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Control of gut development by *fork head* and cell signaling molecules in *Drosophila*

Michael Hoch*, Michael J. Pankratz

Max-Planck-Institut für Biophysikalische Chemie, Abteilung Molekulare Entwicklungsbiologie, Am Fassberg, 37077 Göttingen, Germany

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Abstract

The alimentary canal of most animals can be subdivided into a fore-, mid- and hindgut portion, each gut part possessing distinct physiological functions. The genetic basis underlying the formation of the different gut parts is poorly understood. Here we show that the *Drosophila* genes *hedgehog*, *wingless* and *decapentaplegic*, which encode cell signaling molecules, are required for the establishment of signaling centers that coordinate morphogenesis in the hindgut epithelium. The activation of these genes in the developing as well as in the foregut requires *fork head*, which encodes a transcription factor. Furthermore, we demonstrate that *hedgehog* and *wingless* activities in the gut epithelial cells are required for the expression of the homeobox gene *bagpipe* in the ensheathing visceral mesoderm. These results provide strong evidence that similar principles underlie *Drosophila* fore- and hindgut development, and that the genetic hierarchy of gut development might be conserved between *Drosophila* and vertebrates.

Keywords: Cell signaling in the gut; *hedgehog*; *wingless*; *decapentaplegic*; *fork head*; *bagpipe*; Gut; Morphogenesis

1. Introduction

Nutrition and hydration are basic needs of all organisms. The organ which is required to fulfill these needs in animals is the gut, which most likely belongs to the most conserved organ structures developed during animal evolution. In *Drosophila*, gut formation begins after the segmentation genes have determined the future body plan of the embryo (reviewed in Hoch and Jäckle, 1993; Pankratz and Jäckle, 1993; Skaer, 1993). About 3 h after egg deposition, cells at the anterior/ventral and posterior/dorsal region of the embryo invaginate to form the stomodeum and the proctodeum, respectively. The stomodeum gives rise to the foregut while the proctodeum gives rise to the hindgut. The midgut, which is derived from an anterior and a posterior primordium that abut the stomodeal and proctodeal invaginations, forms between the foregut and the hindgut tubes by fusion of its primordia such that a continuous gut tube is generated. Regional specification then occurs and several gut-associated organs are formed at the junction between the different gut parts.

The genetic basis underlying gut development is only poorly understood. However, a key gene that is required for the development of all gut primordia has been identified: the *fork head* (*fkh*) gene (Jürgens and Weigel, 1988; Weigel et al., 1989a,b). *fkh* encodes a transcription factor that contains an evolutionarily conserved DNA-binding domain termed the fkh-domain (Lai et al., 1990, 1991; Weigel and Jäckle, 1990). The gene is expressed during the initial phase of gut formation in the foregut, the midgut and the hindgut anlagen. In *fkh* loss of function mutants the gut is not formed (Weigel et al., 1989a,b). Target genes *of fkh* that mediate the development of the different gut parts are largely unknown. For the development of the midgut, the gene *serpent* is required along with *fkh* activity, and has been shown to specify midgut versus fore- or hindgut fate (Reuter, 1994). Furthermore, a signaling cascade is known that is required for the formation of the second midgut constriction during the late stages of midgut morphogenesis (reviewed in Bienz, 1994). For the foregut, recent studies revealed that the gene *hedgehog*

* Corresponding author. Tel.: +49 551 2011758; fax: +49 551 2011755; e-mail: mhoch@gwdgvl.dnet.gwdg.de.

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(*hh*), which encodes a secreted protein, and the genes *wingless* (*wg*) and *decapentaplegic* (*dpp*), which encode members of the Wnt and transforming growth factor β (TGFβ) families of growth factors, respectively, define a signaling center within the posterior portion of the foregut which is required for the formation of the proventriculus, a gut-associated organ mediating food passage between fore- and midgut (Pankratz and Hoch, 1995). The least amount of information exists on genes that control hindgut development.

Homologues of the *Drosophila fkh* gene that are expressed in the gut primordia have been identified in a variety of vertebrates as well. These include the family of HNF3 factors, HNF3α, β and γ in mice (Ang et al., 1993; Monaghan et al., 1993; Sasaki and Hogan, 1993). A mouse loss of function mutant has been generated for one member, the HNF3β gene, and these mutants exhibit strong defects in the gut (Ang et al., 1994; Weinstein et al., 1994), giving further support to the notion that this family of genes might play a major role during vertebrate gut development. It has also been demonstrated recently that during the initial steps of hindgut formation in chicken, inductive interactions occur from the gut epithelium towards the visceral mesoderm that surrounds the gut and later forms its musculature: the *Sonic hedgehog* gene, a homologue of the *Drosophila hh* that is expressed in the gut epithelium, was shown to induce Bmp-4, a member of the TGF-β family, and Abd-B-related Hox genes in the developing visceral mesoderm (Roberts et al., 1995).

In this paper we report that epithelial hindgut development requires the activities of the genes *wg*, *hh* and *dpp* which define signaling centers in the hindgut tube where morphogenesis occurs. We show that *fkh* controls the expression of these genes in the hindgut and also in the foregut. We further provide evidence that *wg* and *hh*, expressed in the fore- and hindgut epithelium, are required for the expression of the homeobox gene *bagpipe* in the ensheathing mesoderm. These data reveal, both in terms of developmental strategies and genetic circuitries, close similarities between vertebrate and insect gut development.

2. Results

2.1. Structure of the hindgut epithelium

The hindgut in late embryos is a hollow tube which lies horizontally in the posterior body region and bends at one end such that it displays the shape of a J (Fig. 1A). The characteristic shape of the hindgut can be visualized by monitoring the expression of *fkh*, which is expressed in the entire hindgut (Weigel et al., 1989a,b) (Fig. 1B). The hindgut epithelium is surrounded by the visceral mesoderm which gives rise to the muscle layer surrounding the gut (Fig. 1C).

Little is known about the regionalization of the larval hindgut. Most of the morphological studies have been performed in the adult, with slightly different terminologies for different insects to describe the subdivisions of the hindgut (for example, Strasburger, 1932; Graham-Smith, 1934; Snodgrass, 1935; Miller, 1950). We have adapted the terminology used by Snodgrass (1935) (see legend to Fig. 1 for further discussion) for an idealized insect to describe the regionalization of the larval hindgut of *Drosophila*. This description is based on a combination of morphological observations and on marker gene expression, such as that of *crumbs* (*crb*) (Tepass et al., 1990; see below).

The larval hindgut can be divided into the anterior intestine and the posterior intestine; the posterior intestine is also called the rectum. The anterior intestine itself is composed of two different sub-regions, the small intestine and the large intestine (Fig. 1A). This subdivision can be visualized using *crb* as a marker (Fig. 1D–F). The small intestine is found at the anterior end of the hindgut and lies at the junction to the midgut (Fig. 1D,E). The hindgut is widened in this region and the ureters of the Malpighian tubules evert from this area (Wessing and Eichelberg, 1978) (Fig. 1E). At the transition to the large intestine, there is a narrowing of tissues to form a constriction-like structure in the intestinal wall, which in most of the insects separates the small from the large intestine (Snodgrass, 1935) (Fig. 1D,E). This structure is marked by a circular expression domain of *crb* (Fig. 1D,E). The large intestine is an extended stiff tube due to cells that secrete large amounts of cuticle (Strasburger, 1932). *crb* is expressed in this region in a row of cells on each side (Fig. 1D). At the transition to the rectum, a valve-like structure (rectal valve) occurs which is formed by a constriction of the hindgut tube (Fig. 1D,F). *crb* is expressed in this structure in a circular expression domain as well (Fig. 1D,F). It is at this position where the posterior pair of the Malpighian tubules attach to the abdominal nerve a9 (Hoch et al., 1994). The attachment point of the Malpighian tubules therefore marks the anterior boundary of the rectum and can be used as a landmark. The rectum is characterized by a widening of the hindgut tube. In the adult, this part of the hindgut can be further subdivided into a rectal sac and a rectum proper (Snodgrass, 1935). Our morphological observations do not give clues as to whether this subdivision also exists in the larval rectum. However, some evidence for a potential subdivision comes from regional expression patterns of several genes (see below). The rectum is terminated by the anus.

2.2. Control of hindgut morphogenesis by wg, hh and dpp

The hindgut arises from an anlage of cells located at the posterior pole of the blastoderm embryo (Technau and Campos-Ortega, 1985; Janning et al., 1986). After gastrulation, when the proctodeum has invaginated, the cells of

the hindgut primordium have already formed a hollow tube which lies horizontally in the embryo (Campos-Ortega and Hartenstein, 1985; reviewed in Skaer, 1993). During germband shortening, the hindgut starts to loop back towards the posterior pole; shortly afterwards, the ureters of the Malpighian tubules evert from the anterior end, and the rectum differentiates at the posterior end of the hindgut tube.

wg, *hh* and *dpp* are expressed in the developing foregut and have recently been shown to be required for the formation of the foregut-associated organ, the proventriculus (Pankratz and Hoch, 1995) Strikingly, the three genes are also expressed in restricted domains in the developing hindgut epithelium (Fig. 2A,C,E), suggesting that they could also play a role in hindgut morphogenesis. To further investigate this, we analyzed their expression patterns during hindgut development in more detail. *wg* is expressed initially in the whole hindgut primordium. Its expression becomes restricted to two areas of the developing hindgut tube: to a ring in the small intestine anterior to the outgrowing Malpighian tubules, and to a ring in the posterior region of the rectum (Fig. 2B). Both expression domains persist until the end of embryogenesis. *hh is* also expressed in the hindgut primordium. During germband shortening, *hh* expression becomes restricted to a ring of cells posterior to the outgrowing Malpighian tubules in the future small intestine of the hindgut (Fig. 2D). A second *hh* expression domain is located in the anterior portion of the rectum (Fig. 2D). Those two expression domains are adjacent to the *wg* expression domains and persist until late embryogenesis. *dpp is* expressed in the hindgut primordium and later on one side in the large intestine of the hindgut tube, in between the small intestine and the rectum (Fig. 2F). In summary, the expression domains of *wg*, *hh* and *dpp* subdivide the hindgut tube into a central portion (the large intestine) where *dpp is* expressed, and two flanking regions (the small intestine and the rectum) where *wg* and *hh* are expressed. This is consistent with the tri-partite subdivision of the hindgut observed with morphological markers (Fig. 1). It is in the two flanking regions where morphogenetic processes occur during regionalization of the hindgut.

To correlate these gene expression patterns with function, we examined the hindgut phenotypes of *wg*, *hh* and *dpp* null mutant embryos using anti-Crb, anti-Fkh and anti-MHC antibodies (Fig. 3). In *wg* mutants, the whole hindgut primordium is reduced in size (Fig. 3A). The clearest defects can be seen as a dramatic reduction of cells in the small intestine which can be visualized by anti-Fkh staining (Fig. 3B). The defects are most likely due to an early effect of *wg* on cell proliferation (Skaer and Martinez Arias, 1992). In *hh* mutants, defects arise at both the small intestine and at the rectum, whereas the large intestine is not significantly affected. The small intestine is reduced in size (Fig. 3C); the ureters of the Malpighian tubules, which arise during stage 12 mostly by the outward movement of cells from the region of the small intestine (stages after Campos-Ortega and Hartenstein, 1985) (Fig. 3C), are also drastically shortened in *hh* mutants, suggesting that cell movement cannot occur properly (compare Figs. 1F and 3C). Consistent with this interpretation, *myospheroid* mutant embryos that lack a functional β integrin subunit (MacKrell et al., 1988; Leptin et al., 1989) show a very similar ureter phenotype as *hh* mutants (M. Hoch and M.J. Pankratz, unpublished observation). The rectum is also reduced in size in these mutant embryos (compare Figs. 1D and 3D); consistent with this observation, the posterior pair of the Malpighian tubules now attach to the abdominal nerve 9 at the very posterior end of the embryo (data not shown). In *dpp* mutants, despite gross defects in the morphology, much of the hindgut still forms. In the large intestine, however, there appear to be ectopic cell movements leading to numerous outbuddings in this area (Fig. 3E). This kind of phenotype has also been observed in the foregut of *dpp* mutants (Pankratz and Hoch, 1995). In extreme cases the ectopic outbuddings of the hindgut make contact with the surface of the embryo and produce secondary openings to the outside (Fig. 3F). In addition, the small intestine and the rectum are enlarged and are also characterized by more extensive cell movements as compared to wild type embryos.

To study the regulatory interactions between these genes, we monitored gene expression in various mutant embryos. In *hh* mutants, *wg* expression is still present but at a much reduced level (Fig. 4A) A similar result was obtained monitoring *hh* expression in *wg* mutants (data not shown). In *hh* mutants, *dpp* remains strongly expressed in the large intestine and is almost unchanged as compared to wild type (Fig. 4B). In *dpp* mutants, both *wg* and *hh* are ectopically expressed in the middle portion of the hindgut, in patches of cells undergoing ectopic cell movements (Fig. 4C,D). When we ectopically expressed *wg* and *hh* under heat shock control, we did not see any significant morphological changes in the hindgut, whereas when we ectopically expressed *dpp* by heat shock we observed a reduction of the small intestine and the rectum (Fig. 4E).

The above results suggest that *wg* and *hh* activities are required for differentiation of the small intestine and the rectum, whereas *dpp is* required to suppress these events in the large intestine of the hindgut (Fig. 4F). We note that there are some interesting differences in the roles of these genes during hindgut development as compared to those in eye and leg formation. During eye development, *hh* induces *dpp* expression which is a primary mediator of furrow movement (Heberlein et al., 1993; Ma et al., 1993). During leg formation, *hh* directs the expression of *wg* in the ventral-anterior compartment and *dpp* in the dorsal-anterior compartment (Basler and Struhl, 1994; Diaz-Benjumea et al., 1994). In the hindgut, *hh* also provides positive input to *wg*, but it has no positive effect on

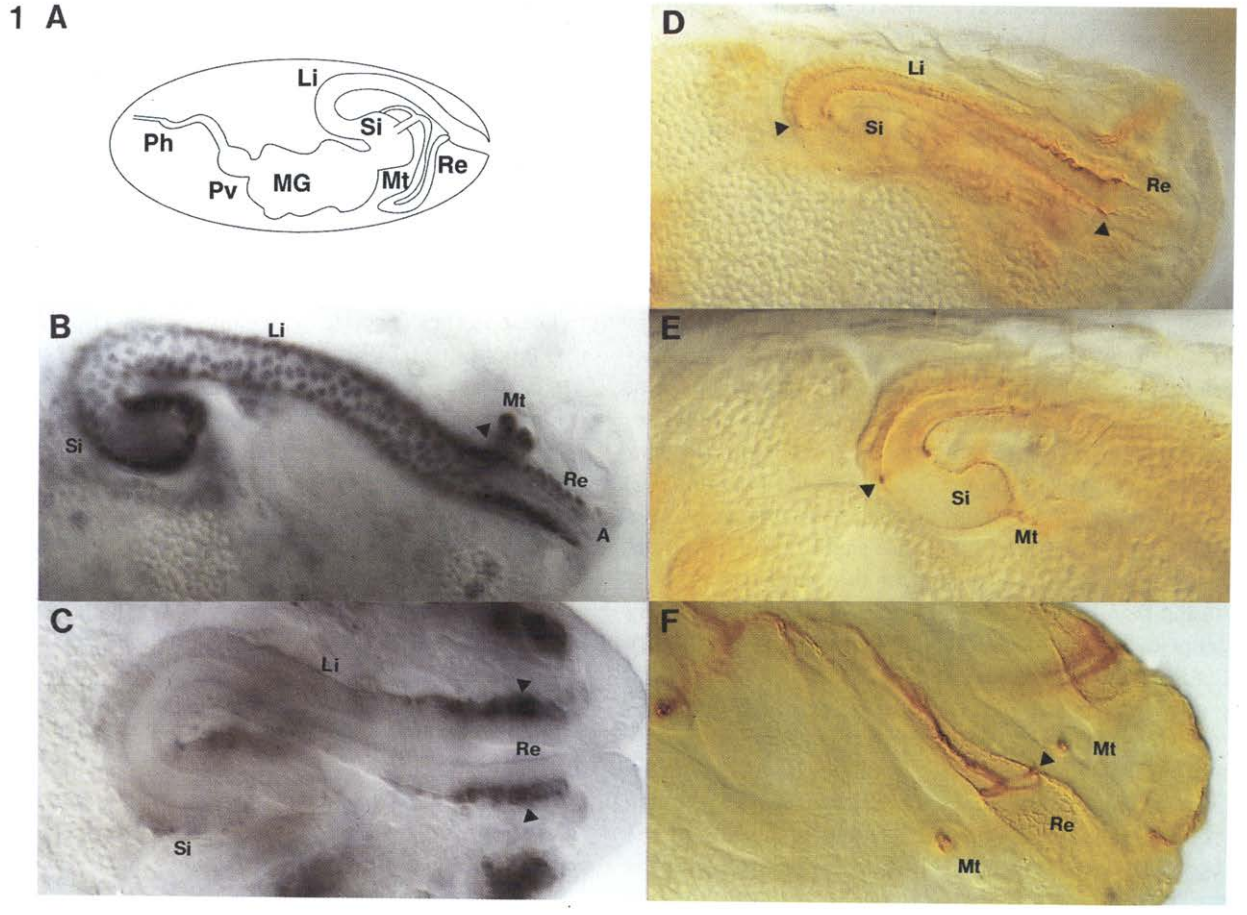
dpp expression. Furthermore, *dpp* acts in the hindgut as a suppressor of morphogenesis, whereas this does not seem to be the case in the other systems. On the other hand, the role of the three signaling molecules in controlling hindgut development is very similar to what has previously been observed for development of the foregut. In the foregut, *wg* and *hh* coordinate morphogenesis of the proventriculus at the posterior region of the foregut, whereas *dpp* suppresses morphogenetic movements in the more anteriorly located esophagus (Pankratz and Hoch, 1995).

2.3. Activation of wg, hh and dpp in the fore- and hindgut epithelium by fkh

How are the components defining these signaling centers in the fore- and hindgut initially activated in the gut primordia? A candidate activator is the region-specific homeotic gene *fkh* (Jürgens and Weigel, 1988), which encodes a transcription factor (Weigel and Jäckle, 1990). *fkh* has been shown to be expressed in the anlagen of the gut in the blastoderm stage under the control of the terminal maternal system (Weigel et al., 1989a,b). Its expression persists in the fore- and hindgut epithelia throughout embryonic and larval development. In *fkh* mutant embryos, the foregut, the midgut and the hindgut epithelia are disrupted. As a first step towards determining a genetic hierarchy in the fore- and hindgut epithelia, we investigated the expression of *wg*, *hh* and *dpp* in *fkh* null mutant embryos. Our results indicate that *fkh* is required for the activation of these genes in the fore- and hindgut primordia (Fig. 5). *fkh* is expressed in the entire foregut and hindgut, whereas *wg*, *hh* and *dpp* are expressed only in restricted subdomains. The spatial domains of expression of these genes, however, appear not to be established through cross-regulatory interactions. For example, in *dpp* mutants we do not observe continuous expression of *wg* and *hh* throughout the large intestine, but rather observe ectopic expression in patches of cells undergoing cell movements (see above). Furthermore, we did not observe changes in the spatial domains of *wg* in *hh* mutants, nor of *hh* in *wg* mutants. Taken together, these results suggest that there must be other factors which act to spatially regulate the *wg*, *hh* and *dpp* expression along the hindgut.

Fig. 1. Structure of the hindgut. (A) The gut of a *Drosophila* larva can be subdivided into a fore-, a mid- and a hindgut portion (Strasburger, 1932; Poulson, 1950; Campos-Ortega and Hartenstein, 1985). Several organs are formed as gut annexes at the junction between the different gut parts. These include the proventriculus (Pv), a valve-like organ regulating food passage at the junction of the fore- and midgut and the Malpighian tubules (Mt), the excretory organs of the larva and the adult, which form at the junction of the mid- and hindgut. To describe the morphology of the larval hindgut we have used the terms small intestine (Si), large intestine (Li) and rectum (Re). This is an adaptation of a terminology that Snodgrass (1935) has used to describe the morphology of the adult hindgut of an idealized insect. (The larval hindgut has not been subdivided based on morphological criteria. Miller (1950) and Snodgrass (1935) subdivide the hindgut of the adult into an anterior and a posterior intestine. The posterior intestine is also referred to as the rectum. Snodgrass further subdivides the anterior intestine into the small intestine and the large intestine and the rectum into the rectal sac and the rectum proper. Strasburger (1932) uses a different terminology to describe the adult hindgut, subdividing it into the proximal hindgut and the rectum. The rectum is in turn subdivided into the proximal, the middle and the distal rectum. The proximal hindgut and the proximal rectum of Strasburger together correspond to the anterior intestine of Snodgrass, whereas the middle and the distal rectum correspond to the rectum. We have adapted the terminology of Snodgrass to describe the *Drosophila* larval hindgut. See text for further discussion). MG, midgut; Ph, pharynx. (B) Higher magnification of the hindgut of a stage 17 embryo stained with anti-Fkh-antibody. *fkh* is expressed in the hindgut epithelium and in the Malpighian tubules whose posterior pair attaches to the rectal valve at the posterior portion of the hindgut (arrowhead). Note that *fkh* is not expressed at the very posterior portion of the hindgut, in the anus region. (C) The hindgut epithelium is ensheathed by the visceral mesoderm, stained with anti-MHC antibody. Note the strong expression of MHC in the visceral mesoderm cells on top of the rectum (arrowheads) and the small intestine, and the weaker expression in the middle portion of the hindgut in the large intestine. (D) Subdivisions of the hindgut visualized with anti-Crb-antibody staining of a stage 17 embryo. Crb is expressed on the apical side of cells in the central portion of the hindgut, i.e. the large intestine, in one row of cells expressed on each side; the central expression domain is bordered by a ring of cells expressed at each side. These landmarks can be used to subdivide the hindgut into three parts: the small intestine at the anterior portion of the hindgut where the two pairs of Malpighian tubules evert, the large intestine which forms a stiff rod, and the rectum at the posterior end of the hindgut. Arrows mark the sphincter-like structures in which Crb is expressed in a circular expression domain at the transitions between the small intestine and the large intestine, and between the large intestine and the rectum. (E) Magnification of the small intestine of a stage 17 embryo stained with anti-Crb-antibody. Note the ring of Crb expression bordering the small intestine (arrowhead). The ureters of the Malpighian tubules evert from the small intestine area. (F) Magnification of the rectum of a stage 17 embryo stained with anti-Crb-antibody. Note the circular expression domain of Crb, which marks the rectal valve (arrowhead) at the transition of the large intestine to the rectum. The posterior pair of the Malpighian tubules are attached to the abdominal nerve a9 in the region of the rectal valve. Embryonic stages according to Campos-Ortega and Hartenstein (1985). Anterior is left and dorsal up.

Fig. 2. Expression patterns of *wg*, *hh* and *dpp* in the developing gut canal of the embryo. (A) Stage 13 embryo showing *wg* expression in signaling centers of the developing gut canal (arrowheads) which coordinate the regionalization of the foregut (the left-most arrowhead) and the hindgut (the two right arrowheads). (B) *wg*/MHC double staining of the hindgut of a stage 16 embryo. *wg* is expressed in the small intestine (Si), towards the anterior side of the Malpighian tubules, and in the posterior portion of the rectum (Re). This region might correspond to the rectum proper of the adult hindgut described by Snodgrass (1935). (C) Stage 13 embryo showing *hh* expression in signaling centers in the foregut (left-most arrowhead) and the hindgut epithelia (the two right arrowheads). (D) *hh*/MHC double staining of the hindgut of a stage 16 embryo. Note a broad ring of *hh*-expressing cells in the small intestine, towards the posterior side of the Malpighian tubules, and a second ring in the anterior portion of the rectum. This region might correspond to the rectal sac of the adult hindgut described by Snodgrass (1935). (E) Stage 13 embryo showing *dpp* expression in signaling centers of the developing fore- and hindgut canal (left and right arrowheads, respectively). (F) *dpp*/MHC double staining of the hindgut of a stage 16 embryo. *dpp* is expressed in the large intestine (Li), abutting the small intestine and rectum. Note that *dpp is* expressed predominantly on one side of the large intestine. Embryonic stages according to Campos-Ortega and Hartenstein (1985). Anterior is left and dorsal up.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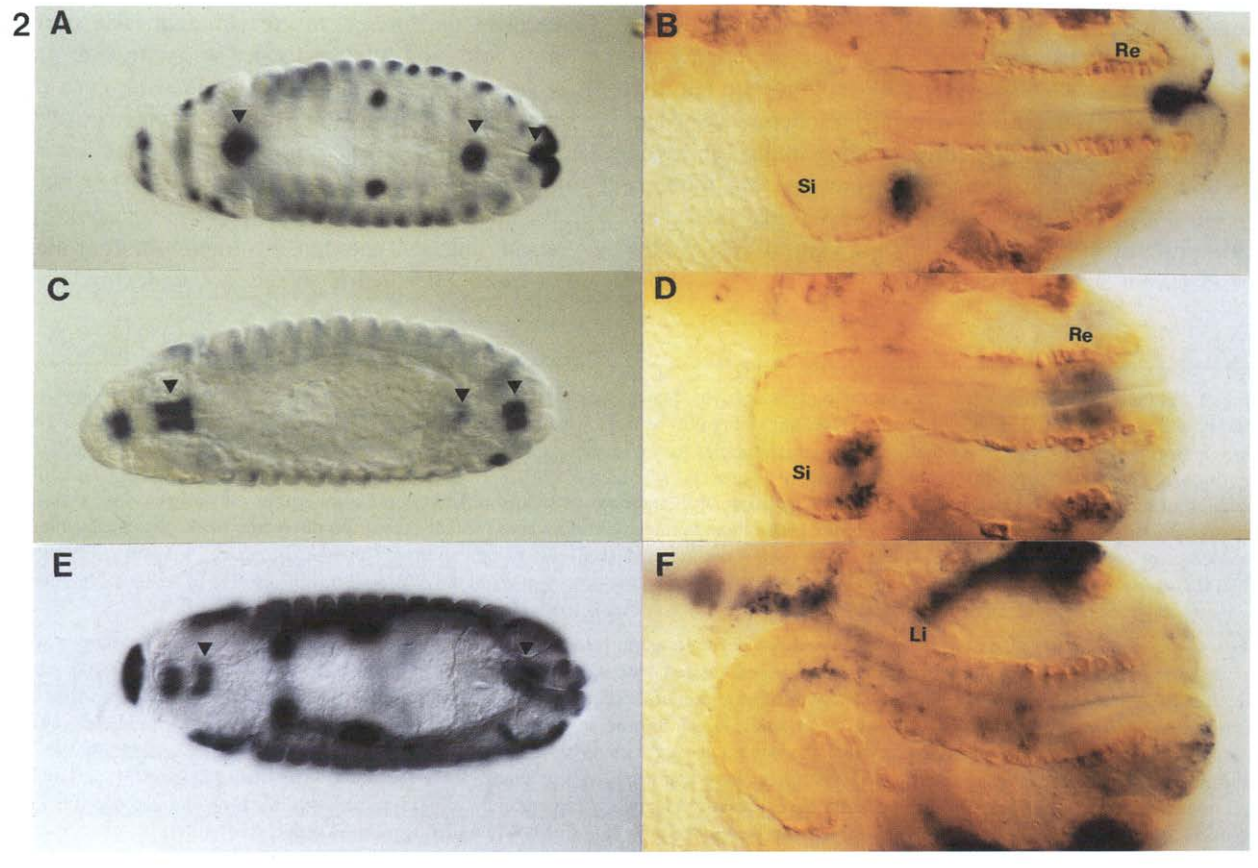
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2.4. Activation of the mesoderm-specific gene bagpipe in the fore- and hindgut by wg and hh

The epithelial tissue of the gut is ensheathed by visceral mesoderm. The mesodermal layer of the fore- and hindgut is gradually assembled around the invaginating stomodeal and proctodeal tubes (Skaer, 1993). This suggests that signaling to the visceral mesoderm from the gut epithelia might occur. A gene that is required for visceral mesoderm differentiation is *bagpipe* (*bap*) (Kim and Nirenberg, 1989; Apiazu and Frasch, 1993; reviewed in Bate, 1993). We first monitored the dynamics of *bap* expression in the foregut and the hindgut, since this has not been previously described in detail. *bap* is strongly expressed in mesodermal cells on top of the proctodeum that will give rise later to the muscles of the hindgut (Fig. 6A). The expression domain then splits to give rise to two subdomains, one around the future small intestine and the other around the future rectum of the hindgut (Fig. 6C). With the beginning of stage 14, *bap* expression gradually appears in the middle region of the hindgut and finally gives rise to a continuous expression domain surrounding the entire hindgut around stage 16 (Fig. 6D). Similar dynamic pattern is observed in the foregut. *bap is* initially expressed in a patch of visceral mesoderm precursor cells on top of the stomodeal invagination (Fig. 6B), which then splits into two subdomains along both ends of the foregut. At around stage 16, *bap* expression covers the entire foregut tube (Fig. 6D).

Strikingly, the *bap* expression domains in the fore- and hindgut are strongest around the signaling centers defined by *wg* and *hh* activities in the underlying gut epithelium. In order to test whether *wg* and *hh* activities are required for *bap* expression in the visceral mesoderm, we examined its expression in the corresponding mutant embryos. In both *wg* and *hh* mutants, *bap* expression was found to be strongly reduced or absent in the visceral mesoderm primordia of the developing hindgut (Fig. 6E,F). Similar results were obtained for the foregut.

3. Discussion

3.1. A genetic hierarchy in the Drosophila gut

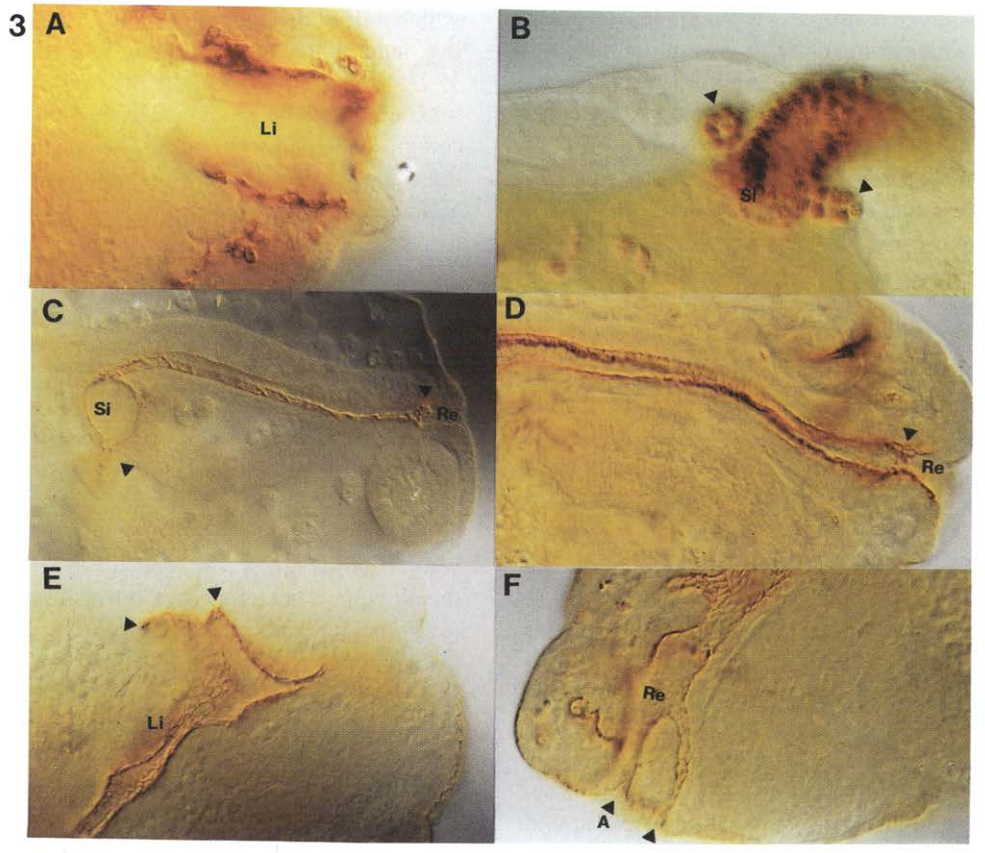
The formation of the larval gut in *Drosophila* requires a cascade of gene activities that can be traced back to oogenesis (Fig. 7): the torso tyrosine kinase signaling pathway activates the terminal gap genes *tailless* and *huckebein* at both poles of the blastoderm embryo (reviewed by Sprenger and Nüsslein-Volhard, 1993). These genes encode zinc-finger transcription factors (Pignoni et al., 1990; Brönner et al., 1994) that activate *fkh*, the key regulator of gut development, in all the gut anlagen during blastoderm stage (Weigel et al., 1989a,b). In the developing midgut, the *serpent* gene is required along with *fkh* and specifies midgut versus fore- or hindgut identity (Reuter, 1994). We have shown in this paper that *fkh* activates the genes *wg*, *hh* and *dpp* in the developing foregut and hindgut primordia. These interactions lead to the establishment of signaling centers which regionalize the fore- and hindgut epithelium and define sites where morphogenesis occurs. *wg* and *hh* then appear to be required for the expression of the mesoderm-specific gene *bap* in the ensheathing visceral mesoderm.

3.2. A common strategy underlying foregut and hindgut development

There is a remarkable similarity both at the morphological level and at the genetic requirements for development of the foregut and the hindgut. The primordia for both fore- and hindgut tissues originate after gastrulation via the invagination of cells at opposite ends of the embryo. At the tips of the invaginating foregut and midgut tubes, there are the primordia for the anterior and posterior parts of the midgut, respectively. After the fusion of the midgut primordia, specific gut-associated organs arise at the junction between the foregut-midgut and midgut-hindgut. These early events are under the control of a

Fig. 3. Hindgut phenotypes of *wg*, *hh* and *dpp* mutant embryos. Stage 17 embryos were stained with anti-MHC-antibody (A), with anti-Fkh antibody (B) or with anti-Crb-antibody (C–F). (A,B) *wg* mutant embryos. The large intestine (Li) and the small intestine (Si) are strongly reduced, and the rectum is nearly absent. Only two of the four Malpighian tubules (arrowheads) evert from the region of the small intestine due to a proliferation defect during early hindgut/Malpighian tubule development (Skaer and Martinez Arias, 1992). (C,D) Hindgut of a *hh* mutant embryos. Note the strong reduction of the rectum (Re; compare to Fig. 1D). The rectal valve (right arrowhead) is at the very posterior end of the hindgut. The small intestine (Si) is strongly reduced as well, and the ureters of the Malpighian tubules (left arrowhead) which evert from the region of the small intestine are strongly reduced size compared to wild type. The large intestine is not affected. (E,F) *dpp* mutant embryo. Note the ectopic cell movements in the large intestine (Li; arrowheads in (E)) leading sometimes to the formation of secondary openings to the outside (arrowheads in (F)). A, anus; Re, rectum. Embryonic stages according to Campos-Ortega and Hartenstein (1985). Anterior is left and dorsal up.

Fig. 4. Regulatory interactions between *wg*, *hh* and *dpp* during hindgut formation. (A) *wg* expression in a stage 14 *hh* mutant embryo. Although initially strongly expressed in the hindgut primordium, *wg* expression decays during later stages of hindgut formation (arrowheads). (B) *dpp* expression in a stage 14 *hh* mutant embryo. *dpp is* still expressed in the region of the large intestine (Li) of the developing hindgut. (C) *wg* expression in a stage 16 *dpp* mutant embryo. Note the ectopic *wg* expression in the large intestine, in patches of cells undergoing cell movements (arrowheads). (D) *hh* expression in a stage 16 *dpp* mutant embryo. Note the ectopic *hh* expression in the large intestine, in patches of cells undergoing cell movements (arrowheads). (E) MHC staining of a stage 17 HS-*dpp* embryo that has been heat shocked (see Section 4). The small intestine (Si) and the rectum (Re) are reduced in size (arrowheads). (F) *wg* and *hh* are expressed in signaling centers in the small intestine and in the rectum and coordinate hindgut regionalization and morphogenesis, whereas *dpp is* expressed in the large intestine where morphogenesis is suppressed.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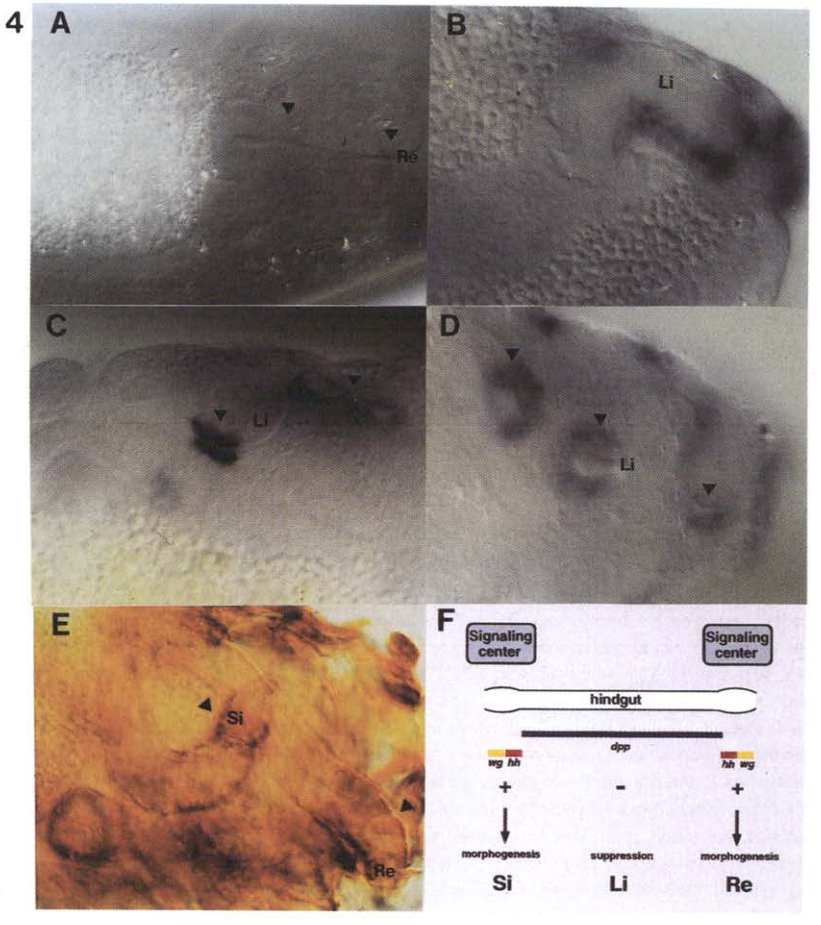
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common maternal pattern-forming system, the terminal system, which is active at the poles of the embryo.

We have previously shown the requirement of *wg*, *hh* and *dpp* in the developing foregut. *wg* and *hh* activities are required for morphogenetic events during proventriculus development, whereas *dpp is* required to suppress these events in the esophagus (Pankratz and Hoch, 1995). We have shown here that *wg* and *hh* are also active at both ends of the developing hindgut, in the small intestine and in the rectum. In these areas morphogenetic events take place: from the region of the small intestine the ureters of the Malpighian tubules evert, and in the rectum the hindgut tube becomes widened and the rectal valve differentiates. These results suggest that *wg* and *hh* define those areas as active zones of morphogenesis. In the large intestine of the hindgut, *dpp* acts to prevent morphogenesis. Furthermore, these genes are activated by a common factor, *fork head*, in both the foregut and the hindgut. As the foregut and hindgut tubes are forming, mesodermal tissues begin to surround these tubes. Again, the modes by which the mesodermal layers invest around the forming tubes are quite similar in the foregut and in the hindgut. *bap is* continuously expressed in the visceral mesoderm of the fore- and hindgut, from very early stages on to the end of embryogenesis, whereas its expression disappears from the midgut mesodermal layer (Apiazu and Frasch, 1993). In addition, both *wg* and *hh* in the fore- and hindgut epithelium may induce the expression of the mesoderm-specific gene *bap* (Fig. 7). This may be a reflection of the way the foregut and the hindgut are generated: the gut epithelia invaginate first and the visceral mesoderm is assembled around the involuting fore- and hindgut tubes. Signaling across germlayers has already been shown to be important for late aspects of midgut development: During the formation of the second midgut constriction, homeotic genes expressed in the visceral mesoderm direct the expression of signaling molecules which induce the activation of the homeotic gene *labial* in the underlaying midgut epithelium (reviewed by Skaer, 1993; Bienz, 1994). Upon this common ground, there must of course be genes which then differentiate between foregut and hindgut tissues. The mechanisms which operate specifically for fore- or hindgut morphogenesis are at this point unknown.

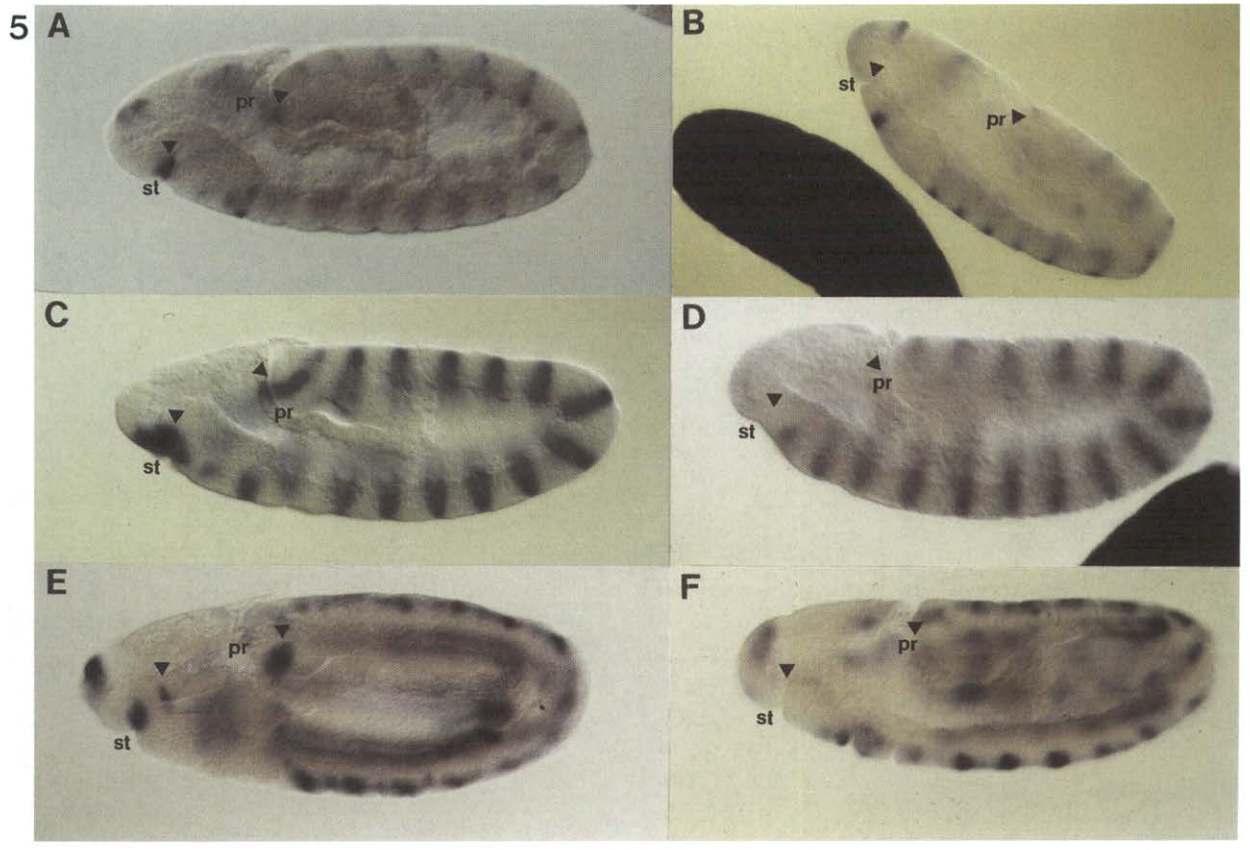
3.3. Conserved developmental strategies and genetic circuitries during vertebrate and insect gut development

There are substantial similarities in the morphogenetic events that lead to fore- and hindgut formation in vertebrates and insects. In chick and mouse embryos the generation of the gut is initiated after gastrulation when cells move from the ventral surface to the interior, forming the anterior intestinal portal (Romanoff, 1960; Snell and Stevens, 1966) (Fig. 7). This structure then lengthens to form the foregut. The future hindgut is formed via a second invagination that occurs posteriorly forming the caudal intestinal portal (Romanoff, 1960; Roberts et al., 1995). In *Drosophila*, fore-and hindgut formation also occurs via the involution of cells at both ends of the embryo (reviewed in Skaer, 1993): the cells of the stomodeal invagination give rise to the foregut epithelium while cells in the proctodeal invagination generate the hindgut.

Despite the striking similarities of fore- and hindgut development in *Drosophila* and vertebrates, the view that gut formation is conserved seems to be hampered by a question of germ layers. The gut in vertebrates is mainly composed of endodermal tissue; the extreme anterior and posterior ends, the mouth and the anus, are considered to be of ectodermal origin (Romanoff, 1960; Snell and Stevens, 1966). In *Drosophila*, however, only the midgut is considered to be of endodermal origin and the fore- and hindgut are considered to be ectodermal tissues (Strasburger, 1932; Poulson, 1950, Campos-Ortega and Harten-

Fig. 5. Activation of *wg*, *hh* and *dpp* expression in the developing gut by *fkh*. Whole mount in situ hybridization of wild type (A,C,E) and *fkh* mutant embryos (B,D,F). (A) *wg* expression in the stomodeum (st) and the proctodeum (pr) of a stage 10 wild type embryo. Note the relatively strong expression domains (arrowheads) compared to the segmental expression in the germband. (B) *wg* expression is absent from the stomodeum and proctodeum of a late stage 11 *fkh* mutant embryo (arrowheads). (C) *hh* expression in a wild type stage 10 embryo (lateral view) in the stomodeum (left arrowhead) and the proctodeum (right arrowhead). (D) *hh* expression is absent in the stomodeum and the proctodeum of stage 10 *fkh* mutant embryos (arrowheads). (E) *dpp* expression in a late stage 11 wild type embryo in the stomodeum and the proctodeum (arrowheads). (F) *dpp* expression is absent from the fore- and hindgut primordium of a stage 11 *fkh* mutant embryo (arrowheads). Embryonic stages according to Campos-Ortega and Hartenstein (1985). Anterior is left and dorsal up.

Fig. 6. Activation of mesodermal *bap* expression by *wg* and *hh*. (A–F) Whole mount RNA in situ hybridization with a *bap* probe. (A) Stage 10 wild type embryo (lateral view). Note the strong *bap* expression domain in the visceral mesoderm primordium of the hindgut (arrowhead) and the segmental expression in the visceral muscle precursors (Apiazu and Frasch, 1993). (B) Early stage 11 wild type embryo (lateral view). Note the upcoming *bap* expression in the visceral mesoderm at the top of the stomodeal foregut tube (arrowhead). (C) Stage 12 wild type embryo (lateral view). The expression of *bap* around the stomodeal foregut invagination has become stronger (left arrowhead). The Malpighian tubules (Mt) evert from the proctodeal invagination. The expression domain of *bap* in the visceral mesoderm on top of the proctodeum begins to split into two subdomains, one around the future small intestine (middle arrowhead) and the other around the future rectum (right arrowhead). (D) Stage 14 wild type embryo (lateral view). The *bap* expression domains in the visceral mesoderm of the hindgut (hg) are strongest around the small intestine and the rectum (middle and right arrowheads). Towards the end of embryogenesis *bap* expression also comes up in the central region of the hindgut visceral mesoderm. In the foregut (fg), *bap is* expressed in the visceral mesoderm around the esophagus (left arrowhead). mg, midgut. (E) *bap* expression in a stage 9 *wg* mutant embryo. Note the strong reduction of *bap* expression in the visceral mesoderm of the hindgut (arrowhead; the segmental expression of *bap* can be used as an internal control). (F) *bap* expression in a stage 9 *hh* mutant embryo. *bap* expression in the visceral mesoderm of the hindgut is drastically reduced (arrowhead).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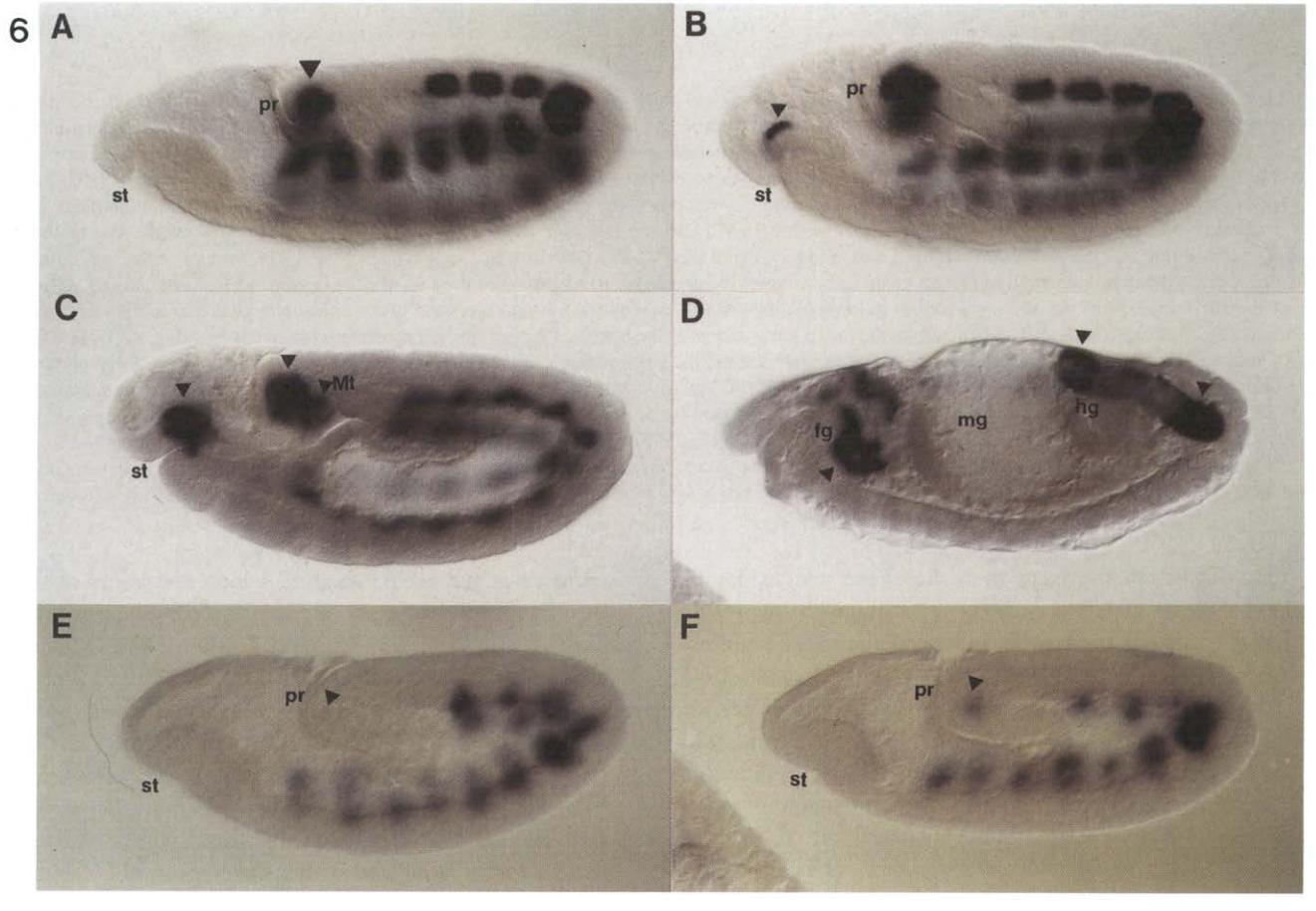
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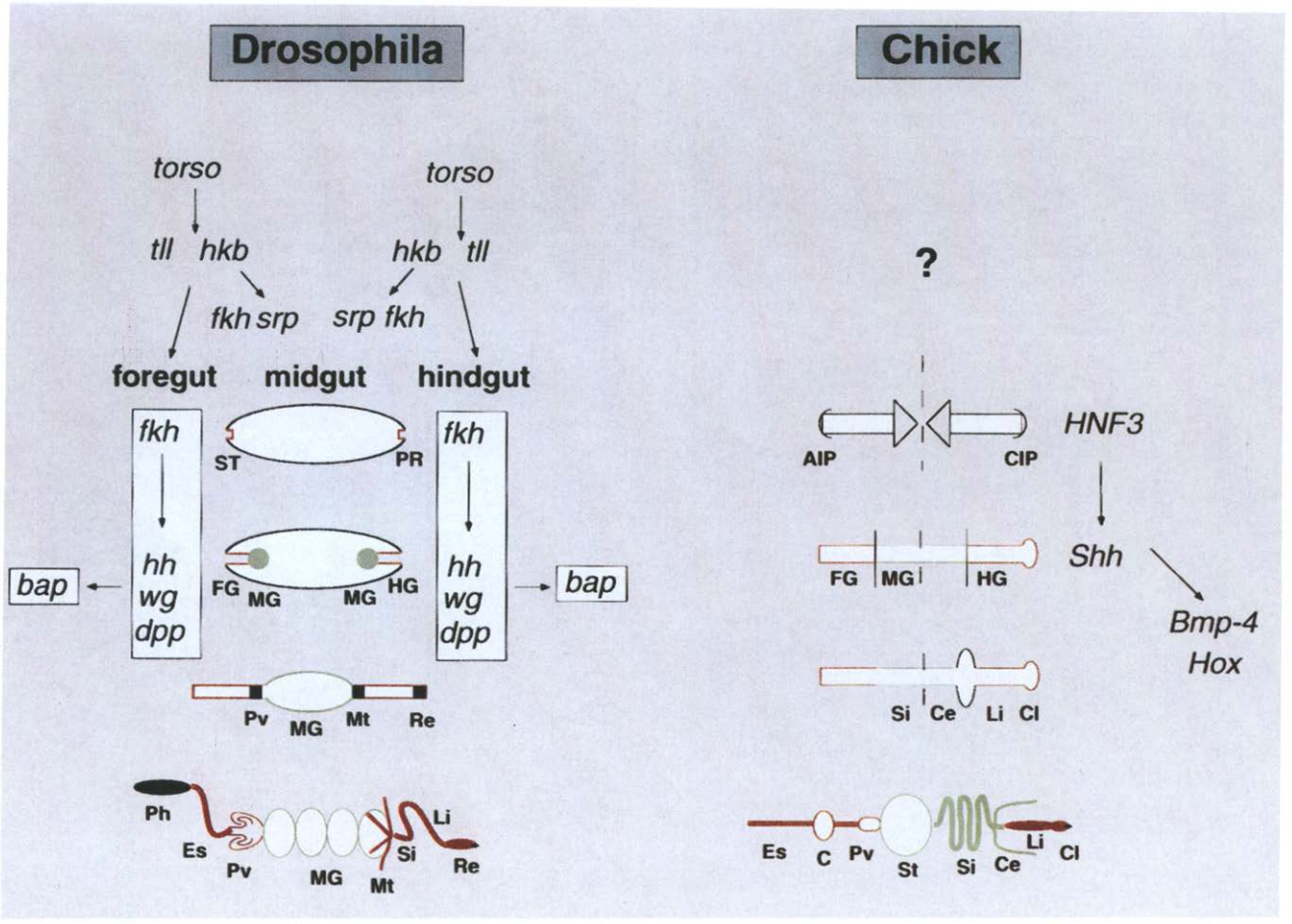

Fig. 7. Genetic circuitries underlying gut formation in *Drosophila* and chick. Schematic representations of *Drosophila* (left side) and chick (right side) gut development. In *Drosophila*, the terminal maternal system controls gut formation via the torso signaling pathway which activates the terminal gap genes *tailless* (*tll*) and *huckebein* (*hkb*) at both poles of the blastoderm embryo (reviewed by Sprenger and Nüsslein-Volhard, 1993). In response to those two gene activities, the region-specific homeotic gene *fkh is* activated in the foregut (FG), the midgut (MG) and the hindgut (HG) anlagen (Jürgens and Weigel, 1988; Weigel et al., 1989a,b). For midgut development the gene *serpent* (*srp*) *is* required in both midgut primordia (green circles) along with *fkh* activity (Reuter, 1994). Our results show that *fkh* activates the genes *hh*, *wg* and *dpp* in signaling centers in the invaginating stomodeum (ST) which comprises the foregut primordium, and the proctodeum (PR) which comprises the hindgut primordium. The activity of the signaling molecules leads to the regionalization of the gut canal and promotes the development of gut-annex organs, like the proventriculus (Pv) and the Malpighian tubules (Mt). In addition, *hh* and *wg* activities in the signaling centers are required for the expression of the homeobox gene *bap* in the visceral mesoderm of the fore- and hindgut. The structure of the larval gut is shown at the bottom. Ph, pharynx; Es, esophagus; Si, small intestine; Li, large intestine; Re, rectum. Gut development in the chick begins at the anterior and the posterior ends of the embryo as well, where the anterior intestinal portal (AIP) and the caudal intestinal portal (CIP), which are the foregut (FG) and hindgut (HG) primordia, respectively, invaginate. It has been suggested that HNF3 factors, which are homologues of the *Drosophila* transcription factor encoded by *fkh*, might activate the expression of *Sonic hedgehog* (*Shh*), a homologue of the *Drosophila hh*, in the gut endoderm (Roberts et al., 1995). During chick hindgut development, *Shh* induces the expression of *Bmp-4* and *Hox* genes in the ensheathing visceral mesoderm (Roberts et al., 1995). The structure of the adult chick gut is shown at the bottom. Ce cecae; Cl, cloaca; C, crop; St, stomach. The schematic drawings of the early stages of chick gut development have been taken and modified after Roberts et al. (1995).

stein, 1985). In the past, there has been a considerable debate about which germlayers contribute to the formation of gut parts in insects (Strasburger, 1932; Snodgrass, 1935; Parks, 1936; Poulson, 1937, 1950; Henson, 1945, 1946; discussed by Skaer, 1993). Also in vertebrates, the definitive endoderm is thought to be derived from ectodermal precursor cells (Levak-Svajger, 1971; Beddington, 1981, 1982). Instead of basing a comparison on germ layer identity, it might, therefore, be more relevant to consider what genes and molecules are active in the primordia of the structures to be compared across species. In vertebrates the HNF3 factors, which are homologues of the *Drosophila fkh* gene, are expressed in the gut primordia (Ang et al., 1993; Monaghan et al., 1993; Ruiz i Altaba et al., 1993; Sasaki and Hogan, 1993). A HNF3β lack of function mutant has been generated in mouse and it was shown that these mice display a strong gut defect (Ang and Rossant, 1994; Weinstein et al., 1994). It has been suggested that the *Shh* gene, a homologue of the *Drosophila hh*, might represent a target gene of the HNF3 factors that is activated in the developing gut epithelium (Weinstein et al., 1994; Roberts et al., 1995). Recently,

Shh was found to induce the expression of the signaling molecule Bmp-4 and of specific homeobox genes in the hindgut mesoderm of the chick gut (Roberts et al., 1995). In *Drosophila*, *fkh* controls the expression of *hh* in the gut epithelium and *hh* in turn is required for the expression of the homeobox gene *bap* in the ensheathing visceral mesoderm. These data suggest that there are not only similar components but that there also could be a common genetic pathway for gut development in vertebrates and insects (Fig. 7).

Taken together, we think that despite the differences in germ layer definition there is a considerable amount of similarity between *Drosophila* and vertebrate gut development, both in terms of the early morphological events leading to the invagination of the fore and hindgut primordia and, as currently emerging, also in terms of the conserved components of the genetic circuitries underlying gut formation.

4. Experimental procedures

4.1. Drosophila stocks

We used the following stocks: Oregon R, wg^{1G22}, hh^{1J35}, dpp^{48}, fkh^{XT6} (provided by the Tübingen and Umea stock centers), HS-*dpp* (a gift from S. Cohen), HS-*hh* (a gift of P. Ingham), HS-*wg* (a gift of S. Cohen) and mys^{XG43} (a gift of M. Affolter). The flies were maintained and embryo collections made according to standard procedures. Mutant embryos were identified through the use of *hb-lacZ* or *elav-lacZ* harboring balancer chromosomes.

4.2. Heat shock protocols

For HS-*dpp*, 0–20 h embryo collections at 18°C were placed at 37°C for 45 min twice, with 3 h at 18°C between each heat shock, allowed to recover for a further 3 h at 25°C, then fixed as described for immunohistochemical staining. The same protocol with wild type, HS-*hh*, or HS-*wg* harboring transgenic strains did not produce hindgut defects.

4.3. Antibody stainings

Antibody staining of whole mount embryos was carried out as described previously (Pankratz and Hoch, 1995), using the Vectastain ABC Elite-horseradish peroxidase system. $NiCl_2$ or $Ni/CoCl_2$ enhancement was used where necessary. The stained embryos were embedded in Araldite in capillaries according to the procedure of Schmidt-Ott and Technau (1992). We used the following antibodies at the dilutions indicated in parentheses: anti-β-galactosidase (Cappel; 1:10 000), anti-myosin heavy chain (MHC) (Kiehart and Feghali, 1986; 1:1000), anti-Fork head (Weigel et al., 1989a; 1:150) and anti-Crumbs (Crb) (Tepass et al., 1990; 1:50).

4.4. In situ hybridization

Whole mount RNA in situ hybridization (*hh*, *wg* and *dpp* as probes) and DNA in situ hybridization were performed essentially as described by Tautz and Pfeifle (1989) with modifications after M. Klinger. The probes used were: *dpp* (gift of S. Cohen), *hh* (a gift of P. Ingham), bap (a gift on M. Frasch), and *wg* (a gift of S. Cohen).

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