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MINIREVIEW

Posterior gut development in *Drosophila*: a model system for identifying genes controlling epithelial morphogenesis

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ABSTRACT

The posterior gut of the Drosophila embryo, consisting of hindgut and Malpighian tubules, provides a simple, well-defined system where it is possible to use a genetic approach to define components essential for epithelial morphogenesis. We review here the advantages of Drosophila as a model genetic organism, the morphogenesis of the epithelial structures of the posterior gut, and what is known about the genetic requirements to form these structures. In overview, primordia are patterned by expression of hierarchies of transcription factors; this leads to localized expression of cell signaling molecules, and finally, to the least understood step: modulation of cell adhesion and cell shape. We describe approaches to identify additional genes that are required for morphogenesis of these simple epithelia, particularly those that might play a structural role by affecting cell adhesion and cell shape.

Key words: *Organogenesis, cell rearrangement, convergent extension, hindgut, Malpighian tubule.*

Advantages of *Drosophila*

Work on Drosophila genetics began 90 years ago, when Thomas Hunt Morgan

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(who later received the Nobel Prize for his work) began studying inheritance in the fruit fly. At that time, the advantage of working with this small organism was that it reproduced rapidly in the laboratory, requiring only a simple growth medium, no special attention, and little expense. As research into this organism continued, other remarkable advantages emerged, namely that it possesses only four chromosomes (allowing each newly identified gene to be quickly mapped to a linkage group). Particularly significant, it possesses giant polytene chromosomes in the salivary glands of the larvae; the detailed and reproducible banding pattern of these chromosomes allows the genetic map to be correlated with the cytological (physical) map.

With the advent of molecular techniques, the polytene chromosomes made it possible to map a particular cloned gene to a specific chromosomal position by using in situ hybridization, and conversely, to clone a gene by knowing its cytological location. The small genome of Drosophila (1.5×10^8 base pairs, only one-twentieth the size of the mammalian genome) makes it relatively much easier to identify a sequence within a *Drosophila* than in a mammalian genomic library (reviewed in[1]).

Another crucial tool used in *Drosophila* molecular genetics is the mobile element P, which can be used as a mutagen (transposon tag), allowing the ready cloning of any gene mutated by insertion of a P element. Furthermore, because it is a mobile element, an appropriately engineered P element can be used as a vector for genomic transformation, allowing a sequence of up to 20 kb to be inserted at random into the *Drosophila* genome[2]. A number of techniques have been developed recently, based on transformation with P elements, that allow generation of clones of marked mutant cells. In this way, one can, in an otherwise wild type organism, examine the effect of removing function of an essential gene in only a small group of cells (reviewed in[3]).

Because of the multiple advantages of Drosophila genetics, investigators are using it to study molecules controlling various cellular processes. We describe here progress made toward understanding genes controlling epithelial morphogenesis, in particular the development of the posterior portion of the alimentary canal. First, we review the development of these organs, and then describe the known genes that control this development.

*Drosoph*ila hindgut and Malpighian tubule development

The mature hindgut and Malpighian tubules are single-layered, polarized epithelia; a thin visceral mesoderm surrounds the hindgut, but not the Malpighian tubules (reviewed in[4]). The hindgut is the most posterior portion of the *Drosophila* gut (alimentary canal) and opens to the outside through the anus; its primary function is water resorption. The four Malpighian tubules connect to the most anterior portion of the hindgut; together they comprise an excretory organ analogous to vertebrate kidneys. The Malpighian tubules excrete nitrogenous waste as uric acid,

which forms insoluble crystals, making the tubules opaque and hence visible in the mature embryo or larva.

The hindgut and Malpighian tubules arise from a ring of committed cells (primordium) at the posterior of the embryo (Fig 1A). This ring of cells invaginates during gastrulation [about 3-4 h after egg laying (AEL)] to form the proctodeum, a short, wide tube (Fig 1, B-E); this invagination is essentially an inward folding of the single-cell layered epithelial cell sheet. Most cells in the proctodeum are further specified as hindgut precursor cells and remain in the proctodeal tube, which continues to grow towards the posterior by cell division and rearrangement (Fig 1F). After completion of germ band shortening (at 9 h AEL), the hindgut bends back towards the posterior pole and reverses its orientation (Fig 1G). The hindgut continues to elongate by a process of cell rearrangement, also known as convergent extension, and forms a J-shaped tube (Fig 1H). The mature hindgut consists of three different sections. The most anterior is the expanded, hook shaped *small intestine* that connects to the posterior midgut, in the central part is the elongated *large intestine*, and most posterior is the corrugated, bell-shaped rectum ([5]; Fig 1H). Overall, by the process of convergent extension, the hindgut increases in length at least three-fold ([4]; Fig 1, F-H).

At 6 h AEL, the four buds of the Malpighian tubules evaginate from a region at the most anterior part of the proctodeum that connects to the posterior midgut. This bud evagination is first a process of cell sheet folding, and then cell recruitment, during which the newly specified Malpighian tubule cells migrate from the proctodeum into the forming buds (Fig 2A). At the distal end of each bud, a "tip cell" is further specified by 9 h AEL, and signals to the neighboring cells in the bud[6, 7]. The cells in the bud continue to proliferate under the control of the tip cell, and concomitantly undertake another morphogenetic process, bud extension. By this process, the cylindrical bud extends and becomes narrower both proximally (where it inserts into the hindgut) and distally (near the tip cell), forming a crescent shape ([8]; Fig 2B). After the cessation of cell proliferation (10 h AEL), the tubules continue to elongate at least four-fold, with the initial 12-14 cells that surround the lumen rearranging by convergent extension to only two cells around the lumen ([9]; Fig 2, B-D). Finally, the cells of the Malpighian tubules differentiate into two morphologically different cell types[4].

Identifying genes controlling hindgut and Malpighian tubule development

Nuesslein-Volhard and Wieschaus[10] established the paradigm of using mutagenesis followed by screening of thousands of mutant embryos to identify most of the genes controlling patterning, development and differentiation of the embryonic epidermis. The significance of this work, which identified many critical genes required for development, including genes encoding components of the Hedgehog,

Wnt, BMP-4 and Notch signaling pathways, was recognized by the Nobel Prize in 1995. Subsequently, more detailed examination of the mutants identified in this screen demonstrated that some also affect development of internal organs, including the hindgut and Malpighian tubules. Additional screens have since been carried out to identify more genes controlling development of internal organs. Eberl and Hilliker[11] screened the X chromosome by .examining lethal mutant embryos for genes controlling various structures, including midgut and Malpighian tubules, and identified the genes *mummy (mmy) and yolky (yok)*. By using antibody staining to screen a collection of deficiencies and known mutants, we screened for mutants affecting Malpighian tubule and hindgut development[12]. From this screen, and work of others, two loci were identified, *brachyenteron (byn) and bowel (bowl)*, that are required for hindgut development[12-14]. Recently, we completed a screen of

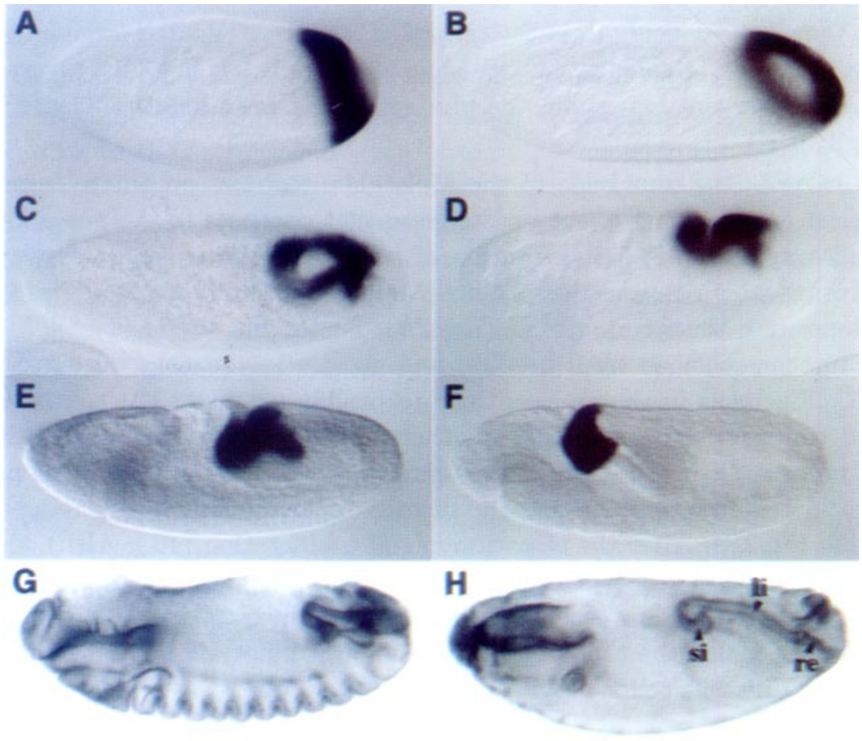


Fig 1. Development of the Drosophila hindgut. Expression of *byn*, detected by *in situ* hybridization, is used as a marker for the proctodeal primordium. This primordium is established at about 3 h AEL (A). During gastrulation, it forms a ring that moves dorsally (B) and draws together (C, D), sinking inward (E) and becoming fully internalized by 5 h AEL (F). The anti-Crb antibody outlines the apical (luminal) surface of the hindgut, which is a J-shaped tube by 10 h AEL (G), and a more elongated tube with three compartments, small intestine (*si*), intestine (*li*) and rectum (*re*), by about 15 h AEL (H).

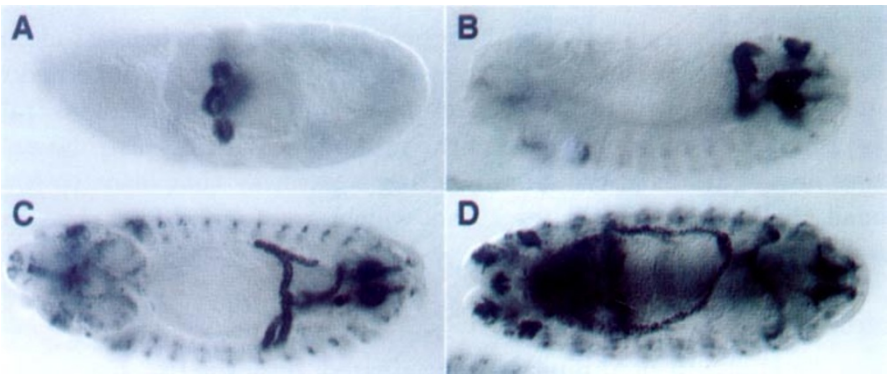


Fig 2. Development of the *Drosophila* Malpighian tubules. Staining with the anti-Cut antibody labels the nuclei of the Malpighian tubule cells. The buds evaginate at 6h AEL (A), extend until 10 h AEL (B); then the tubules further elongate (C) until 15 h AEL, at which point they reach their full length (D).

several thousand lethal mutant *lines* by examining Malpighian tubule morphology in the living embryo and identified two novel loci *walrus (wal)* and *drumstick (drm)*[8].

Genetic pathways controlling hindgut and Malpighian tubule development

Molecular, genetic and phenotypic analysis of mutants affecting posterior gut development indicates that most genes corresponding to these mutants can be placed into pathways that control different steps in organogenesis of these two structures. Below we discuss first genes required to establish the proctodeal primordium (which gives rise to both hindgut and Malpighian tubules), next genes controlling hindgut development, and finally, genes controlling Malpighian tubule development.

Establishing the proctodeal primordium

Most of the genes required to establish proctodeal cell fate are known and encode transcription factors; the genetic hierarchy of their interaction is summarized in Fig 3A, and has been reviewed in[4, 15, 16]. The proctodeal primordium is established, primarily by expression of *tailless (tll)*, as a ring of cells at the posterior of the blastoderm stage embryo. Transcription factors encoded by various segmentation genes define the anterior, and Huckebein defines the posterior border of this primordium. The cell signaling molecule encoded by the *wingless (wg)* gene also plays an undefined role in commitment to the proctodeal fate.

Hindgut development

Invagination

After the initial specification of the proctodeal domain in the blastoderm embryo, localized expression of the putative cell signaling molecule Fog (turned on by the combined activities of the Tailless, Huckebein, Fork head and Caudal transcription factors) is required for invagination of the primordium during gastrulation (Fig 3B). Fog coordinates gastrulation by stimulating a pathway that leads to activation of the small GTPases Concertina and Rho, which are required for apical constriction of the invaginating cells[17, 18].

Maintaining the primordium

Once the hindgut primordium has invaginated, both the Brachyenteron and Fork head transcription factors are required for its maintenance and to promote its further morphogenesis and differentiation (Fig 3C). Tailless and Huckebein regulate expression of Brachyenteron and Fork head, but these latter two transcription factors function independently of each other. In the absence of either factor, cells of the primordium undergo apoptosis ([15, 19]; Singer J, Lengyel J. unpublished data). Given the smaller size of the hindgut *in bowl* mutant embryos[14], it seems likely that the transcription factor encoded by this gene is also required to maintain and further specify the hindgut primordium.

Compartmentation and morphogenesis

The genes *wg, hh* and *dpp* (encoding the Drosophila homologs of Wnt-1, Hedgehog and Bone Morphogenetic Protein-4) play interacting roles in morphogenesis of the hindgut[5], wg and hh are each expressed in adjacent and mutually reinforcing

▷ **Fig 4.** Genes controlling Malpighian tubule development. The Malpighian tubule primordium is specified as a region that arises from the posterior of the proctodeum (*pr*) (hatched region with yellow color in A; derivatives of this region are shown in yellow in B, C and D). The tubule buds (*mt*) evaginate form the proctodeum about 6 h AEL, at the junction of the hindgut (*hg*) and the midgut (mg) (B). Cells within the bud continue to divide under the control of the tip cell (*tc*), and rearrange, causing extension of the bud, until 10 h AEL (C). After cessation of cell proliferation, the tubules continue to elongate by cell rearrangement until 15 h AEL (D). The arrows in the structures indicate the direction of cell movement. Genes required for different steps in tubule development are shown below, with green arrow and red line plus bar indicating positive and negative regulation, respectively. The type of protein encoded by each molecularly characterized gene is indicated by a superscript: transcription factor (*T*), signaling molecule (*S*), adhesion molecule (*A*), and molecule involved in cell division (D). Genes are also classified into groups (in parentheses) according to their apparent functions in tubule development, such as cell adhesion (A), cell division (D), and cell signaling (S). The dotted lines associated with wg and thr indicate multiple developmental steps affected in these two mutants.

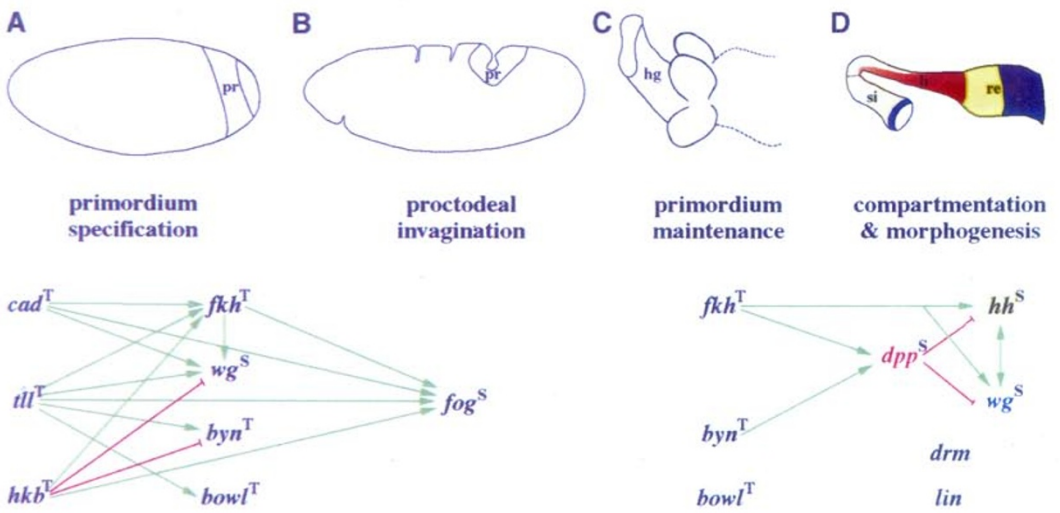
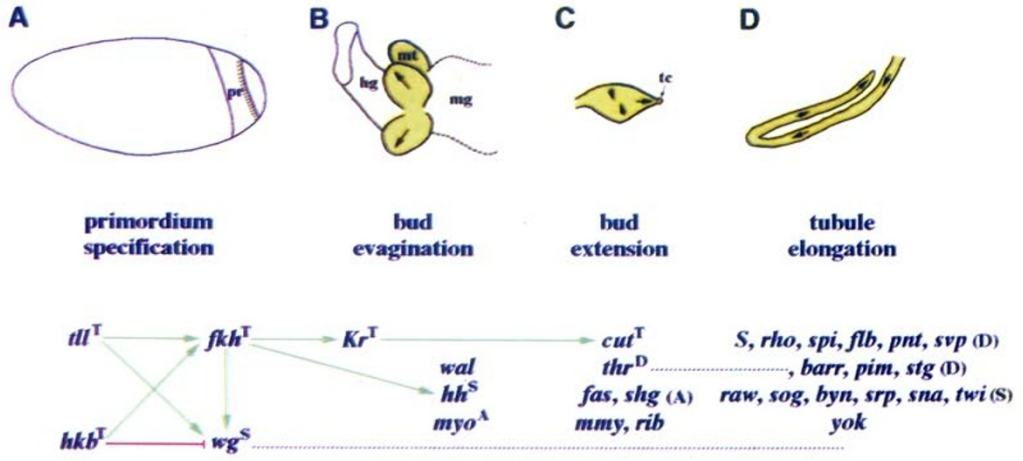


Fig 3. Genes controlling hindgut development. The proctodeal primordium (pr) is specified by expression of the genes shown in the hierarchy below (A, 3 h AEL embryo). Invagination of the proctodeum is coordinated by localized expression of the secreted protein encoded by *fog* (B, gastrulating embryo at 3.5 h AEL). Continued expression of *fkh*, byn and bowl is required to maintain the primordium and promote its further morphogenesis (C, posterior gut primordium of 6 h AEL embryo modified from[29]; *hg*, hindgut). Compartmentation and morphogenesis of the three parts of the hindgut, small intestine (*si*), large intestine (*li*) and rectum (*re*), is regulated by localized and non-overlapping expression of the genes hh (yellow), wg (blue) and dpp (red) (D, outline of hindgut of 10 h AEL embryo shown in Fig 1G). Note that there is a posterior to anterior gradient in both intensity and circumferential extent of *dpp* expression in the large intestine. In all drawings anterior is left and dorsal is up. Regulatory interactions are indicated by green arrows (positive regulation) and red lines with bar (negative regulation). The type of protein encoded by each molecularly characterized gene is indicated by a superscript: transcription factor (T) and signaling molecule (S).



stripes in the most anterior (*small intestine*) and most posterior (rectum) regions of the hindgut, while *dpp* is expressed in the intervening large intestine (Fig 3D). Ectopic expression and analysis of mutants reveals that *dpp* suppresses wg and hh expression in the large intestine. It has been proposed that expression of wg and hh in the small intestine and rectum makes these regions of the hindgut "zones of morphogenesis" that differentiate into more complex structures, while dpp expression in the large intestine suppresses morphogenesis[5]. It should be noted, however, that there is a significant amount of cell rearrangement within the large intestine itself, leading to its dramatic elongation. Thus, while suppressing morphogenesis characteristic of the *small intestine* and *rectum*, Dpp is likely promoting the morphogenesis (convergent extension) characteristic of the *large intestine*.

Two genes that have not yet been characterized molecularly, *lines (lin)* and *drm*, affect morphogenesis of the entire hindgut. In contrast to *byn*, *fkh* and *bowl* mutants, the number of cells in the hindgut does not appear to be severely reduced in *lin* or *drm* mutant embryos; rather the shape of the hindgut is radically altered in these two mutants. In the *lin* mutant, the hindgut appears stretched and bloated, with a thin epithelium[12], while in contrast, in *drm* mutant embryos, the hindgut is short and wide, with a thick epithelium[8].

Malpighian tubule development

Bud evagination

Expression of the transcription factor *Krnppel (Kr)* in the proctodeum at its junction with the posterior midgut is required for the evagination of the Malpighian tubule buds from the proctodeum[20, 21]. In addition, Wingless is required for the evagination of four tubule buds (only two buds form in the wg mutant) [9]. While Kr and wg are required for early steps in bud evagination, other genes are required for completion of this process (Fig 4). Thus, Hedgehog, the integrin receptor encoded by the *myospheroid (myo)* gene and the novel gene wal are required for various aspects of the outmigration of cells from the proctodeum into the tubule buds[5, 8].

Bud extension

Genes required for this process are cut, which encodes a transcription factor, *shotgun (shg)*, which encodes the *Drosophila* E-cadherin, *faint sausage (fas)*, which encodes an extracellular protein with immunoglobulin-like domains, *three rows (thr)*, which encodes a protein necessary for chromatid segregation, *mmy* and *ribbon (rib)*, which have not been molecularly characterized[8, 12, 22-25].

Tubule elongation

After completion of cell division (at 10 h AEL), the tubules continue to elongate by cell rearrangement for another 6 h (Fig 4D). Even though cell division is completed before tubule elongation, it is still essential that it have taken place in

order for elongation to occur normally. Genes that regulate cell division globally within the embryo, such as *barren (bar)*, *pimple (pim)*, *string (stg)*, and *three rows (thr)*, are required for tubule elongation[8]. In addition, signaling by both Wingless and a *Drosophila* EGF-like ligand (produced by the tip cells) is required for normal proliferation within the tubules and their subsequent elongation[7, 9, 12].

A number of genes have been identified that, although they do not appear to be expressed in the tubules themselves, are required for tubule elongation. This suggests that there is (as yet undefined) signaling between tubule and non-tubule cells required for tubule elongation. Among this group of genes are sog, which encodes a secreted protein that antagonizes activity of the BMP4 homolog Dpp[12, 26], and raw, which encodes a novel protein required for the cell signaling involved in dorsal closure[8, 24, Letsou A. personal communication]. In addition, the transcription factor encoding genes *byn* and *serpent (srp)* are required for tubule elongation, even through they are not expressed in the elongating tubules[13, 15, 27]. It is possible that the early expression of *byn* and *srp* in the proctodeal primordium has a later effect on tubule elongation. Another possibility is that signaling from the hindgut and posterior midgut, which are affected in *byn* and *srp* mutants, respectively, is necessary for tubule elongation. Finally, cell-cell interactions necessary for tubule elongation may also occur between the Malpighian tubules and nearby mesoderm, since embryos mutant for *twist* and *snail*, which lack mesoderm, do not elongate their tubules normally[28].

Implications for organogenesis

As outlined above, many genes are required to control the development of the single layered epithelial organs of hindgut and Malpighian tubule in *Drosophila*. Based on what has been demonstrated for spatial patterning of the early *Drosophila* embryo, and what we have learned about development of the posterior gut in *Drosophila*, we can make the following generalizations about organogenesis.

The first step in the development of an organ is the patterning of a cell group by differential expression of transcription factors. Thus, in a field of cells that are initially equivalent, something disrupts this equivalency, resulting in expression of different transcription factors in different cells (e.g., *tll* and *hkb* are expressed in the posterior while other gap genes are expressed in the anterior of the embryo). This then leads to regulation of downstream transcription factors that subdivide the field and promote further differentiation (e.g., *tll* and *hkb* determine the narrow stripe of *byn* expression, which is required for hindgut maintenance and compartmentation). There is much overlap in the domains of transcription factor expression. The combined action of multiple transcription factors is required for the expression of downstream genes (e.g., Caudal, Tailless, Huckebein and Fork head are necessary for the posterior stripe of wg expression). These principles have been established for the segmentation patterning of the early Drosophila embryo; the work reviewed

here demonstrates that they also apply to patterning the regions of the embryo that gastrulate and give rise to internal organs.

In addition to regulating other transcription factors, the transcription factors expressed at various stages in the hierarchy regulate expression of signaling molecules that control morphogenesis and cell division. We have described one example in which the transcription factors Tailless, Huckebein, Fork head and Caudal together regulate the expression of the secreted molecule Fog. By activating a pathway involving the small GTPases Concertina and Rho, Fog coordinates the cell shape change that drives the proctodeal invagination. Another example is the regulation by Brachyenteron and Fork head of the genes *wg*, *hh* and *dpp*, which encode signaling molecules playing as yet uncharacterized roles in morphogenesis of the three compartments of the hindgut.

Both cytoskeleton and cell-cell contacts (adhesion) must undergo significant alteration to drive cell shape changes and cell rearrangements during morphogenesis. Yet among the genes discovered to date that control tubule morphogenesis, only three (*myo, fas,* and *shg*) encode cell adhesion molecules (an integrin receptor, an Ig-like extracellular protein, and E-cadherin) that play a structural role in morphogenesis. Genes encoding components of the cytoskeleton have not yet been described among the mutants affecting tubule morphogenesis.

Why have so few molecules that provide the structural basis of morphogenesis been identified in genetic screens? One possibility is that many such gene products are provided both maternally and zygotically in the Drosophila embryo, and thus have not been identified in screens based on removal only of the zygotic activity. Screens involving germline clones might make it possible to identify such molecules. A second possibility is that, if any such genes function only in the gut, they might (like drm) not be embryonic lethal. Future screens to detect such genes might require direct observation of living embryos or larvae that express a suitable marker in the gut such as green fluorescent protein. Finally, genes controlling cell structure during morphogenesis might be more likely to be redundant (as are genes encoding actin, tubulin, etc.), thus, mutations in any one such gene might not result in a defective mutant phenotype. Identification of such genes might be possible by observing their expression pattern, rather than mutant phenotypes.

Another important question about molecules that cause changes in cell shape and cell adhesion is whether they are specifically expressed within regions undergoing morphogenesis, or globally present but only function in those regions as a response to local signals. Molecular characterization of already identified genes (e.g., *drm* and *wal*) as well as of genes that remain to be identified in future screens should provide insight into this question.

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