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REVIEW

It Takes Guts: The *Drosophila* Hindgut as a Model System for Organogenesis

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The *Drosophila* hindgut is fruitful territory for investigation of events common to many types of organogenesis. The development of the *Drosophila* hindgut provides, in microcosm, a genetic model system for studying processes such as establishment (patterning) of an epithelial primordium, its internalization by gastrulation, development of left–right asymmetric looping, patterning in both the anteroposterior and dorsoventral axes, innervation, investment of an epithelium with mesoderm, reciprocal epitheliomesenchymal interactions, cell shape change, and cell rearrangement. We review the genetic control of these processes during development of the *Drosophila* hindgut, and compare these to related processes in other bilaterians, particularly vertebrates. We propose that *caudal*/Cdx, *brachyenteron*/Brachyury, *fork head*/HNF-3, and *wingless*/Wnt constitute a conserved "cassette" of genes expressed in the blastopore and later in the gut, involved in posterior patterning, cell rearrangement, and gut maintenance. Elongation of the internalized *Drosophila* hindgut primordium is similar to elongation of the archenteron and also of the entire embryonic axis (both during and after gastrulation), as well as of various tubules (e.g., nephric ducts, Malpighian tubules), as it is driven by cell rearrangement. The genes *drumstick*, *bowl*, and *lines* (which encode putative transcriptional regulators) are required for this cell rearrangement, as well as for spatially localized gene expression required to establish the three morphologically distinct subregions of the hindgut. Expression of signaling molecules regulated by *drumstick*, *bowl*, and *lines*, in particular of the JAK/STAT activator Unpaired at the hindgut anterior, may play a role in controlling hindgut cell rearrangement. Other cell signaling molecules expressed in the hindgut epithelium are required to establish its normal size (Dpp and Hh), and to establish and maintain the hindgut visceral mesoderm (Wg and Hh). Both maternal gene activity and zygotic gene activity are required for asymmetric left–right looping of the hindgut. Some of the same genes (*caudal* and *brachyenteron*) required for embryonic hindgut development also act during pupation to construct a new hindgut from imaginal cells. Application of the plethora of genetic techniques available in *Drosophila*, including forward genetic screens, should identify additional genes controlling hindgut development and thus shed light on a variety of common morphogenetic processes.

Key Words: **tubule; gut; digestive tract; Malpighian tubules; *caudal*/Cdx; *fork head*/HNF-3; *brachyenteron*/Brachyury; *wingless*/Wnt.**

INTRODUCTION

Following the completion of gastrulation and generation of germ layers, development of the various internal organs of the body begins. Until fairly recently, this process of organogenesis has been understood primarily at the level of morphology and tissue interactions. In the past 10 years, however, the explosion in genetic information and molecular technology has provided the foundation for a corresponding expansion in our understanding of the molecular genetic mechanisms required for the development of different organs.

Many organs develop from a primordium consisting of an epithelial lining and surrounding mesenchyme; interaction between these tissue layers is essential for construction of

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the organ. In vertebrates, this type of organogenesis is seen for the endodermal derivatives gut, lung, liver, and pancreas, and some mesodermal derivatives such as the kidney (Wolpert, 1998). The role of specific genes in processes such as primordium specification, tissue signaling, and outgrowth, required for development of these vertebrate organs, has been reviewed recently (Grapin-Botton and Melton, 2000; Roberts, 2000; Cardoso, 2000; Warburton *et al.*, 2000; Zaret, 1999, 2000; Kuure *et al.*, 2000). In *Drosophila*, the foregut, midgut, and hindgut similarly develop as simple epithelia surrounded by visceral mesoderm (reviewed by Skaer, 1993; Bienz, 1994; Lengyel and Liu, 1998; Murakami *et al.*, 1999). Recent studies make it timely to review development specifically of the *Drosophila* hindgut, as a genetically tractable model for tubular epithelial morphogenesis. We describe here the genes known to be required for early patterning, internalization via gastrulation, division into subregions, epitheliomesenchymal interaction, left–right asymmetry, and elongation by cell rearrangement of the *Drosophila* hindgut. We discuss how each of these processes may be related to similar processes occurring throughout the bilateria, including vertebrates.

Drosophila HINDGUT AS A MODEL FOR ORGANOGENESIS

All multicellular organisms must ingest and digest food, absorb nutrients, and expel undigested waste. These common requirements, taken together with anatomical similarities, suggest that the gut is one of the most evolutionarily ancient and conserved organs. In organisms with both a mouth and an anus (most of the bilateria), the digestive tract is composed of three parts: the foregut (esophagus in humans) functions to ingest food, the midgut (stomach and intestine in humans) to digest and absorb food, and the hindgut (colon and rectum in humans) to resorb water and ions.

The hindgut can range from very simple to very complex. In *Caenorhabditis elegans*, a protostome, the origin and fate of every cell is known; the worm hindgut (rectum plus anus) is composed of only 12 epithelial cells and 2 muscle cells, and is innervated by a single neuron (White, 1988). In *Drosophila*, also a protostome, the hindgut is a single-layered, ectodermally derived epithelium surrounded by an innervated, thin circular visceral musculature (Fig. 1; Skaer, 1993; Campos-Ortega and Hartenstein, 1997; Tepass and Hartenstein, 1994a; Kusch and Reuter, 1999). In sea urchin, a primitive deuterostome, the hindgut is a simple epithelium derived from the posterior portion (last to invaginate) of the archenteron (Gustavson and Wolpert, 1967). In humans, also deuterostomes, the colon is 1.5 m in length and consists of four layers, each of which has its own complex substructure and constituent cell types; it is served by the blood, lymphatic, and nervous systems. Of primary interest here is that the mammalian colon is lined with a simple columnar epithelium and encircled by visceral musculature (Ross *et al.*, 1995). In all of these diverse organisms, the hindgut constitutes the most posterior portion of the digestive system and is lined with a simple epithelium; in arthropods and chordates, this epithelium is surrounded by an innervated visceral musculature.

The hindgut epithelium is described as arising from endoderm in vertebrates, ectoderm in insects, and either ectoderm or endoderm in *C. elegans* (Campos-Ortega and Hartenstein, 1997; Wolpert, 1998; Sulston, 1988). While the significance of the germ layer distinction remains unresolved, evidence for homology of the hindgut among different organisms is best demonstrated by conservation of gene expression (Skaer, 1993; Hoch and Pankratz, 1996; Kalb *et al.*, 1998), as will be discussed below.

To investigate the molecular genetic basis of organogenesis, it is useful to have a system in which genes controlling component processes can be readily identified and manipulated. *Drosophila*, with its small, clear, rapidly developing embryos, plethora of genetic tools, and sequenced genome, is a useful experimental organism for this approach (reviewed by Lengyel and Liu, 1998). In particular, the *Drosophila* hindgut provides a useful model for development of a mesenchyme-invested, tubular epithelial organ. As is summarized in Fig. 1, in a period of only 22 h, a small group of cells is committed to the primordium, internalized by invagination, subdivided into Malpighian tubules (insect kidney) and hindgut proper, invested with mesoderm, and innervated (reviewed by Skaer, 1993; Campos-Ortega and Hartenstein, 1997). During its development, the hindgut epithelium undergoes limited proliferation, is divided into morphologically distinct subregions, develops a left–right loop, undergoes cell shape change, elongates by cell rearrangement, and forms distinct cell types. The genes and pathways required for these processes, which are common to most types of organogenesis, can be characterized in *Drosophila* by analysis of mutant phenotypes. More than 20 *Drosophila* genes have been identified that, when mutant, result in hindgut defects (Fig. 2; Table 1). Characterization of these genes and their mutant phenotypes provides insight into the steps required to form the hindgut. Here we review the events of *Drosophila* hindgut development in terms of required gene function, and relate these to hindgut development in other organisms.

ESTABLISHING THE PRIMORDIUM: PATTERNING AT THE BLASTODERM STAGE

Patterning, the commitment of cells in a field to different future fates, is initially observed as a difference in spatial expression of various genes, particularly those encoding transcription factors or cell signals (reviewed by Davidson, 2001). In *Drosophila*, a highly refined pattern of gene expression at the blastoderm stage is required to establish segmental ectoderm cell fates, as well as mesoderm, endoderm, and internal ectoderm fates (reviewed by Lawrence, 1992). We discuss here, for a number of different

organisms, the location of the prospective hindgut cells in the early embryo, and the unique patterns of gene expression that are involved in establishing this primordium. At least four genes or gene families—HNF-3, Cdx, Wnt, and Brachyury—show conserved expression in the hindgut primordium of many organisms; in a later section, we discuss the possible conserved role of these genes in hindgut development.

In *Drosophila,* the hindgut arises from a group of cells at the posterior of the blastoderm stage embryo, referred to as the proctodeal primordium, or proctodeal ring (Fig. 1; Campos-Ortega and Hartenstein, 1997). Commitment of cells to the hindgut fate is initiated by activation of the maternally provided Torso receptor tyrosine kinase at the posterior of the embryo (reviewed by St. Johnston and Nüsslein-Volhard, 1992). This results in transcriptional activation, in overlapping posterior caps, of the transcription factor encoding genes *tailless* (*tll*) and *huckebein* (*hkb*). The smaller cap of *hkb* expression repressively establishes the posterior border of the proctodeal ring (Brönner *et al.*, 1994). *tll* acts positively to establish the proctodeal ring by activating expression of *brachyenteron* (*byn, Drosophila* Brachyury), *fork head* (*fkh, Drosophila* HNF-3), *bowel* (*bowl,* a *Drosophila* relative of Odd-skipped-related), and *wingless* (*wg,* a *Drosophila* Wnt), all of which are required for hindgut development (Fig. 3B; Mahoney and Lengyel, 1987; Pignoni *et al.*, 1990; Weigel *et al.*, 1990; Kispert *et al.*, 1994; Singer *et al.*, 1996; Wang and Coulter, 1996; Diaz *et al.*, 1996; Wu and Lengyel, 1998). Independent of the terminal system and of *tll, caudal* (*cad, Drosophila* Cdx) is expressed first (maternally) as a posterior-to-anterior gradient and second (zygotically) in the proctodeal ring (Wu and Lengyel, 1998).

GASTRULATION: INTERNALIZATION OF THE HINDGUT PRIMORDIUM

Gastrulation is a complex process of cell internalization that can involve different types of movements, often acting in concert (reviewed by Wolpert, 1998). During **ingression,** cells individually move inside the embryo; in **invagination,** coordinated apical constriction of cells in an epithelium results in its infolding; in **involution,** cells roll over the lip of the blastopore. The elongation of a group of cells in the anteroposterior (AP) axis is driven by a process of mediolateral cell **rearrangement,** or **intercalation.**

In the nematode, gastrulation begins at the 28-cell stage, when the two intestinal precursor cells sink inward; they are followed by the cells that will give rise to the musculature, pharynx, and hindgut (Wood, 1988). In the sea urchin, invagination of the vegetal plate (a few hundred cells) forms the initial archenteron (primitive gut), which is subsequently lengthened by involution (Fig. 3C; reviewed by Wessel and Wikramanayake, 1999). Once all cells have been internalized in the sea urchin embryo, the hindgut forms from the posterior third of the archenteron (Gustavson and Wolpert, 1967). In frog embryos, gastrulation takes place by involution of cells over the lip of the blastopore; the most internal cells form the endodermal archenteron, the most posterior of these form the hindgut epithelium (Fig. 3D). In both sea urchin and frog embryos, the archenteron, once formed, is elongated by cell rearrangement (Keller *et al.,* 1985; Wessel and Wikramanayake, 1999).

Similar to what occurs in sea urchin and *Xenopus,* the hindgut in many chordates develops from the last cells to be internalized during gastrulation (reviewed by Holland, 2000). In birds and mammals, endodermal cells ingress during gastrulation through the primitive streak (Figs. 3D and 3E); the hindgut then arises from an invagination of the posterior endoderm (Fig. 3E; Roberts *et al.,* 1995; Roberts, 2000). The cells in the hindgut primordium proliferate, contributing to an increase in overall size and length; the anus then forms as an ectodermal invagination that joins the hindgut.

Internalization during gastrulation of the posterior gut primordium in *Drosophila* appears morphologically similar to early steps in sea urchin gastrulation. First, the posterior midgut primordium invaginates by apical constriction; next, the proctodeal ring, consisting of several hundred cells, draws together at the surface of the embryo and is internalized by involution (Fig. 1; Harbecke and Janning, 1989; Campos-Ortega and Hartenstein, 1997; Wu and Lengyel 1998; reviewed by Leptin, 1999). Expression of *folded gastrulation* (*fog,* encoding a novel cell signaling molecule) in the posterior gut primordium is required for its invagination (Costa *et al.,* 1994; Morize *et al.,* 1998), suggesting that, as in sea urchin gastrulation, invagination is driven by a cell autonomous process. Fog is postulated to activate a cell signaling pathway leading to actin cytoskeleton reorganization, thereby effecting the cell shape changes required for invagination (Costa *et al.,* 1994; Barrett *et al.,* 1997; Häcker and Perrimon, 1998; reviewed by Leptin, 1999). Since expression of *fog* at the posterior of the embryo requires *tll, hkb, cad,* and *fkh* (Costa *et al.,* 1994; Wu and Lengyel, 1998), the first step in hindgut morphogenesis, invagination, depends on correct patterning of the blastoderm.

cad, byn, fkh, AND *wg:* AN EVOLUTIONARILY CONSERVED "CASSETTE" INVOLVED IN GASTRULATION

Although *tll* and *hkb* are not known to play conserved roles in gastrulation or gut formation outside the arthropods, the overlapping expression and required functions of *cad, fkh, byn,* and *wg* resemble the expression and required function, during gastrulation and gut development in other organisms, of the Cdx, HNF-3 (FoxA), Brachyury (T), and Wnt genes (Fig. 3). These four genes (or gene families) are expressed in the blastopore equivalent, namely the *Drosophila* amnioproctodeal invagination (which gives rise to the posterior midgut, hindgut and Malpighian tubules), the sea urchin and *Xenopus* blastopore, the zebrafish blastopore

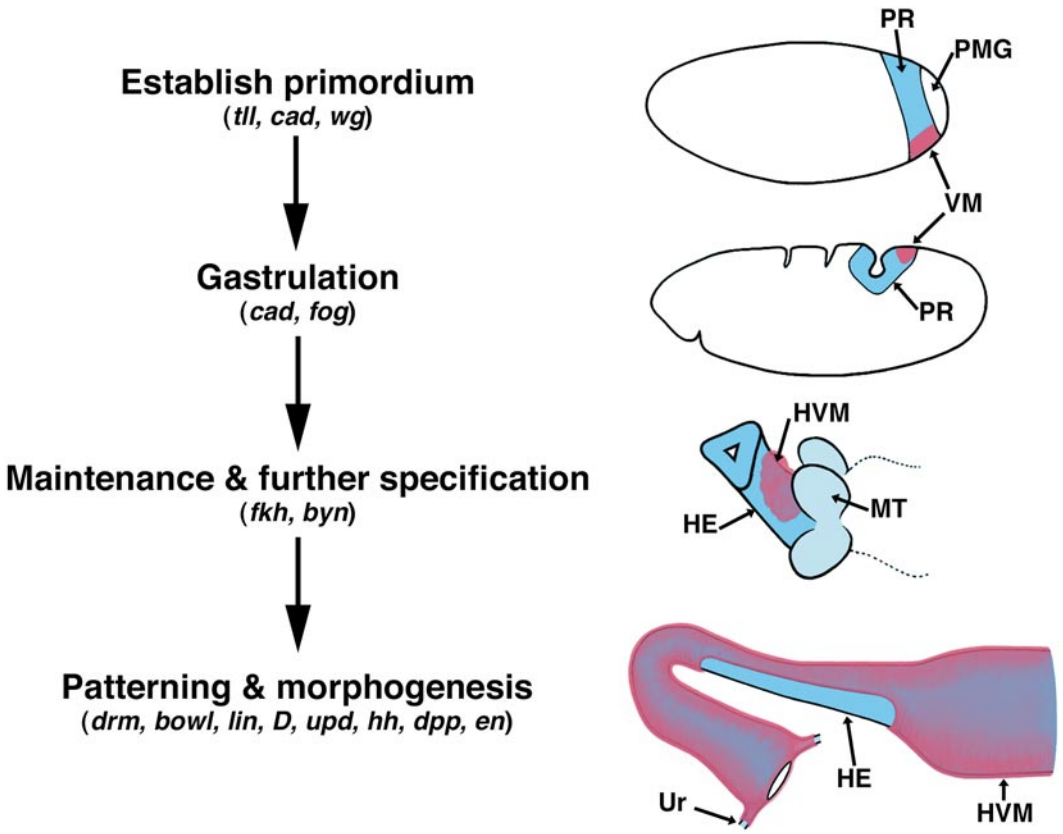

FIG. 1. Steps in *Drosophila* hindgut development. Cells are committed to the proctodeal ring (PR) at the blastoderm stage (stage 5); the most ventral cells of this ring will become visceral mesoderm (VM). During gastrulation, the posterior midgut (PMG) primordium is internalized by invagination, and the proctodeal ring follows by involution (stage 7/8). After the completion of germband extension, and during segmentation (stage 10/11), the Malpighian tubules (MT) evaginate from the proctodeal primordium; the hindgut visceral mesoderm (HVM) is associated with the hindgut epithelium (HE) and begins to migrate around it. By the end of germband shortening (stage 13), the hindgut has elongated significantly by mediolateral cell rearrangement, and is completely surrounded by the HVM; a cutaway shows the underlying hindgut epithelium. The two ureters (Ur) connect the four Malpighian tubules to the hindgut. Hindgut epithelium (or its primordium) is shown in blue, visceral mesoderm (or its primordium) is shown in red.

ring, and the amniote primitive streak. Their overlapping expression and related function (where characterized) suggest that these genes have a shared ancestral function.

cad/Cdx. In the *Drosophila* embryo, *cad* transcripts are present first in an anteroposterior (AP) gradient (highest at the posterior), and then in the hindgut primordium, in a ring that disappears after gastrulation (Fig. 3B; Wu and Lengyel, 1998). Mutant analysis shows that *cad* is necessary for internalization and maintenance (prevention of apoptosis) of the hindgut primordium; at least part of this effect is due to the required role of *cad* in expression of *wg*, *fkh*, and *fog* in the hindgut primordium (Wu and Lengyel, 1998). *cad* is also expressed later, in a similar AP gradient (of unknown function) at the posterior of the midgut (Fig. 3B) (Wu and Lengyel, 1998).

In *C. elegans*, the *cad* homolog *pal-1* is expressed maternally in posterior cells of the early embryo, and zygotically in mesoderm cells of the posterior gut (Fig. 3A; Edgar *et al.*, 2001). In vertebrates, the three Cdx genes are expressed in a graded AP pattern during gastrulation, and then later in the gut (Figs. 3D and 3E; reviewed by Freund *et al.*, 1998). Vertebrate Cdx genes are believed to be derived from the most posteriorly expressed gene of a Hox cluster-like "ParaHox" complex (Brooke *et al.*, 1998). The graded AP expression of Caudal genes in the posterior of many embryos at the beginning of gastrulation (Fig. 3) suggests that the ancestral function of Caudal, as the most posteriorly expressed ParaHox gene, was in patterning the most posterior portion of the embryo (Brooke *et al.*, 1998). The graded expression, and required function of Caudal genes in the gut may be a continuation, or co-option, of their earlier AP patterning role.

byn/Brachury. In *Drosophila*, *byn* is expressed in the ring of cells that will be internalized to form the hindgut, and continues to be expressed in the hindgut throughout embryogenesis (Fig. 3B; Kispert *et al.*, 1994). In *byn* mu-

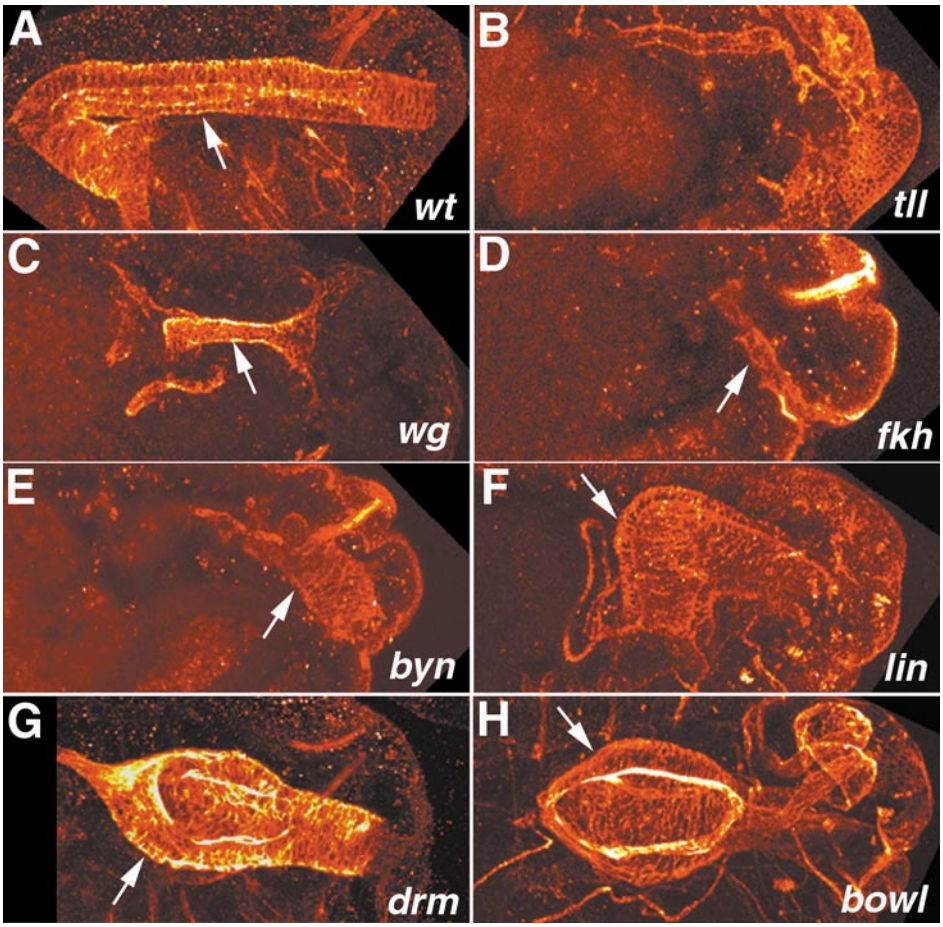

FIG. 2. Mutants affecting *Drosophila* hindgut development. Stage 16 embryos were incubated with the primary antibodies anti-Crumbs [α-Crb, which labels the apical (luminal) surface of the hindgut epithelium (Tepass *et al.*, 1990)], and anti-Connectin [α-Con, which labels the hindgut visceral mesoderm (Nose *et al.*, 1992)]; staining with Cy3-labeled secondary antibody was visualized by confocal microscopy. Genotypes are wild type (A) and null alleles of: *tll* (B), *wg* (C), *fkh* (D), *byn* (E), *lin* (F), *drm* (G), and *bowl* (H). The hindgut, or hindgut remnant, is indicated by an arrow. No hindgut is detectable in *tll* (B); the hindgut is very short and narrow in *wg*, *fkh*, and *byn* (C, D, and E, respectively). The hindgut is shorter and much wider in *lin*, *drm*, and *bowl* (F, G, and H, respectively). HVM is not detected in *wg* and *fkh* (C and D).

tants, the proctodeum forms and invaginates normally, but begins undergoing apoptosis by the end of germband extension, resulting in a short hindgut remnant that lacks its central subdomain, the large intestine (Fig. 2E; Singer *et al.*, 1996; J.A.L., unpublished). At least part of the role of *byn* in specification and maintenance of the *Drosophila* hindgut may be attributed to its early function of activating *orthopedia* (*otp*, a homeodomain gene) throughout the primordium, and its later function of activating *dpp* and *engrailed* (*en*) in the large intestine (Singer *et al.*, 1996; J.A.L., unpublished).

Brachyury is expressed in the blastopore equivalent throughout the bilateria; once cells have been internalized by gastrulation, expression continues either in the hindgut (ecdysozoans, echinoderms, and hemichordates) or in the notochord (cephalochordates and vertebrates) (Fig. 3; reviewed by Technau, 2001; Shoguchi *et al.*, 1999; Harada *et al.*, 1995; Peterson *et al.*, 1999a,b; Tagawa *et al.*, 1998; Holland *et al.*, 1995; Herrmann and Kispert, 1994). While notochord and gut are distinct later during development, it is worth noting that these tissues are continuous during early development in both amphioxus and mouse (Conklin, 1933; Sasaki and Hogan, 1993). In *Xenopus*, Brachyury is required for convergent extension (Conlon and Smith, 1999), the midline convergence and radial plus mediolateral cell intercalation that drives gastrulation and elongation of the embryonic AP axis (Keller *et al.*, 1985; Warga and Kimmel, 1990). The conserved expression of Brachyury in the blastopore equivalent, and subsequently in tissue undergoing elongation by cell rearrangement (notochord, hindgut), argues that the ancestral role of Brachyury was in the early morphogenetic movements of internalization and cell rearrangement (Wu and Lengyel, 1998; Holland, 2000; McGhee, 2000).

TABLE 1

Genes Involved in *Drosophila* Hindgut Development

Gene	Homology/molec. function	Hindgut phenotype of mutant	Role in hindgut	Ref.
tll	Nuclear receptor/transcription	No primordium	Establish primordium	1
fkh	HNF-3/transcription	Degeneration starting st. 12	Maintain primordium	2, 3
byn	T-domain/transcription	Large intestine degeneration, beginning st. 11	Gene regulation throughout hindgut; maintain, specify large intestine	4–6
bowl	Zn finger/transcription	Shorter, wider	Establish small intestine, control elongation	7, 8
drm	Zn finger/transcription	Shorter, wider	Establish small intestine, control elongation	8–10
lin	Novel, nuclear localized/ transcription?	Shorter, distended	Repress small intestine, control elongation	8, 11, 12
D	Sox/transcription	Variably shorter, wider	Control large intestine gene expression, elongation	13
en	Homeodomain/transcription	Morphology normal, no boundary cells	Establish boundary cells	11, 14
otp	Homeodomain/transcription	[a]	Gene regulation throughout hindgut?	15
bap	Homeodomain/transcription	Shorter, wider	Visceral mesoderm function	16
dri (retn)	ARID domain/DNA binding	Disorganized boundary cells	Gene regulation in primordium & boundary cells?	17
Sox 100B	Sox/transcription	[a]	Gene regulation in boundary cells?	18
wg	Wnt/cell signaling	Very short	Establish and maintain both epithelium and visceral mesoderm	11, 19, 20, 21
Ser	Notch ligand	Normal	?	22
Dl	Notch ligand	Extremely defective	Establish boundary cells	23
hh	Hh/cell signaling	Reduced rectum and small intestine	Establish primordium? Maintain small intestine and rectum	14, 19
upd	Cell signaling activates JAK/ STAT	Shorter, wider	Elongation	24, 25
dpp	BMP4/cell signaling	Shorter large intestine	DNA endoreplication in large intestine	14, 19
crb	Notch-like/cell surface	Some degeneration	Establish apical polarity, maintain epithelium	26, 27
fas	Ig-type/cell adhesion	Shorter, narrower large intestine	Cell–cell interaction, maintain large intestine	9, 28
thr	Novel/sister chromatid separation	Shorter, wider	Cell division	9, 29, 30
raw	Novel	Large intestine shorter, constricted	Maintain, specify large intestine	9, 31–33
mmy	Not determined	Large intestine shorter, constricted	Maintain, specify large intestine	11
rib	BTB/POZ domain transcription	Cells more squamous	Control cell shape/movement	31, 32, 34, 35
crn (yok)	Spliceosome assembly	Shorter, w/ narrow rugose lumen[b]	Elongation?	11, 36
phm (exo)	Not determined	Longer, more curved[c]	Control cell number and/or elongation?	11

Note. References: Pignoni *et al.*, 1990 (1); Weigel *et al.*, 1989a,b (2, 3); Kispert *et al.*, 1994 (4); Murakami *et al.*, 1995 (5); Singer *et al.*, 1996 (6); Wang and Coulter, 1996 (7); Iwaki *et al.*, 2001 (8); Liu, X., *et al.*, 1999 (9); Green *et al.*, manuscript in preparation (10); Harbecke and Lengyel, 1995 (11); Hatini *et al.*, 2000 (12); Sanchez-Soriano and Russell, 2000 (13); Takashima and Murakami, 2001 (14); Simeone *et al.*, 1994 (15); Azpiazu and Frasch, 1993 (16); Shandala *et al.*, 1999 (17); Loh and Russell, 2000 (18); Hoch and Pankratz, 1996 (19); Skaer and Martinez Arias, 1992 (20); San Martin and Bate, 2001 (21); Thomas *et al.*, 1991 (22); Iwaki and Lengyel, manuscript submitted (23); Harrison *et al.*, 1998 (24); Johansen and Lengyel, manuscript in preparation (25); Tepass and Knust, 1990 (26); Skaer, 1993 (27); Lekven *et al.*, 1998 (28); D'Andrea *et al.*, 1993 (29); Philp *et al.*, 1993 (30); Jack and Myette, 1997 (31); Blake *et al.*, 1998 (32); Byars *et al.*, 1999 (33); Bradley and Andrew, 2001 (34); Shim *et al.*, 2001 (35); Chung *et al.*, 1999 (36).

[a] No known mutant alleles.

[b] Characterized in the *yolky (yok)* allele.

[c] Characterized in the *exocephalon (exo)* allele.

fkh/HNF-3. In *Drosophila, fkh* is expressed in the gut primordia prior to gastrulation; this expression is maintained in the developing gut (foregut, midgut, hindgut) during embryogenesis (Fig. 3B). The gut primordium forms and is internalized normally in *fkh* mutants but then begins to undergo apoptosis; by late embryogenesis, there is only a very short hindgut remnant (Fig. 2D; Weigel *et al.,* 1989a; Wu and Lengyel, 1998). *fkh* is required for activation of all genes (except for *byn*) known to be expressed in the gut epithelium (Weigel *et al.,* 1989a; Kispert *et al.,* 1994; Hoch and Pankratz, 1996). Thus *fkh,* rather than specifying particular domain(s) of the *Drosophila* gut, acts to maintain gene expression (and thereby prevent apoptosis) throughout the entire gut epithelium.

HNF-3, like Brachyury, is expressed in the blastopore equivalent of bilaterians; once cells have been internalized by gastrulation, expression of HNF-3 continues in the gut (ecdysozoans, echinoderms, and hemichordates) or in the gut endoderm, plus notochord and floorplate (urochordates, cephalochordates, and vertebrates) (Weigel *et al.,* 1989b; Azzaria *et al.,* 1996; Horner *et al.,* 1998; Kalb *et al.,* 1998; Harada *et al.,* 1996; Olsen and Jeffery, 1997; Shimauchi *et al.,* 1997; Di Gregorio *et al.,* 2001; Taguchi *et al.,* 2000; Shimeld, 1997; Strähle *et al.,* 1993; Ruiz i Altaba *et al.,* 1993; Monaghan *et al.,* 1993; Sasaki and Hogan, 1993; reviewed by Harada *et al.,* 1996). HNF-3 is required for proper formation of the node (blastopore equivalent) in mouse, and for gut development in *C. elegans* (Ang and Rossant, 1994; Weinstein *et al.,* 1994; Mango *et al.,* 1994). While HNF-3β is required for foregut and midgut development in mouse, it is not required for hindgut development, likely due to overlapping expression of HNF-3α (Dufort *et al.,* 1998). Consistent with the results of mutant analysis in flies, worms, and mice, *in vivo* footprinting studies suggest that HNF-3 proteins confer competence for gene expression in the developing gut (Zaret, 1999). Thus the conserved function of HNF-3 appears to be in establishing and maintaining cells that internalize during gastrulation.

wg/Wnt. In *Drosophila, wg* is expressed throughout the hindgut primordium prior to and during gastrulation; this early expression then rapidly disappears (Fig. 3B; Hoch and Pankratz, 1996; Wu and Lengyel, 1998). Analysis of loss-of-function and temperature-sensitive mutants indicates that this early *wg* expression is required for establishment of the hindgut primordium, for postblastoderm mitoses, and for maintenance of the hindgut (Skaer and Martinez Arias, 1992; Harbecke and Lengyel, 1995; Hoch and Pankratz, 1996; K. A. Johansen and J.A.L., unpublished data).

Like Brachyury and HNF-3, members of the Wnt family of cell signaling proteins are expressed in the bilaterian blastopore equivalent. Wnt signaling is observed in the sea urchin vegetal plate (Logan *et al.,* 1999), and multiple Wnts are expressed in the vertebrate blastopore/primitive streak (Figs. 3C through 3E). At least two vertebrate Wnts are required for aspects of gastrulation. Wnt3, expressed in the primitive streak of mouse, is required for formation of the primitive streak, mesoderm, and node (Liu, P., *et al.,* 1999). Wnt11, expressed in the blastopore of both *Xenopus* and zebrafish, appears to be involved in controlling convergent extension movements of gastrulation (Tada and Smith, 2000; Wallingford *et al.,* 2000; Heisenberg *et al.,* 2000). In addition to the overlap of Wnt and Brachyury expression, there are required regulatory interactions among these genes in the blastopore equivalent: in mouse, Wnt3 upregulates Brachyury; in *Drosophila, byn* is required for *wg* expression; in *Xenopus*, Brachyury activates Wnt11 expression (Yamaguchi *et al.,* 1999; Wu and Lengyel, 1998; Tada and Smith, 2000). A conserved role of Wnt signaling in the blastopore may thus be to coordinate cell movement during gastrulation.

A cassette involved in axial cell rearrangement. The overlapping expression and cross-regulation of the *cad*/Cdx, *byn/Brachyury*, fkh/HNF-3, and *wg*/Wnt genes in the bilaterian blastopore equivalent are striking. Where mutant analysis has been carried out, these genes/gene families are revealed to be required for processes of gastrulation (formation of primitive streak and node, convergent extension) and maintenance (proper gene expression, elongation, prevention of apoptosis) of the internalized tissue (gut, notochord). The accumulation of expression and mutant phenotype data (described above) supports our earlier speculation that the Cdx, Brachyury, HNF-3, and Wnt genes constitute an evolutionarily conserved "cassette" functioning in gastrulation and attendant axial elongation (Wu and Lengyel, 1998).

Recently, evidence has been obtained on gene expression in cnidarians (diploblastic, phylogenetically basal metazoans) that provides further support for the idea of such a cassette, and additionally suggests that the cassette is evolutionarily very ancient. The cnidarian *Hydra,* which does not undergo gastrulation, expresses Brachyury, HNF-3, and Wnt during budding (head formation) (Technau and Bode, 1999; Martinez *et al.,* 1997; Hobmayer *et al.,* 2000). Another cnidarian, *Nematostella* (a sea anemone), does undergo gastrulation and expresses Brachyury in the blastopore (Technau, 2001). The ancestral function of the Cdx/Brachyury/HNF-3/Wnt cassette could thus have been in gastrulation, but possibly in an even more basal, or primordial process. We propose that this ancestral function was to control cell rearrangement (connected to an opening) that elongates the body axis (head–foot in *Hydra,* oral–aboral in echinoderms, AP in other bilateria). The genes of the ancestral "axial cell rearrangement" cassette likely had the functions of regulating gene expression to establish and maintain the primordium, establishing cell polarity to orient the rearrangement, and modulating adhesion of the rearranging cells. These properties are collectively retained by the present-day constituent genes of the cassette.

EPITHELIAL/VISCERAL MESODERM INTERACTIONS IN THE HINDGUT

In vertebrates, interaction between epithelium and investing mesodermal mesenchyme has long been known to be required for proper morphogenesis and differentiation of

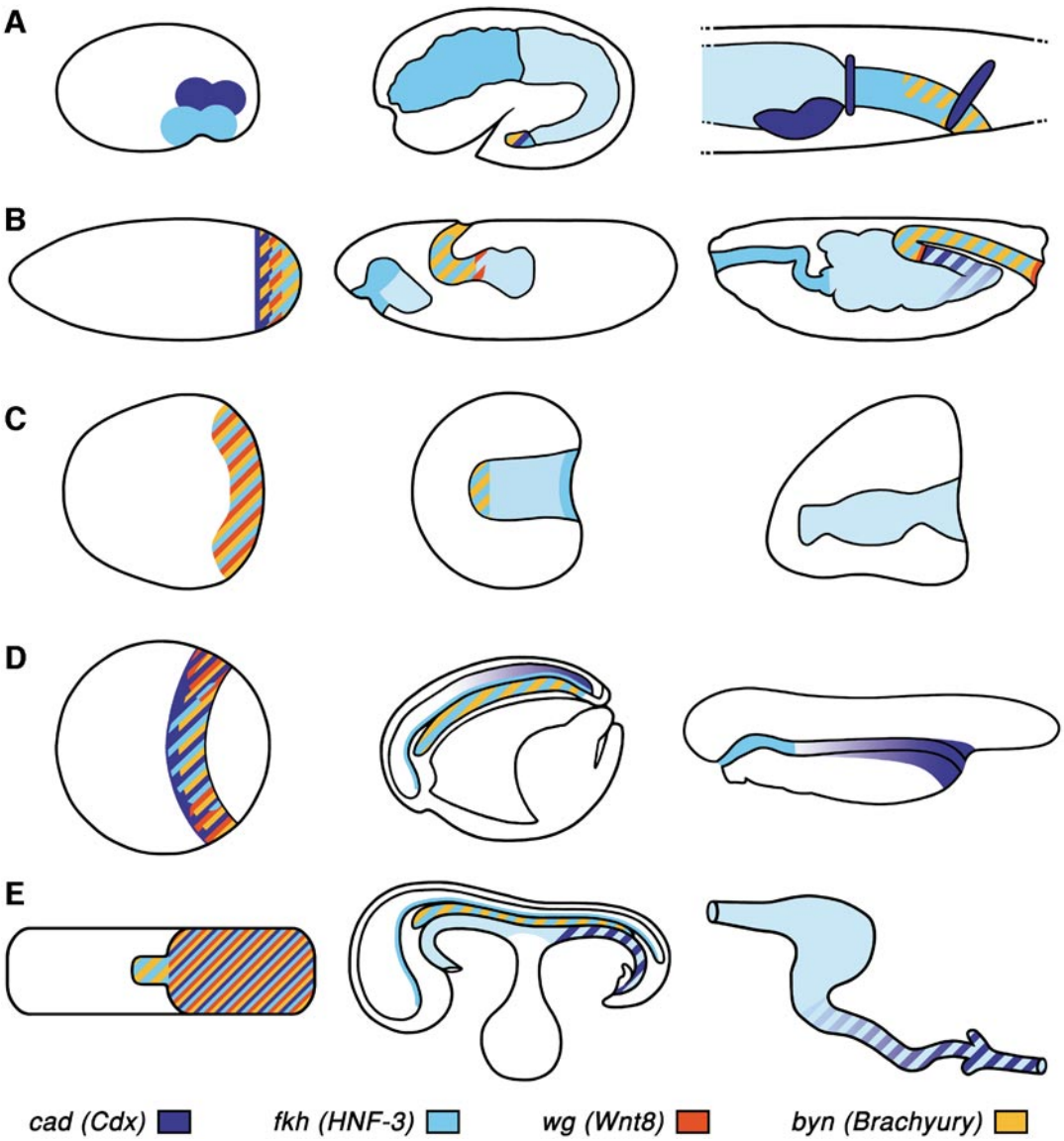

FIG. 3. Conserved gene expression during hindgut development. Three stages in hindgut development are shown for: *C. elegans* (A), *Drosophila* (B), sea urchin (C), *Xenopus* (D), and mouse (E). For each organism, a superficial view of the embryo at the initiation of gastrulation (left column), and silhouettes of the gut at the completion of gastrulation (central column) and in the mature embryo/larva (right column) are shown. For the mouse at the beginning of gastrulation, the right-hand side of the diagram indicates the primitive streak; its leftward protrusion indicates the developing notochord. In the right column, only the posterior gut of *C. elegans* and the gut beginning with the stomach for mouse are shown. Expression domains are color-coded: *cad*/Caudal, purple; *fkh*/HNF-3, blue; *wg*/Wnt, red; *byn*/Brachyury, gold. References: (A) *C. elegans: cad/pal-1* (Edgar *et al.*, 2001), *fkh/Ce-fkh-1/pha-4* (Azzaria *et al.*, 1996; Horner *et al.*, 1998; Kalb *et al.*, 1998), Brachyury/*mab-9* (Woollard and Hodgkin, 2000); (B) *Drosophila: cad, wg* (Wu and Lengyel, 1998; Hoch and Pankratz, 1996); fkh (Weigel *et al.*, 1989a,b); byn (Kispert *et al.*, 1994; Singer *et al.*, 1996); (C) sea urchin: HNF-3, Brachyury (Harada *et al.*, 1995, 1996), Wnt (Angerer and Angerer, 2000) (D) *Xenopus:* Xcad (Pillemer *et al.*, 1998); HNF-3 (Ruiz i Altaba *et al.*, 1993; Lef *et al.*, 1996), Brachyury (Smith *et al.*, 1991; Cunliffe and Smith, 1994), XWnt-8, XWnt11 (Moon *et al.*, 1993; Du *et al.*, 1995; Tada and Smith, 2000); (E) mouse: Caudal (James and Kazenwadel, 1991; Meyer and Gruss, 1993; Gamer and Wright, 1993; Freund *et al.*, 1998); HNF-3β (Ang *et al.*, 1993; Monaghan *et al.*, 1993); Brachyury (Kispert and Herrmann, 1994); Wnt3 (Liu, P. *et al.*, 1999).

many endodermal epithelial organs (reviewed by Roberts, 2000). These interactions are reciprocal and depend on secretion by both tissues of cell signaling molecules. Vertebrate lung morphogenesis, for example, depends on both FGF-10 expression in the mesoderm and BMP4 and Hh expression in the endoderm (reviewed by Warburton *et al.*,

2000; Cardoso, 2000). In the vertebrate hindgut, Sonic hedgehog (Shh) expression in the epithelium induces expression of BMP4 and Abd-B-like Hox genes in the visceral mesoderm; Shh, Hoxa-13, and Hoxd-13 are required for normal rectal/anal development (reviewed by Roberts, 2000; Grapin-Botton and Melton, 2000). In *Drosophila,* Dpp and Wg expression (turned on by HoxC genes) in the midgut mesoderm is required to signal to the epithelium and control its expression of *labial* (reviewed by Bienz, 1994).

In *Drosophila*, the hindgut visceral mesoderm (HVM) arises from the most ventral portion of the proctodeal ring, which is internalized during gastrulation (Fig. 1; Kusch and Reuter, 1999; San Martin and Bate, 2001). At germ band extension, the HVM progenitors form a group of cells on the future ventral side of the hindgut epithelium that begin to express the cell adhesion protein Connectin and the homeodomain protein encoding *bagpipe* (*bap*) (Figs. 1 and 4C; Nose *et al.,* 1992; Azpiazu and Frasch, 1993; Skaer, 1993; Hoch and Pankratz, 1996; San Martin and Bate, 2001). The HVM cells migrate around the hindgut epithelium, completely surrounding it by the end of germ band retraction and developing into a single-layered ring of circular muscles around the mature hindgut (Figs. 1, 2A, and 4D; Bate, 1993; Kusch and Reuter, 1999; San Martin and Bate, 2001).

Multiple lines of evidence reveal a required interaction between the *Drosophila* hindgut epithelium and surrounding HVM. In *wg* mutants, as indicated by absence of both *bap* and Connectin expression, there is no HVM (Fig. 2C; Hoch and Pankratz, 1996; San Martin and Bate, 2001). In *fkh* mutants, the HVM disappears (Fig. 2D), concomitant with apoptosis in the hindgut epithelium; this is presumably due to reduced *wg* expression in the hindgut epithelium of *fkh* mutants (Hoch and Pankratz, 1996; San Martin and Bate, 2001; J.A.L., unpublished data). In *hh* mutants, expression of *bap* in the HVM is reduced, revealing an induction that may be evolutionarily related to the Shh-based induction of Hox-C genes in the vertebrate hindgut mesoderm (Hoch and Pankratz, 1996; Roberts, 2000). Taken together, these data indicate that signaling from the hindgut epithelium (in the form of Wg, Hh, and possibly other molecules) is required to establish and maintain the HVM. There is likely also signaling from the HVM to the hindgut epithelium, suggested by fact that the HVM-defective mutants *twist* and *bap* have defects in hindgut morphogenesis (San Martin and Bate, 2001; J.A.L., unpublished data). The tools available in *Drosophila* should make it possible to further dissect the genetic bases of these reciprocal epitheliomesenchymal interactions in the hindgut.

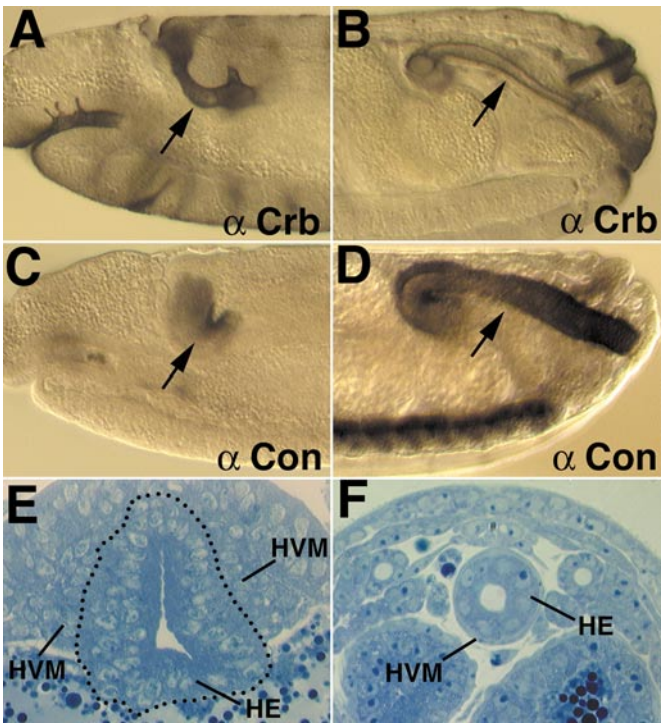

FIG. 4. Cell rearrangement, cell shape change, and visceral mesoderm investment of *Drosophila* hindgut. Embryo whole mounts are shown at completion of germband extension (stage 11) (A, C, and E) and at the completion of organogenesis (stage 16) (B, D, and F). Arrows indicate the apical surface of the hindgut epithelium (HE) stained with α-Crb (A and B), and the hindgut visceral mesoderm (HVM) stained with α-Con (C and D). Light micrographs of cross sections of the hindgut at stages 11 and 16 (same magnification for both) are shown in E and F; cells of the HE and HVM are indicated.

CELLULAR BASIS OF *Drosophila* HINDGUT MORPHOGENESIS: CHANGES IN CELL SIZE, CELL SHAPE, AND CELL REARRANGEMENT

Cellular processes of migration, apoptosis, proliferation, changes in shape and size, and rearrangement can individually or collectively contribute to morphogenesis and organogenesis. During development of the *Drosophila* embryo hindgut, the last two processes play primary roles. The cells of the hindgut (in contrast to those of the midgut) remain in a coherent single-layered epithelium throughout embryogenesis (Tepass and Hartenstein, 1994b; Campos-Ortega and Hartenstein, 1997), ruling out a contribution of cell migration to embryonic hindgut development. Hindgut size and morphology appear normal in embryos lacking apoptosis genes (Iwaki *et al.,* 2001), indicating that programmed cell death does not play a major role in molding the embryonic hindgut. Finally, in contrast to the situation in vertebrates, where regulated cell proliferation is an important contributor to organ shape, cell proliferation does not contribute significantly to *Drosophila* embryonic hindgut size or shape. The cells of the proctodeal ring arise not by proliferation, but as a result of the early syncytial nuclear divisions and cellularization that establish the blastoderm. After the blastoderm stage, there are two rounds of mitosis, in which most of the cells of the hindgut primordium divide

(Campos-Ortega and Hartenstein, 1997). In *string* mutants, which lack the postblastoderm divisions, the hindgut appears normal; thus the postblastoderm mitoses reduce cell size, but do not affect morphology or total cell volume of the hindgut (Hartenstein and Posakony, 1990; J.A.L., unpublished).

Processes that do play a significant role in the morphogenesis of the *Drosophila* hindgut epithelium are changes in cell shape and size, and cell rearrangement, as described below. The characterization of mutants has identified genes and molecules essential for these processes.

Cell shape change: Columnar to cuboidal. As the hindgut elongates, the initially tall and columnar epithelial cells become dramatically shorter and thicker in cross section, leading to an increase in epithelial surface area (Figs. 4E and 4F; Campos-Ortega and Hartenstein, 1997; Iwaki *et al.*, 2001). The cells forming the large intestine become cuboidal, while those forming the small intestine become flatter and more squamous (Iwaki *et al.*, 2001). Analysis of cell shape in mutants with defective hindgut morphology suggests that establishment and maintenance of correct cell size and proportions play a role in hindgut morphogenesis. *raw, ribbon* (*rib*), and *Dichaete* (*D*) mutants have irregular, abnormally squamous epithelial cells (Jack and Myette, 1997; Blake *et al.*, 1998; Sanchez-Soriano and Russell, 2000). *lines* (*lin*) mutants have squamous epithelial cells throughout the hindgut, rather than just in the small intestine, while *drumstick* (*drm*) mutants fail to carry out the columnar to cuboidal hindgut cell shape change (Figs. 2F and 2G; Iwaki *et al.*, 2001).

Cell size: Decrease then increase. As mentioned above, the two postblastoderm mitoses reduce cell size throughout the embryo, including the hindgut. Genes required zygotically for postblastoderm mitoses throughout the embryo are *string* (*stg*), *three rows* (*thr*), *barren* (*barr*), *pebble* (*pbl*), and *pimples* (*pim*) (Edgar and O'Farrell, 1989; Hime and Saint, 1992; D'Andrea *et al.*, 1993; Philp *et al.*, 1993; Bhat *et al.*, 1996). All of these, when mutant, have dramatically larger cells than the wild-type embryo (and also affect Malpighian tubule morphogenesis), but only *thr* appears to be required for normal hindgut morphogenesis (Hartenstein and Posakony, 1990; Harbecke and Lengyel, 1995; Liu, X., *et al.*, 1999; Jack and Myette, 1999). Thus, neither the increase in cell number nor the reduction in cell size brought about by the two postblastoderm mitoses is essential for normal hindgut morphogenesis.

While the reduction of cell size brought about by the postblastoderm mitoses is not essential for normal hindgut morphogenesis, a later increase in cell size increases the length of the hindgut. After stage 13, large intestine cells undergo a roughly twofold endoreplication of their DNA (Smith and Orr-Weaver, 1991). Regulated positively by *dpp* and negatively by *knirps* and *knirps-related*, this endopolyploidization results in an increase in cell size and a corresponding increase in the length of the large intestine (Fuss *et al.*, 2001; Takashima and Murakami, 2001).

Cell rearrangement drives AP elongation. This key component of *Drosophila* hindgut elongation shares features with other embryonic processes. During gastrulation in *Xenopus* and zebrafish, cells converge toward the midline and intercalate between each other mediolaterally, causing elongation of the embryo AP axis (Keller *et al.*, 1985; Warga and Kimmel, 1990). A partial molecular pathway controlling this process of convergent extension has been elucidated. Expression of Brachyury/T-box transcription factors in the blastopore regulates expression of a Wnt (Wnt11) and a cadherin (PAPC) (Yamamoto *et al.*, 1998; Tada and Smith, 2000). Wnt11 activates the planar cell polarity (PCP) pathway, which orients mediolateral cell intercalation; PAPC is proposed to mediate transient associations between intercalating cells (Heisenberg *et al.*, 2000; Wallingford *et al.*, 2000; Yamamoto *et al.*, 1998).

In addition to its role in gastrulation and embryonic axis elongation, cell rearrangement contributes to the AP elongation of a number of epithelial tubes, including the sea urchin archenteron, axolotl nephric duct, *C. elegans* intestine, and insect Malpighian tubule (Ettensohn, 1985; Poole and Steinberg, 1981; Saxen, 1987; Leung *et al.*, 1999; Skaer, 1992). Cell rearrangement also drives elongation of the *Drosophila* germ band, ovarian terminal filaments, leg and spiracle, and the *C. elegans* dorsal epidermis. The transcriptional regulators Even-skipped, Bric-a-brac, Grain, and DIE-1, respectively, are required for each of these processes, but the targets mediating their effects have not been defined (Irvine and Wieschaus, 1994; Godt and Laski, 1995; Brown and Castelli-Gair, 2000; Heid *et al.*, 2001).

The *Drosophila* hindgut also elongates, from a short, thick tube to a long, narrow cylinder, by cell rearrangement (Figs. 1, 4A, and 4B; Singer *et al.*, 1996; Campos-Ortega and Hartenstein, 1997; Iwaki *et al.*, 2001). Mutants in a number of genes that might be expected to play a role in cell rearrangement, i.e., those encoding E-cadherin and other cell surface proteins (*shotgun, crumbs, faint sausage*), have only weak and variable effects on hindgut morphogenesis, presumably due to the maternal provision of their products (Table 1; Tepass and Knust, 1990; Knust *et al.*, 1993; Wodarz *et al.*, 1995; Uemura *et al.*, 1996; Tepass, 1996; Tepass *et al.*, 1996; Grawe *et al.*, 1996; Lekven *et al.*, 1998; Liu, X., *et al.*, 1999). Mutations in several genes, however, namely *drm, bowl,* and *lin,* have a dramatic effect on cell rearrangement in the hindgut (Figs. 2F through 2H; Harbecke and Lengyel, 1995; Wang and Coulter, 1996; Liu, X., *et al.*, 1999; Hatini *et al.*, 2000; Iwaki *et al.*, 2001; Green *et al.*, manuscript in preparation). Drm, Bowl, and Lin are putative transcriptional regulators and, as is discussed below, are required to pattern the developing hindgut into three morphological domains with distinct patterns of gene expression. At least one of the genes regulated by *drm, bowl,* and *lin* encodes a signaling molecule that may be involved in controlling cell rearrangement in the hindgut.

In conclusion, cell shape change and cell rearrangement drive *Drosophila* hindgut morphogenesis. The genes *drm, bowl,* and *lin* control these processes and encode putative

transcriptional regulators that control gene expression in the hindgut. The role of this patterned gene expression in hindgut morphogenesis is described below.

MORPHOLOGICAL SUBDIVISION OF THE HINDGUT

The hindgut in most organisms is divided into a number of morphologically distinct regions along the AP axis, and is delimited by a sphincter at each end. The simple, 12-cell C. *elegans* hindgut epithelium has an intestinal rectal valve (two cells) at the anterior and an anus (one cell) at the posterior; each valve is associated with a single muscle cell (White, 1988). At the other extreme of complexity, the developing hindgut in vertebrates forms the pouch-like cecum at its anterior end, the large intestine (ascending, transverse, descending, and sigmoid colon in humans), and the cloaca (in birds) or rectum (in mammals) at its posterior end (reviewed by Roberts *et al.,* 1995; Ross *et al.,* 1995). Two sphincters define the ends of the vertebrate hindgut: the ileocaecal sphincter at the junction with the small intestine, and the anal sphincter at the posterior terminus. Along the length of the mammalian colon, some histological differences are seen: a change in the ratio of absorptive to mucus-secreting epithelial cells, and a difference in the organization of the longitudinal musculature (Ross *et al.,* 1995).

In *Drosophila,* the invaginated proctodeum gives rise to the Malpighian tubules and the epithelium of the hindgut proper. The four Malpighian tubule buds evaginate from the proctodeum at its junction with the posterior midgut (Fig. 1; Skaer, 1993); intriguingly, the development of these finger-like protrusions at the midgut/hindgut junction appears superficially similar to the formation of the avian ceca and mammalian appendix at the intestine/hindgut junction. This prompts the speculation that the juxtaposition of two differently fated epithelial domains, i.e., posterior midgut and hindgut in *Drosophila,* or intestine and hindgut in vertebrates, constitutes an organizing center for the evagination of tubules.

The developing *Drosophila* hindgut becomes subdivided in the AP axis, as is common for most hindguts, and also, unusually, in the dorsoventral (DV) axis. Along its AP axis, the elongating hindgut forms three morphologically distinct regions: small intestine, large intestine, and rectum (Fig. 5A; Hoch and Pankratz, 1996; Takashima and Murakami, 2001; Iwaki *et al.,* 2001). The small intestine is the most anterior region of the hindgut and connects to the posterior midgut. At the most anterior of the small intestine are two ureters, each of which drains a pair of Malpighian tubules (Skaer, 1993); just posterior to the insertion of the ureters are the cells of the imaginal ring, which will develop into the anterior of the adult hindgut epithelium (Robertson, 1936) (see below). The major part of the small intestinal epithelium becomes surrounded by a thick muscular layer and forms the larval pyloric sphincter, a contractile valve between the posterior midgut and hindgut (Snodgrass, 1935; Murakami and Shiotsuki, 2001). Posterior to the small intestine is the narrowest and longest region of the hindgut, the large intestine. As mentioned earlier, cells of the large intestine epithelium are larger (due to endopolyploidization) and taller than cells of the small intestine.

The large intestine is the only portion of the *Drosophila* gut for which differentiation along the dorsoventral (DV) axis has been observed; three distinct regions are formed. The dorsal region (large intestine–dorsal, or li-d) constitutes the outer portion of the hindgut loop and becomes specialized for energy-dependent absorption of water and ions (Murakami and Shiotsuki, 2001); the ventral region (large intestine–ventral, or li-v) constitutes the inner portion of the loop (Fig. 5A). The boundary cells (bc) are organized as two rows between the li-d and li-v domains (where they are elongated in the AP axis), and as two rings at the anterior and posterior borders of the large intestine (Fig. 5A; Murakami *et al.,* 1999; Takashima and Murakami, 2001; Iwaki and Lengyel, manuscript submitted). At the most posterior of the hindgut is the rectum; in the larva the rectal epithelium is convoluted and surrounded by well-developed sphincter muscles (Murakami and Shiotsuki, 2001). Although the common function of the insect rectum is water resorption (Noble-Nesbitt, 1998), membrane specializations characteristic of this function are not observed in the *Drosophila* larval rectum (Murakami and Shiotsuki, 2001). The apical surface of the hindgut is covered with a secreted cuticle; this is thickest in the region of the rectum (Murakami and Shiotsuki, 2001).

Like the amniote hindgut, the simple-appearing *Drosophila* hindgut develops distinct regions along its AP axis; cells within each of these regions have different characteristic morphologies. The *Drosophila* hindgut is unusual in also differentiating morphologically distinct cell types in its DV axis. We discuss below the differential gene expression that plays a role in establishing these subregions.

PATTERNED GENE EXPRESSION IN THE HINDGUT

Commitment of cells to different gut fates, and thus to participation in different morphogenetic events, depends on finely localized gene expression. In chick and mouse, development of morphologically distinct regions along the gut AP axis depends on spatially restricted expression of homeodomain transcription factors in both epithelium (endoderm) and mesenchyme (mesoderm). Similar to their expression in other tissues, genes of the Hox and ParaHox complexes are expressed in ordered patterns along the gut AP axis that roughly reflect their order along the chromosome (reviewed by Grapin-Botton and Melton, 2000; Roberts, 2000; Beck *et al.,* 2000). Required roles for some of these genes have been determined by generation of knockout mice; for example, early expression of Pdx-1 (a putative ParaHox gene) in endoderm posterior to the stomach is

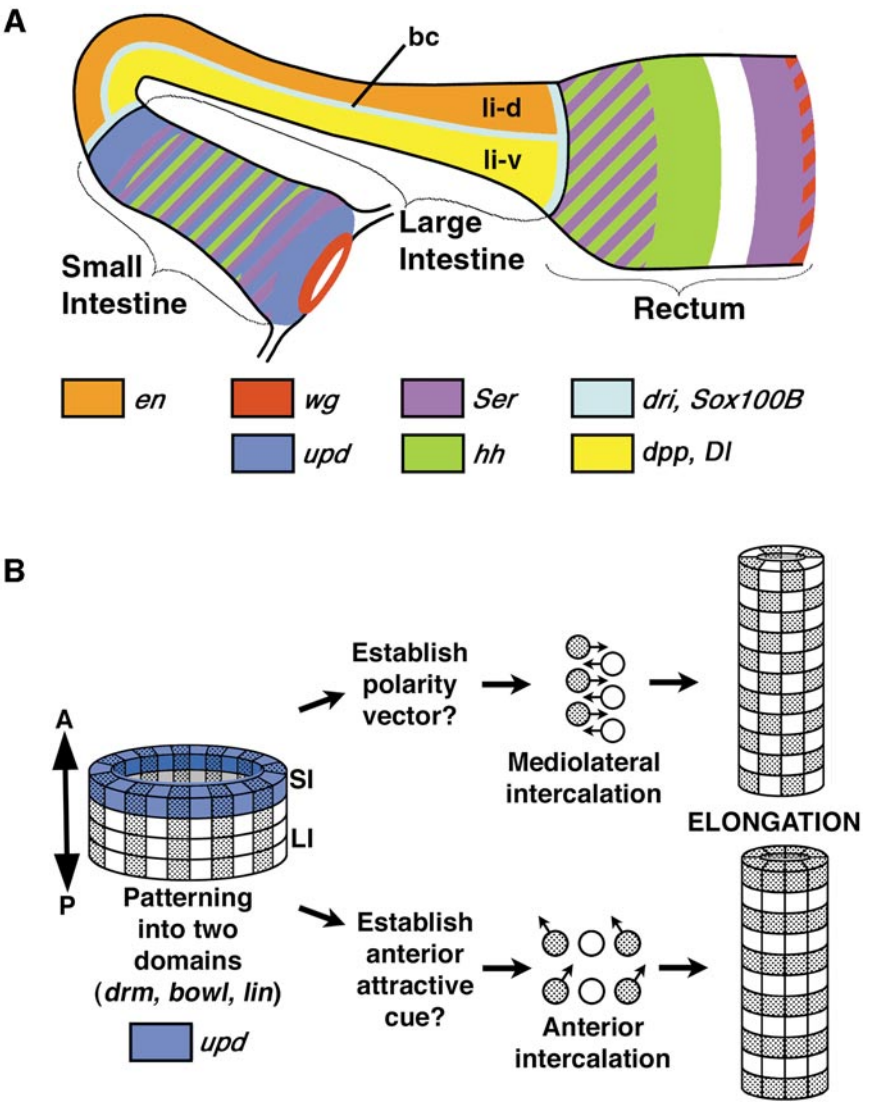

FIG. 5. Patterning of the *Drosophila* hindgut epithelium and speculations on cell rearrangement. At stage 13, distinct domains within the hindgut epithelium are recognized by the localized expression of *upd*, *hh*, *wg*, *Ser*, *dpp*, and *Dl*, which encode cell signaling molecules, and *en*, *dri*, and *Sox100B*, which encode transcriptional regulators (A, references in Table 1). The hindgut in (A) is drawn in lateral view, with anterior on the left (cf. Figs 2A and 4B); when viewed at stage 13, li-d lies on the left, and the small intestine on the right of the embryo (Campos-Ortega and Hartenstein, 1997; Hayashi and Murakami, 2001). Patterning of the hindgut into domains with distinct gene expression (e.g., *upd* in the small intestine, but not in the large intestine) might orient cell rearrangement, either by establishing a polarity vector or by providing an attractive cue (see text) (B). bc, boundary cells; li-d and li-v, dorsal and ventral domains of large intestine, respectively; SI, small intestine; LI, large intestine.

required for development of the pancreas. Both expression patterns and mutant phenotypes suggest that HoxD complex genes demarcate different regions along the AP axis, and that junctions of expressing and nonexpressing domains are required for development of the sphincters (e.g., ileocaecal and anal). In addition to these differences in the AP axis, differences in gene expression in the DV axis of the developing amniote gut are required for the establishment of organs that arise from (mostly ventral) endodermal outbuddings. For example, ventrally localized expression of the homeodomain protein Nkx2.1 is required for formation of lung and thyroid (reviewed by Roberts, 2000).

Although extensively deployed to pattern the AP axis of the vertebrate gut and the *Drosophila* midgut, Hox-C genes do not appear to play significant roles in the development of the *Drosophila* hindgut (reviewed by Bienz, 1994). A number of non-HoxC homeodomain genes are, however, expressed in the *Drosophila* hindgut; these include *cad*,

bagpipe (*bap*), *otp*, and *engrailed* (*en*) (Table 1). As described earlier, *cad* plays an early role in invagination and maintenance of the hindgut primordium. *otp* is expressed early throughout the hindgut epithelium under control of *byn*, but its function is not known, as no mutants have been described. *bap* is expressed throughout the hindgut visceral mesoderm (Simeone *et al.*, 1994; Azpiazu and Frasch, 1993); analysis of *bap* mutants reveals a requirement in hindgut morphogenesis (J.A.L., unpublished data). The only homeodomain gene known to be expressed in a subregion of the hindgut (li-d) is *engrailed* (*en*), which is required to establish the boundary cells (bc) (Fig. 5A; Takashima and Murakami, 2001). Thus in the hindgut, *en* is required not for AP, but rather for DV patterning.

A number of genes have been identified that, on the basis of morphological and gene expression criteria, are required to pattern the *Drosophila* hindgut along the AP axis into small intestine, large intestine, and rectum (Table 1, Fig. 5A). Proper gene expression in and maintenance of the large intestine requires *byn*, *D*, *raw*, *lin*, and *mummy* (*mmy*), while gene expression in and maintenance of the small intestine requires *drm* and *bowl* (Table 1; Harbecke and Lengyel, 1995; Singer *et al.*, 1996; Jack and Myette, 1997; Blake *et al.*, 1998; Liu, X., *et al.*, 1999; Sanchez-Soriano and Russell, 2000; Iwaki *et al.*, 2001). All of these genes (with the exception of *mmy*) have been molecularly characterized and encode known or proposed transcriptional regulators (Table 1). Their function in establishing different hindgut subdomains is therefore most likely due to a role in setting up and maintaining restricted patterns of target gene expression.

Of these putative transcriptional regulators controlling hindgut patterning, the function in hindgut morphogenesis of *drm*, *bowl*, and *lin* has been characterized in greatest detail. *drm*, *bowl*, and *lin* are required not only for patterning of the hindgut into small intestine and large intestine, but (as described previously) also for the cell rearrangement that elongates and narrows the hindgut (Iwaki *et al.*, 2001). This suggests that interaction between two correctly patterned domains (small intestine and large intestine) is required for cell rearrangement.

Mutations in *drm*, *bowl*, and *lin* affect the spatially localized expression of the signaling molecules Ser (a Notch ligand), Wg (a Wnt), Hh, Dpp (*Drosophila* BMP2/4) and Upd (a ligand activating the JAK/STAT pathway) (Fig. 5A). This raises the question of whether any of these signaling molecules is involved in mediating the required function of *drm*, *bowl*, and *lin* in hindgut cell rearrangement. Characterization of mutant phenotypes reveals that *Ser* and *wg* expression in the small intestine and rectum is not required for normal hindgut morphogenesis (Thomas *et al.*, 1991; Johansen, K. A., and J.A.L., manuscript in preparation). *hh* (expressed throughout the hindgut primordium and then in small intestine and rectum) is required for aspects of hindgut development (normal overall size and maintenance of rectum), but does not appear to be required to generate a hindgut of normal diameter (Hoch and Pankratz, 1996; Takashima and Murakami, 2001). Similarly, *dpp* (expressed in li-v) is required for the DNA endoreplication in the large intestine that increases hindgut size, but does not appear to be required for the coupled elongation and narrowing of the hindgut (Smith and Orr-Weaver, 1991; Takashima and Murakami, 2001). *upd* (expressed very early, and only in the small intestine) is unique among the signaling genes known to be expressed in the hindgut: its absence results in a defect in both narrowing *and* elongation of the hindgut (Johansen and Lengyel, ms. in preparation). While this suggests that Upd plays a required role in cell rearrangement, the weakness of the *upd* phenotype relative to that of *drm*, *bowl*, and *lin* suggests that additional targets of *drm*, *bowl*, and *lin* required for hindgut cell rearrangement remain to be discovered.

How might the juxtaposition of the patterned small intestine and large intestine, which is regulated by *drm*, *bowl*, and *lin*, control cell rearrangement throughout the hindgut? Two possible mechanisms are summarized in Fig. 5B. The juxtaposition of two different regions might set up an AP vector; by analogy to events occurring during convergent extension during vertebrate gastrulation (Wallingford *et al.*, 2000), cells might orient to such a vector and intercalate mediolaterally (Fig. 5B). Another possibility, analogous to cell movements described in the elongating *Drosophila* stigmatophore, is that, attracted by anteriorly produced molecule(s), cells might move and intercalate in an anterior direction (Brown and Castelli-Gair Hombria, 2000). Expression of the JAK/STAT ligand Upd in the small intestine could be relevant to either of these mechanisms, since Upd contributes to establishing polarity in the *Drosophila* eye imaginal disc (Zeidler *et al.*, 1999), and cell signaling that activates the JAK/STAT pathway is important in hematopoietic stem and tumor cell migration (Vila-Coro *et al.*, 1999; reviewed by Moore, 2001). Analysis of cell movements *in vivo*, using constructs that express green fluorescent protein (GFP) in the hindgut, is required to test these models.

LEFT–RIGHT ASYMMETRY OF HINDGUT

Most of the visceral organs of vertebrates, including the gut, are left–right (LR) asymmetric and/or situated a symmetrically along the LR axis. Thus, the heart forms by asymmetric LR looping, the morphology of the stomach is LR asymmetric, and the colon crosses the body from right to left to connect to the rectum. In vertebrates, a few conserved genes (Nodal, Pitx2) have been identified that are expressed specifically on the left side of the early embryo and appear to play a role in the development of LR asymmetry (reviewed by Yost, 1999). Even before these genes are expressed, however, the developing early embryo must integrate information from the AP and DV axes to define the LR axis; the genetic basis for this decision is under active investigation. In *C. elegans*, LR asymmetry arises as a result of skewing of mitotic spindles; this leads to LR asymmetric Notch-based signaling, which causes LR twist-

ing of the intestine (reviewed by Wood, 1998; Hermann *et al.*, 2000). The extent to which the establishment of left–right asymmetry is conserved among bilaterians is an open question that requires investigation in a variety of diverse organisms.

The *Drosophila* gut develops a stereotyped, LR asymmetric pattern of loops; this asymmetry appears first in the foregut and the hindgut (Strasburger, 1932; Campos-Ortega and Hartenstein, 1997). During germband shortening, the bend in the hindgut at its junction with the midgut (Fig. 4A) becomes more pronounced and is shifted to the left side of the embryo; this converts the DV asymmetry of Dpp and Engrailed expression in the large intestine into the earliest described LR asymmetrical gene expression in the *Drosophila* embryo (Fig. 5A). The genetic basis for hindgut LR asymmetry in *Drosophila* has only recently come under investigation. A high level of LR randomization in the ectopic hindguts formed at the anterior of embryos from *bicoid* mothers implies that there is input from some posterior feature of the egg; failure of the hindgut loop to form in *huckebein* mutants suggests there is a later required input from the midgut/hindgut junction (Hayashi and Murakami, 2001).

ADULT HINDGUT

During pupation, the epithelium and musculature of the *Drosophila* larval hindgut undergo histolysis; the adult hindgut epithelium is formed by proliferation of the cells of the imaginal ring (these are hindgut epithelial cells lying just posterior to the point of insertion of the Malpighian tubules) and of the genital imaginal disc (Robertson, 1936). Like the larval hindgut, the adult hindgut is a single-layered epithelium surrounded by a circular visceral musculature; it also has an anterior LR loop and a posterior muscular rectum. The large bulbous rectum, bounded anteriorly by the rectal valve and posteriorly by the anus, differentiates four rectal papillae that may be involved in water resorption (Strasburger, 1932; Miller, 1950).

Some of the same genes known to be involved in embryonic hindgut formation have been found to play a role in adult hindgut development: *cad* is required in the genital imaginal disc to activate expression of *byn* and *even-skipped;* this gene activity initiates hindgut (as opposed to anal plate) differentiation (Moreno and Morata, 1999). Thus, at least two transcription factor encoding genes, *cad* and *byn*, act early in the development of both the embryonic and adult hindgut. Whether other processes observed in the embryonic hindgut, such as cell proliferation, cell rearrangement, envelopment by visceral mesoderm, and establishment of LR asymmetry and of AP subdomains, are controlled by similar genetic mechanisms during both embryonic and adult hindgut development remains to be determined.

SCREENING FOR ADDITIONAL GENES CONTROLLING HINDGUT MORPHOGENESIS

Genes that affect gut development in various organisms have been identified by two distinct approaches. In chick and mouse, research has focused primarily on known genes that have expression patterns in the gut (reviewed by Grapin-Botton and Melton, 2000; Roberts, 2000; Beck *et al.*, 2000). This approach has provided important insights, but does not provide a means of identifying novel players in this process. In *C. elegans*, *Drosophila*, and zebrafish, not only can known genes expressed in the gut be studied (Kispert *et al.*, 1994; Murakami *et al.* 1995; Wang and Coulter, 1996), it is also possible to carry out forward genetic screens for mutations affecting gut morphology (Harbecke and Lengyel, 1995; Bilder and Scott, 1995; Pack *et al.*, 1996; Chamberlin *et al.*, 1999; Liu, X., *et al.*, 1999). Since only a relatively small number of mutants have been identified in these nonsaturating screens, more genes controlling gut development remain to be identified. In *Drosophila*, mutant screens for genes controlling hindgut morphogenesis will be aided by the hindgut-specific expression of GFP (Iwaki and Lengyel, manuscript submitted), which allows assessment of morphology *in vivo*, and also by the fully sequenced genome (Adams *et al.*, 2000), which allows ready mapping and molecular characterization of mutant loci.

CONCLUSIONS AND FUTURE PROSPECTS

The genetic tools available in *Drosophila* have made possible significant progress toward understanding development of a simple tubular epithelial organ, the hindgut. Early acting genes that are required to establish and maintain the primordium have been identified and characterized. The genes *cad*, *fkh*, *byn*, and *wg* are expressed in the primordium and play roles related to those of their vertebrate homologs Cdx, HNF-3, Brachyury, and Wnt, suggesting that these genes function in an evolutionarily conserved cassette involved in gastrulation, cell rearrangement, and axis elongation. Further study of the genetic control of gastrulation should ultimately connect cell signaling (via *fog* and other molecules) with the actin cytoskeletal modulation that leads to apical constriction and hence invagination (reviewed by Leptin, 1999). Expression in the hindgut epithelium of the Wg and Hh signaling molecules is required for interaction between epithelium and visceral mesoderm. The full set of signaling molecules expressed in the *Drosophila* hindgut epithelium and mesenchyme, as well as their required roles in hindgut morphogenesis, remains to determined.

A key concept that emerges is that patterning along the AP axis of the hindgut epithelium is essential for the cell rearrangement that brings about elongation of the hindgut. Early subdivision of the hindgut is first evident from finely regulated gene expression patterns (Fig. 5A); these are then

translated into the morphological subdivisions of small intestine, large intestine, and rectum. The genes *drm*, *bowl*, and *lin*, which encode putative transcriptional regulators, are required both for establishment of the adjacent small intestine and large intestine domains, and for the cell rearrangement that drives hindgut elongation and narrowing. This suggests that juxtaposition in the AP axis of the small intestine and large intestine, which express different cell signaling genes (in particular, the JAK/STAT activator *upd*), might be important for orienting cell rearrangement. Analysis of additional genes that control patterning of the hindgut, such as *D*, *mmy*, and *raw*, might shed further light on the apparent required relationship between hindgut patterning and its elongation by cell rearrangement.

In addition to its utility for studying cell rearrangement and tubular elongation, the *Drosophila* hindgut constitutes an extremely attractive model system for study of a variety of problems in organogenesis. In less than 24 h, the development of this relatively small and simple organ brings into play many processes of great interest to those studying vertebrate organogenesis. These include not only cell rearrangement, but also investment of epithelium with mesenchyme, reciprocal interaction between epithelium and visceral mesoderm, left–right looping, and gut innervation. The availability of a sequenced genome, a large number of genetic mutations, and a well-developed technology of gene manipulation via germline transformation means that these problems can be profitably investigated at the molecular genetic level in *Drosophila*. Characterization of already known, and newly discovered genes, and the pathways in which they act, should make important contributions to our understanding of these processes required for organogenesis that are readily studied in the *Drosophila* hindgut.

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