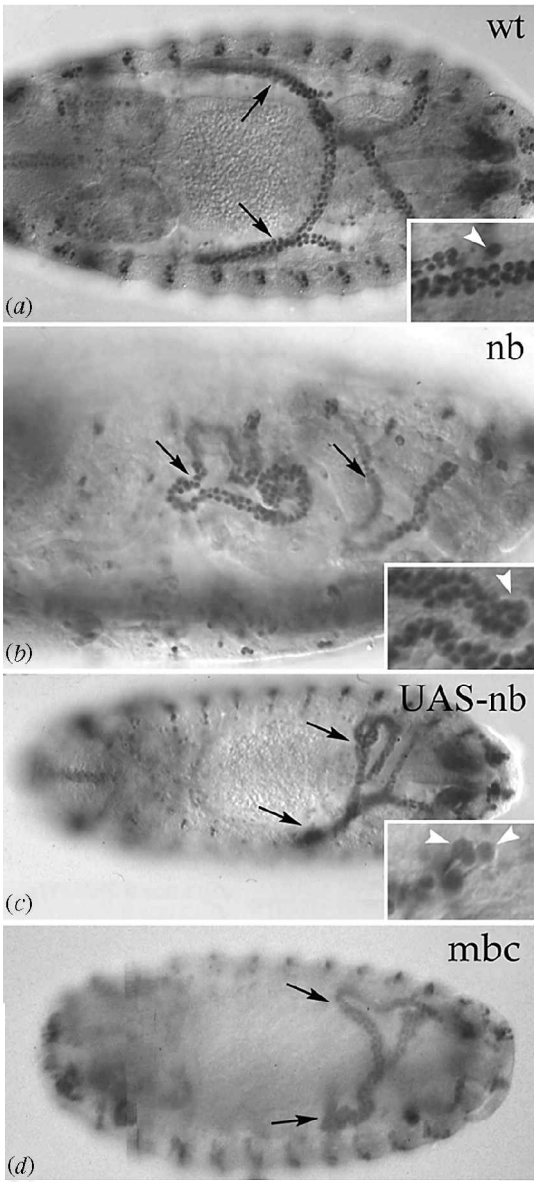

Figure 4. Three-dimensional arrangement of the tubules. Dorsal views of stage 16 embryos stained with an antibody to Cut. (*a*) Wild-type showing the typical arrangement of the tubules (anterior tubules arrowed). Insert shows the single tip cell (white arrowhead) at the distal end of the tubule. (*b*) In embryos mutant for *numb*^{796} the tubules (arrows) do not take up their normal positions. Insert shows that the tubules lack tip cells (white arrowhead). (*c*) When *numb* is overexpressed in the tubules, two tip cells develop (insert, white arrowheads) and the tubules (arrows) are not arranged normally. (*d*) In mutants for *myoblast city*, the tubules are also abnormally arranged (arrows). In all figures anterior is to the left. (*a*, *c*, *d*) Dorsal view; (*c*) lateral view.

(b) *Specification of the tip cell lineage is required for cell division and tubule morphogenesis*

Soon after it has been specified the tip cell expresses two genes, *rhomboid* and *Star*, that encode factors known to be required for the processing of a secreted, and therefore active, form of the ligand for the *Drosophila* epidermal growth factor receptor (reviewed in Freeman 1997; Kerber *et al*. 1998). Activation of this pathway in cells close to the tip cells results in cell division (Kerber *et al*. 1998; Baumann & Skaer 1993). We therefore anticipated that embryos mutant for *numb* that lack tip cells would also have tubules with a reduced number of cells. To our surprise we found that the tubules in these embryos had a completely wild-type number of cells. The same was true when *numb* was overexpressed and the tubules had two tip cells but no sibling cell (Wan *et al*. 2000). The tip cell and its sibling are therefore both capable of driving mitosis in their neighbours and in accordance with this, we find that both cells express *rhomboid* and are therefore able to secrete active ligand (P. S. Vikram and H. Skaer, unpublished data).

These data indicate that it is the specification of the tip mother cell and its lineage that is critically important in establishing the normal programme of cell division in the Malpighian tubules. What then is the significance of separating the fates of the tip cell and its sibling? We sought an answer to this question by examining the phenotype of embryos lacking or overexpressing *numb*. We found that tubules with either two tip cells or two sibling cells showed a pronounced defect in morphogenesis. Although the cells rearranged normally so that the tubules elongated, they did not course through the body cavity in the highly stereotyped fashion typical of wild-type embryos. Instead they appeared to lose their way, so that the distal ends did not achieve their final positions but were randomly arranged, often resulting in the tubules clumping together (figure 4*b*,*c*). Thus the normal targeting of the distal ends of the tubules requires the presence of both the tip cell and its sibling. In contrast to their overlapping roles in the regulation of cell division, these cells cannot replace each other in controlling the spatial arrangement of the growing tubules.

The challenge is to identify the genes that are differentially expressed in these two cell types to regulate tubule outgrowth and so link the specification of particular cell types to tubule morphogenesis. One approach is to find mutants showing defective tubule organization but without failure of cell division or tubule elongation. We find that embryos mutant for *myoblast city* (*mbc*) show this phenotype (figure 4*d*). This finding is of interest for two reasons. First, *mbc* is a member of a gene family, with homologues in *Caenorhabditis elegans* (CED-5) and mammals (DOCK180) (Wu & Horvitz 1998). These genes encode regulators of Rac (Klyokawa *et al*. 1998; Nolan *et al*. 1998), a Rho-GTPase that is active in reorganization of the actin cytoskeleton (Ridley *et al*. 1992; Hall 1998). *mbc* is therefore a candidate regulator whose activity might be controlled by tip-cell–sibling-cell activity, to mediate the changes in cell shape and movement required for tip cell navigation or tubule migration. Second, CED-5 is required for a tissue migration in the nematode that is remarkably similar to the tip-cell–sibling-cell-dependent navigation of the Malpighian tubules. At the distal end of each gonad arm in *C. elegans* is a distal tip cell, which leads the outgrowth of each arm to produce the bilaterally hooked structure characteristic of the mature tissue (Wu & Horvitz 1998). This indicates that the activity of specific cells in guiding tissue morphogenesis may be a conserved feature of organogenesis.

The distal tip cells of the gonad and tip cells of the Malpighian tubules show other strikingly parallel features, both in their distinctive morphology and position at the tip of a growing tubular structure and in

having a mitogenic function (Kimble & White 1981; Austin *et al*. 1989; Henderson *et al*. 1994). It seems possible that the activity of genes, shown to be expressed in the distal tip cells of *C. elegans* and required for gonad outgrowth, will be conserved in the tubule tip cells. A recently identified candidate is the metalloprotease GON-1 (Blelloch & Kimble 1999). GON-1 is a member of the proteolytic enzyme family that degrades extracellular matrix components and so prepares the way for cell migration and tissue remodelling, including branching morphogenesis in mammalian lung, mammary tissue and kidney (Talhouk *et al*. 1992; Nakanishi *et al*. 1986; Lelongt *et al*. 1997; Moerman 1999).

4. CONCLUSION

There are several stages during the development of *Drosophila* Malpighian tubules that illustrate the connection between cell specification and a resulting change in tissue morphogenesis. We are just beginning to understand the networks of gene activity that act to specify particular cell types and thus, directly or indirectly, have profound effects on cell adhesion, shape and movement which together drive morphogenesis. Our task now is to make the link between the early changes in gene expression in cells, as they take on new fates, with the resulting changes in the expression and activity of proteins that alter their behaviour and so drive morphogenesis.

We thank C. Rushlow, I. Rebay, R. Reuter, W. Chia, M. Bate and M. Bienz for reagents and fly stocks and S. Vikram for help in preparing the figures. This work is supported by the UK Medical Research Council (S.W.) and the Wellcome Trust (C.A. and H.S.).

REFERENCES

Abel, T., Michelson, A. M. & Maniatis, T. 1993 A *Drosophila* GATA family member that binds to Adh regulatory sequences is expressed in the developing fat body. *Development* **119**, 623–633.

Ainsworth, C. 1999 The allocation of the Malphighian tubule cells of *Drosophila melanogaster*. DPhil thesis, Oxford University, UK.

Austin, J., Maine, E. & Kimble, J. 1989 Genetics of intercellular signalling in *C. elegans*. *Development* (*Suppl*.) 53–57.

Baumann, P. & Skaer, H. le B. 1993 The *Drosophila* EGF receptor homologue (DER) is required for Malpighian tubule development. *Development* (*Suppl*.) 65–75.

Blelloch, R. & Kimble, J. 1999 Control of organ shape by a secreted metalloprotease in the nematode *Caenorhabditis elegans*. *Nature* **399**, 586–590.

Brand, A. & Perrimon, N. 1993 Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**, 401–415.

Bronner, G. & Jäckle, H. 1991 Control and function of terminal gap gene activity in the posterior pole region of the *Drosophila* embryo. *Mech. Dev.* **35**, 205–211.

Cadigan, K. M. & Nusse, R. 1997 Wnt signaling: a common theme in animal development. *Genes Dev.* **11**, 3286–3330.

Freeman, M. 1997 Cell determination strategies in the *Drosophila* eye. *Development* **124**, 261–270.

Gaul, U. & Jäckle, H. 1987 Pole region-dependent repression of the *Drosophila* gap gene *Krüppel* by maternal gene products. *Cell* **51**, 549–555.

Gaul, U., Seifert, E., Schih, R. & Jäckle, H. 1987 Analysis of *Krüppel* protein distribution during early *Drosophila* development reveals posttranslational regulation. *Cell* **50**, 639–647.

Gloor, H. 1950 Schädigungsmuster eines Letalfaktors (Kr) von *Drosophila melanogaster Arch. Julius Klaus-Stift. Vererbungsforsch. Sozialanthropol. Rassenhyg.* **25**, 38–44.

Guo, M., Jan, L. Y. & Jan, Y. N. 1996 Control of daughter cell fates during asymmetric division: interaction of Numb and Notch. *Neuron* **17**, 27–41.

Hall, A. 1998 Rho GTPases and the actin cytoskeleton. *Science* **279**, 509–514.

Harbecke, R. & Janning, W. 1989 The segmentation gene *Krüppel* of *Drosophila melanogaster* has homeotic properties. *Genes Dev.* **3**, 114–122.

Hartenstein, V. 1993 *Atlas of* Drosophila *development*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Hartenstein, V., Technau, G. M. & Campos-Ortega, J. A. 1985 Fate-mapping in wild-type *Drosophila melanogaster*. III. A fate map of the blastoderm. *Roux's Archiv. Devl Biol*. **194**, 213–216.

Henderson, S., Gao, S., Lambie, E. & Kimble, J. 1994 *lag-2* may encode a signaling ligand for the GLP-1 and LIN-12 receptors of *C. elegans*. *Development* **120**, 2913–2924.

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

Kerber, B., Fellert, S. & Hoch, M. 1998 Seven-up, the *Drosophila* homolog of the COUP-TF orphan receptors, controls cell proliferation in the insect kidney. *Genes Dev.* **12**, 1781–1786.

Kimble, J. & White, J. 1981 On the control of germ cell development in *Caenorhabditis elegans*. *Devl Biol.* **81**, 208–219.

Klyokawa, E., Hashimoto, Y., Kobayashi, S., Sugimura, H., Kutata, T. & Matsuda, M. 1998 Activation of Racl by a Crk SH3-binding protein, DOCK180. *Genes Dev.* **12**, 3331–3336.

Kozopas, K. M., Samos, C. H. & Nusse, R. 1998 *DWnt-2*, a *Drosophila* wnt gene required for the development of the male reproductive tract, specifies a sexually dimorphic cell fate. *Genes Dev.* **12**, 1155–1165.

Lawrence, P. A. 1992 *The making of a fly: the genetics of animal design*. Oxford, UK: Blackwell.

Lelongt, B., Trugnan, G., Murphy, G. & Ronco, P. M. 1997 Matrix metalloproteases MMP2 and MMP9 are produced in early stages of kidney morphogenesis but only MMP9 is required for renal organogenesis *in vitro*. *J. Cell Biol.* **136**, 1363–1373.

Leptin, M. 1999 Gastrulation in *Drosophila*: the logic and the cellular mechanisms. *EMBO J.* **18**, 3187–3192.

Liu, S. & Jack, J. 1992 Regulatory interactions and role in cell type specification of the Malpighian tubules by *cut*, *Krüppel* and *caudal* genes of *Drosophila*. *Dev. Biol.* **150**, 133–143.

Liu, S., McLeod, E. & Jack, J. 1991 Four distinct regulatory regions of the *cut* locus and their effect on cell type specification in *Drosophila*. *Genetics* **127**, 151–159.

Moerman, D. G. 1999 Organ morphogenesis: a metalloprotease prepares the way. *Curr. Biol.* **9**, R701–R703.

Nakanishi, Y., Suguira, J., Kishi, I. & Hayakawa, T. 1986 Collagenase inhibitor stimulates cleft formation during early morphogenesis of mouse salivary gland. *Devl Biol.* **113**, 201–206.

Nolan, K. M., Barrett, K., Lu, Y., Hu, K.-Q., Vincent, S. & Settleman, J. 1998 Myoblast city, the *Drosophila* homolog of DOCK180/CED-5, is required in a Rac signaling pathway utilized for multiple developmental processes. *Genes Dev.* **12**, 3337–3342.

Ridley, A. J., Paterson, H. F., Johnston, C. L., Diekmann, D. & Hall, A. 1992 The small GTP-binding Rac regulates growth factor-induced membrane ruffling. *Cell* **70**, 389–399.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Skaer, H. 1989 Cell division in Malpighian tubule development in *Drosophila melanogaster* is regulated by a single tip cell. *Nature* **342**, 566–569.

Skaer, H. 1993 Development of the alimentary canal. In *The development of* Drosophila (ed. M. Bate & A. Martinez Arias), pp. 941–1012. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Skaer, H. & Martinez Arias, A. 1992 The *wingless* product is required for cell proliferation in the Malpighian tubule anlage of *Drosophila melanogaster*. *Development* **116**, 745–754.

Spana, E. & Doe, C. 1996 Numb antagonises Notch signalling to specify sibling neuron cell fates. *Neuron* **17**, 21–26.

Talhouk, R. S., Bissel, M. J. & Werb, Z. 1992 Coordinated expression of extracellular matrix-degrading proteinases and their inhibitors regulates mammary epithelial function during involution. *J. Cell Biol.* **118**, 1271–1282.

Wan, S., Cato, A.-M. & Skaer, H. 2000 Multiple signalling pathways establish cell fate and number in *Drosophila* Malpighian tubules. *Devl Biol.* **217**, 153–165.

Wu, Y.-C. & Horvitz, R. 1998 *C. elegans* phagocytosis and cell-migration protein CED-5 is similar to human DOCK180. *Nature* **392**, 501–504.

Zhong, W., Feder, J., Jiang, M., Jan, L. Y. & Jan, Y. N. 1996 Asymmetric localization of mammalian Numb homolog during mouse cortical neurogenesis. *Neuron* **17**, 43–53.

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