Genomic Regions Required for Morphogenesis of the Drosophila Embryonic Midgut

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Manuscript received June 10, 1995
Accepted for publication August 8, 1995

ABSTRACT

The Drosophila midgut is an excellent system for studying the cell migration, cell–cell communication, and morphogenetic events that occur in organ formation. Genes representative of regulatory gene families common to all animals, including homeotic, TGFβ, and Wnt genes, play roles in midgut development. To find additional regulators of midgut morphogenesis, we screened a set of genomic deficiencies for midgut phenotypes. Fifteen genomic intervals necessary for proper midgut morphogenesis were identified; three contain genes already known to act in the midgut. Three other genomic regions are required for formation of the endoderm or visceral mesoderm components of the midgut. Nine regions are required for proper formation of the midgut constrictions. The *E75* ecdysone-induced gene, which encodes a nuclear receptor superfamily member, is the relevant gene in one region and is essential for proper formation of midgut constrictions. *E75* acts downstream of the previously known constriction regulators or in parallel. Temporal hormonal control may therefore work in conjunction with spatial regulation by the homeotic genes in midgut development. Another genomic region is required to activate transcription of the homeotic genes *Antp* and *Scr* specifically in visceral mesoderm. The genomic regions identified by this screen provide a map to novel midgut development regulators.

A full understanding of how organs are formed will involve learning how genes shape tissues and how these tissues acquire the specific properties of organs necessary for physiological function. The genetic approaches available in model organisms have contributed significantly where systematic searches for relevant genes are possible, particularly for external structures (NÜSSLEIN-VOLHARD and WIESCHAUS 1980; HORVITZ and STERNBERG 1991; DICKSON and HAFEN 1993). Identification of genes important for organogenesis in model organisms is increasingly desirable in light of recent results demonstrating the astonishing conservation of regulatory genes involved in development of the fly eye and heart and the corresponding organs in vertebrates (SCOTT 1994).

Organogenesis requires definition of primordia, movements of cells to bring primordia together, inductive interactions between tissue layers, and spatially and temporally controlled cell differentiation. All these processes are exemplified by the formation of the Drosophila embryonic midgut, where our research is focused. The midgut is derived from two tissues, endoderm and visceral mesoderm (reviewed in BATE 1993; SKAER 1993). The endoderm component arises from two mesenchymal primordia that form at the anterior and posterior terminalia and then migrate through the center of the embryo to fuse together, surrounding the yolk. The visceral mesoderm component is an early segregant from the mesoderm precursors forming in the ventral furrow of the early embryo. Visceral mesoderm cells move dorsally to enclose the tube of endoderm in a thin sheath. This simple structure is soon elaborated by the formation of three constrictions in specific positions along the anterior-posterior axis of the tube, followed by the evagination of four pockets of tissue, called the gastric caeca, from the anterior midgut. The landmark events of midgut development—segregation of the visceral mesoderm, establishment and migration of the endodermal primordia, and formation of the midgut constrictions—are well described and highly replicable. These features make the development of the embryonic midgut an excellent system for studying organogenesis.

Most of the genes implicated in fly midgut development were isolated because mutations in them affect patterning of the embryonic cuticle. The posterior midgut primordium, for example, is determined by the terminal class genes *tailless* (*tll*) and *huckebein* (*hkb*), which together activate the *forkhead* (*fkh*) gene in the cells of the primordium (WEIGEL *et al.* 1990). In *fkh* mutants, the midgut primordia do not invaginate and ultimately decay (WEIGEL *et al.* 1989). Formation of the three midgut constrictions and gastric caeca is dependent on the homeotic (Hox class) genes *Sex combs reduced* (*Scr*), *Antennapedia* (*Antp*), *Ultrabithorax* (*Ubx*), and *abdominal-A* (*abd-A*) (reviewed in BIENZ 1994). The expression of each homeotic gene defines a discrete nonoverlapping

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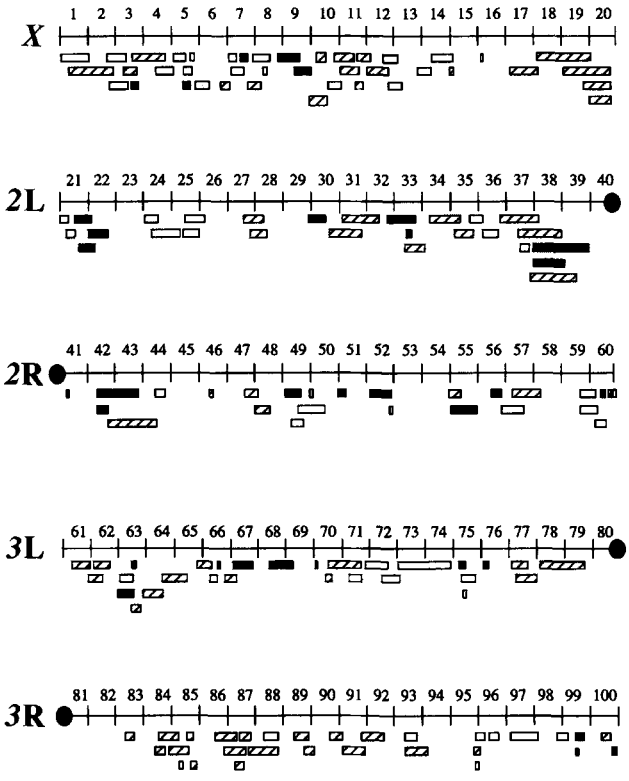

FIGURE 1.—Results of deficiency screen for midgut phenotypes. Deficiencies examined are indicated by a box representing the approximate extent of chromsomal bands deleted in each stock, below an outline of the five major chromosome arms. The stocks cover in sum ~58% of the genome. Twenty-one percent of the genome appears to be zygotically dispensable for wild-type midgut morphogenesis (□), whereas genes involved in early developmental processes prevent us from assaying 25% (▨). Thirty-one stocks show midgut defects (■). Fifteen of these are fully penetrant (see Table 2A), whereas 16 are variably penetrant (see Table 2B and MATERIALS AND METHODS).

domain along the anterior-posterior axis of the visceral mesoderm. Embryos lacking one of the homeotic gene functions fail to develop the constriction in the region where the gene is normally expressed. The domains of Hox gene expression in vertebrate gut mesoderm, which are reminiscent of the pattern seen in the fly midgut (D. J. ROBERTS and C. J. TABIN, personal communication), suggest an evolutionary link in the regulation of anterior-posterior gut differentiation.

The homeotic genes exert their effects on the midgut constrictions by activating downstream targets such as the patterning genes *decapentaplegic* (*dpp*), *wingless* (*wg*), and *teashirt* (*tsh*) (IMMERGLÜCK *et al.* 1990; REUTER *et al.* 1990; MATHIES et al. 1994). *dpp*, which encodes a member of the TGFβ family of secreted signaling proteins, and *wg*, which encodes a Wnt class secreted protein, are required for signaling between different regions of the mesoderm to activate the transcription factor *tsh* in the central constriction (MATHIES *et al.* 1994). Signal transduction components of *dpp* and *wg* pathways have also been implicated (AFFOLTER *et al.* 1994; KLINGENSMITH *et al.* 1994; NELLEN *et al.* 1994; PENTON *et al.* 1994). These studies have made the midgut a system of choice for examining how homeotic genes act on downstream targets to dictate pattern formation at the cellular level.

The actions of known regulators are insufficient to control all the cellular and developmental events that produce the midgut. What proteins mediate the migration of the endodermal primordia along the visceral mesoderm? What gene products distinguish visceral mesoderm from somatic, cardiac, and fat body mesoderm? What factors cause homeotic proteins to regulate their tissue-specific targets? What unknown genes are regulated by the homeotic genes in specifying the constrictions and other aspects of anterior-posterior identity in the midgut? What cytoskeletal factors are ultimately responsible for the midgut constrictions?

A genetic approach may reveal new components of the genetic hierarchy directing midgut development. We describe the phenotypes caused by loss of 12 genomic regions not previously known to contain midgut regulators. Regulatory regions acting both upstream and downstream of the homeotic genes were found, as was evidence linking hormonal control to embryonic organogenesis.

MATERIALS AND METHODS

Stocks: All deficiency stocks were obtained from the Bloomington Stock Center, except *Df(3R)X3F*, which was obtained from J. WARMKE (Merck, Rahway, NJ) and *Df(3R)E40* from G. REUTER (University of Pennsylvania). Cytology was taken from Flybase and LINDSLEY and ZIMM (1992). The *CyO/Sco, TM3/TM6B, CxD/TM6*, and *sqh/FM7* balancer stocks were from the SCOTT laboratory; *ru h th st cu ea/TM8, SM5/In(2LR)bw[v1]*, and *S/In(2L+2R)Cy* were obtained from BLOOMINGTON. *sry* δ^{14} mutations were obtained from A. VINCENT (Toulouse). *E75*^{e213} was provided by W. SEGRAVES (Yale) and rebalanced over a *TM6B* chromosome containing a *Ubx-lacZ* reporter construct to allow for identification of homozygous embryos.

Embryo fixation and immunohistochemical staining: Flies were allowed to lay eggs for 8 hr on molasses agar caps at room temperature. Caps were aged 10 hr before fixation. Embryos were fixed and stained as previously described (MATHIES *et al.* 1994). Briefly, embryos were dechorionated in 50% bleach and fixed in a 1:1 solution of 4% formaldehyde in HME (50 mM HEPES pH 6.9, 1 mM EGTA, 2 mM $MgSO_4$) and heptane for 30 min. Devitellinization was accomplished by replacing the formaldehyde stage with methanol and shaking the embryos vigorously. Embryos were washed in methanol, 1:1 MeOH/PBSTB (1× PBS, 0.1% Triton X-100, 0.2% bovine serum albumin) and four 20-min washes in PBSTB. Incubation with primary antibody was for 3 hr, followed by six washes in PBSTB, 3-hr incubation in secondary antibody and six further washes. Staining solution was 0.5 mg/ml diaminobenzidene in 100 mM Tris pH 7.5, with 0.03% hydrogen peroxide; for double immunolabeling, 0.6% NiCl was added to the staining solution to create a dark reaction product. The reaction was terminated by two rapid washes in PBSTB. Stained embryos were dehydrated in increasing dilutions of ethanol and mounted in methyl salicylate. The antimuscle myosin antibody was kindly provided by D. KIEHART (Duke)

TABLE 1

Deficiencies that permit wild-type midgut development

Deficiency stock	Cytology	Deficiency stock	Cytology
Df(1)BA1	1A1-2A	*Df(2L)E55*	37D2-E1; 37F5-38A1
Df(1)64c18	2E1-2; 3C2	*Df(2R)M41A4*	41A
Df(1)JC70	4C15-16; 5A1-2	*Df(2R)44CE*	44C4-5; 44E2-4
Df(1)C149	5A8-9; 5C5-6	*Df(2R)CX1*	49C1-4; 50C2-3
Df(1)N73	5C2; 5D5-6	*Df(2R)vg-B*	49D3-4; 49F15-50A3
Df(1)JF5	5E3-5; 5E8	*Df(2R)Jp8*	52F5-9; 52F10-53A1
Df(1)G4e	5E3-8; 6B	*Df(2R)AA21*	56F9-17; 57D11-12
Df(1)ct-J4	7A2-3; 7C1	*Df(2R) or-BR6*	59D5-10; 60B3-8
Df(1)ct4b1	7B2-4; 7C3-4	*Df(2R)vw*	59D6-E1; 60C
Df(1)KA14	7F1-2; 8C6	*Df(2R)Px4*	60B; 60D1
Df(1)1z90b24	8B5-8; 8B8-9	*Df(3L)HR370*	63A1; 63D1
Df(1)HA85	10C1-2; 11A1-2	*Df(3L)66C-G28*	66B8-9; 66C9-10
Df(1)RK2	12D2-E1; 13A2-5	*Df(3L)BK10*	71C; 71F
Df(1)RK4	12F5-6; 13A9-B1	*Df(3L)brm11*	71F1-4; 72D1-10
Df(1)sd72b	13F1; 14B1	*Df(3L)st-f13*	72C1-D1; 73A3-4
Df(1)r-D1	14B6; 15A2	*Df(3L)81K19*	73A3; 74F
Df(1)B	16A2; 16A6	*Df(3L)Cat*	75B8; 75C
Df(2L)PMF	21A1; 21B7-8	*Df(3L)W4*	75B10; 75C1-2
Df(2L)al	21B8-C1; 21C8-D1	*Df(3R)red1*	88B1; 88D3-4
Df(2L)ed1	24A3-4; 24D3-4	*Df(3R)e-R1*	93B3-5; 93D2-4
Df(2L)sc19-8	24C2-8; 25C8-9	*Df(3R)XS*	96A1-7; 96A21-25
Df(2L)ch-h3	25D2-4; 26B2-5	*Df(3R)XTA1*	96B; 96D
Df(2L)GpdhA	25D7-E1; 26A8-9	*Df(3R)T1-P*	97A; 98A1-2
Df(2L)r10	35E1-2; 36A7-8	*Df(3R)3450*	98E3; 99A6-8
Df(2L)H2	36A8-9; 36E1-2		

and used at a dilution of 1:1000. The secondary antibody used was goat anti-rabbit horseradish peroxidase (Jackson Labs, West Grove, PA), used at 1:500. Anti-Scr, anti-Antp, anti-abdA and anti-tsh antibodies were used as previously described (ZENG *et al.* 1993; MATHIES *et al.* 1994).

Microscopy and photography: Embryos were examined under a Nikon Optiphot using DIC optics and photographed using a 20× lens with Ektachrome 64T slide film (Kodak). Slides were scanned on a Nikon Coolscan, and figures were assembled using Adobe Photoshop and Illustrator. Figures were modified only by adjusting contrast, brightness, and color balance.

Evaluation of midgut morphology: The chromosomal deficiencies, obtained as stocks from the Bloomington Drosophila stock center, are maintained over balancer chromosomes. One quarter of the embryos from each stock will be deficiency homozygotes and one quarter will be balancer homozygotes. To ascertain whether observed midgut defects could be due to embryos homozygous for balancer chromosomes, additional stocks containing the balancers *FM7, CyO, SM5, In(2LR)bw, In(2L+2R)Cy, TM3, TM6B,* and *TM8* (see above) were also examined. No midgut defects were seen in these stocks, suggesting that midgut phenotypes observed in deficiency stocks are due to the nonbalancer chromosome. Embryos from *T(Y;2)G100* (LINDSLEY *et al.* 1972) also did not show midgut defects, suggesting that the interstitial deficiency produced by *Df(2R)G100-L141* is the source of the observed midgut phenotype.

Midgut morphology was evaluated by staining embryos from each stock with an antibody to myosin heavy chain. This antibody, which recognizes the visceral musculature as well as the somatic musculature, highlights the morphology of the midgut. In addition, the well-defined array of somatic muscles serves as an indicator of defects in body patterning outside the midgut, and the position of the pharyngeal muscles can be used to evaluate head involution. Head involution and development of the somatic musculature were used to stage embryos according to CAMPOS-ORTEGA and HARTENSTEIN (1985).

For each stock, >100 stage 12–16 embryos were examined and midgut morphology was evaluated. Deficiency stocks in which all embryos have wild-type midgut morphology are considered wild-type (Table 1). Stocks that produce nearly one quarter embryos with aberrant midguts are categorized as "defective" (Table 2A). Stocks from which 5% to 14% of embryos have aberrant midguts ($n > 200$) are classified as "variably defective" (Table 2B); this class encompasses stocks from which additional embryos were uninterpretable as well as stocks from which all other embryos were wild-type. Stocks in which midgut morphology could not be determined because of significant defects in development before stage 16 are classified as "uninterpretable" (Table 3).

RESULTS

Midgut morphology was assayed in embryos collected from 155 stocks carrying chromosomal deficiencies (Figure 1). Collectively, the stocks allow 58% of the genome to be screened. The deficiencies causing interpretable phenotypes (see MATERIALS AND METHODS) cover 25% of the genome. Homozygous deficiencies encompassing 21% of the genome have no visible defect (Table 1), suggesting that genes in the chromosomal regions covered by these deficiencies are not required in the zygotic genome for proper midgut morphogenesis.

Among the regions of the genome tested, 15 are re-

TABLE 2

Deficiencies displaying midgut defects

Deficiency stock	Cytology	Phenotype
	A. Deficiencies causing visible midgut phenotypes	
Df(1)sqh	5D1-2; 5E	Ectopic constriction forms anterior to normal central constriction
Df(2L)dp79b	22A2-3; 22D5-E1	Visceral mesoderm does not develop
Df(2L)30C	29F7-30A1; 30C2-5	Midgut primordia do not migrate from terminalia
Df(2L)TW161	38A6-B1; 40A4-B1	Variable anterior and absent central constriction (*tsh*)
Df(2R)cn88b	42C; 42E	Anterior and posterior constrictions fail to form, though small invaginations present at appropriate sites
Df(2R)pk78s	42C1-7; 43F5-8	Central constriction is absent (*sax*)
Df(2R)trix	51A1-2; 51B1-6	Central and posterior constrictions absent (*Asx*)
Df(2R)G100-L141	56D; 56F	Anterior constriction absent
Df(3L)GN50	63E1-2; 64B17	Gastric caeca do not develop; hypertrophy of anterior midgut
Df(3L)AC1	67A2; 67D7-13	Central constriction absent
Df(3L)vin7	68C8-11; 69B4-5	No constrictions form; midgut epithelium is thin and fragile
Df(3L)W10	75B3; 75C1	Posterior constriction forms anterior to normal site; anterior constriction fails to form
Df(3L)VW3	76A3; 76B2	Visceral mesoderm does not develop
Df(3R)X3F	99D	No constrictions form
Df(3R)B81;Dp(3;1)67A	99D3; 99D9-E1	Anterior and posterior constrictions and gastric caeca fail to form
	B. Deficiencies showing variably penetrant defects in midgut morphogenesis	
Df(1)dme75e19	3C11; 3E4	Midgut smaller
Df(1)C128	7D1; 7D5-6	Dorsal closure of gut incomplete
Df(1)C52	8E; 9C-D	Incomplete constriction formation
Df(1)v-L15	9B1-2; 10A1-2	Incomplete constriction formation
Df(2L)S2	21C6-D1; 22A6-B1	Incomplete constriction formation
Df(2L)ast2	21D1-2; 22B2-3	No constrictions form
Df(2L)pr1	32F1-3; 33F1-2	Variable constrictions absent
Df(2L)esc10	33A8-B1; 33B2-3	Incomplete constriction formation
Df(2L)TW1	38A7-B1; 39C2-3	Incomplete constriction formation
Df(2R)vg135	49A-B; 49D-E	Incomplete constriction formation
Df(2R)Jp5	52A13-B3; 52F10-11	Posterior constriction absent
Df(2R)PC4	55A; 55F	Variable constrictions absent
Df(2R)Px2	60C5-6; 60D9-10	Central constriction forms posterior to normal site
Df(3L)M21	62F; 63D	Variable constrictions absent
Df(3L)HR232	63C1; 63D3	Variable constrictions absent
Df(3R)E40	100C5-D1; 100F	Central constriction absent

quired for midgut morphogenesis (Table 2A). Three deficiencies cause specific defects in early stages of midgut morphogenesis. Twelve others cause defects in the formation of one or more of the midgut constrictions. In three deficiency stocks identified as constriction-defective (*Df(2L)TW161, Df(2R)pk78s, Df(2R)trix,* Table 2A), the midgut phenotype can be attributed to absence of a gene previously known to be involved in midgut morphogenesis. The screen is therefore successful in detecting expected gene functions. In nine stocks, no midgut phenotype for mutations within the interval has been described. The phenotypes caused by loss of the newly discovered regulatory regions are described below.

Deficiencies causing defects in early midgut formation: In stage 10 wild-type embryos, the visceral mesoderm segregates from the somatic mesoderm and forms two bands on the ventrolateral surface of the embryos, interior to the somatic muscle precursors. At this time, only the visceral mesoderm and the pharyngeal muscles contain muscle myosin. The midgut endodermal primordia are present as large mesenchymal cell masses at the anterior and posterior of the embryo. These primordia travel along the bands of visceral mesoderm to fuse in the center of the embryo during stage 12. Subsequently, the endoderm and visceral mesoderm migrate dorsally together to enclose the yolk. At stage 14, stained myosin outlines the visceral mesoderm that has formed a continuous sheet around the midgut tube (Figure 2A).

Homozygous deletions for either of two genomic intervals cause a striking loss of all of the visceral mesoderm of the midgut. In stage 10 embryos homozygous for either *Df(2L)dp79b* or *Df(3R)VW3,* visceral mesoderm cannot be detected with antibodies against either muscle myosin (Figure 2B) or Fas III (data not shown),

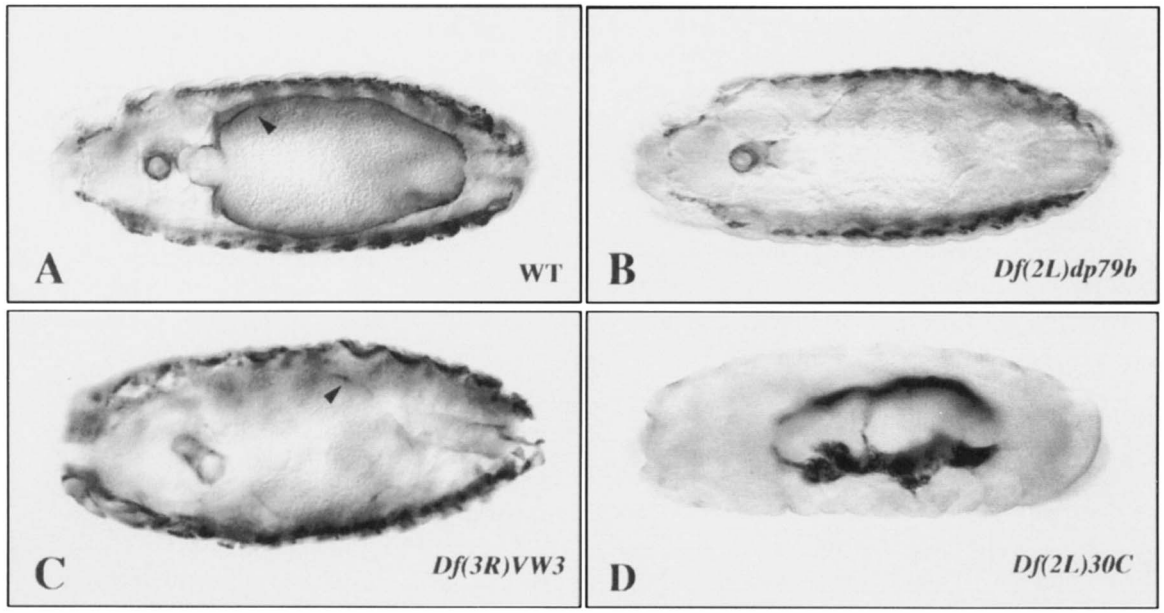

FIGURE 2.—Early midgut defects shown by deficiency stocks. Unless otherwise noted, all embryos are stained with an antibody to muscle myosin to reveal somatic and visceral musculature. Anterior is to left in all figures. (A) Wild-type embryo at stage 14. At this stage the endoderm forms a simple tube of epithelium, and the visceral musculature (arrow) has surrounded it. (B) Stage 13 *Df(2L)dp79b* embryo. No visceral mesoderm is present, although the somatic musculature and visceral musculature covering the foregut and hindgut is developing normally. (C) Stage 16 *Df(3L)VW3* embryos show a phenotype identical to *Df(2L)dp79b* (see B above). By this stage, the endoderm has spread around the yolk sac but has not enclosed it. A few small myosin-positive cells can be seen attatched to the midgut endoderm (arrow). (D) Stage 11 *Df(2L)30C* embryo. No endoderm can be seen in the center of the embryo. Visceral mesoderm cells are seen migrating dorsally and ventrally in a disorganized fashion in the absence of the endoderm.

an antigen expressed on visceral mesoderm after its segregation from somatic mesoderm (PATEL *et al.* 1987). Migration of the midgut primordia is delayed, although endoderm can be seen around the margin of the yolk by stage 16. Also at stage 16, occasional muscle myosin-positive cells are found adhering to the midgut (Figure 2C). Except for these cells, the midgut is devoid of visceral mesoderm, retains a rounded structure, and never attains the heart shape of the wild-type stage 15 gut. No evidence of midgut constrictions is seen in these embryos. Despite the lack of midgut visceral mesoderm, the visceral mesoderm surrounding the foregut and hindgut is present in these embryos, as is the mesodermal component of the dorsal vessel (data not shown).

In embryos homozygous for *Df(2L)30C,* the bands of visceral mesoderm cells at stage 10 appear normal. However, by stage 12, the visceral mesoderm cells migrate dorsally in a disorganized manner rather than as a coherent sheet (Figure 2D). Close examination of these embryos reveals that the migrating midgut primordia arrest shortly after invaginating at the anterior and posterior poles. Disorganization of the visceral mesoderm seems to result from an attempt by the mesoderm cells to migrate properly in the absence of the endoderm.

Deficiencies causing defects in the formation of midgut constrictions: In wild-type embryos, three constrictions form in the midgut, each oriented perpendicular to the anterior-posterior axis of the midgut tube. The constrictions appear to originate by a local contraction of visceral mesoderm cells, which causes the endoderm to compress the yolk (REUTER and SCOTT 1990). The central midgut constriction is the first to form and can be seen from late stage 15. The posterior constriction forms shortly thereafter, followed by the formation of the anterior constriction by the end of stage 16. These three constrictions divide the midgut into four approximately equal compartments (Figure 3A). During late stage 16, the gastric caeca appear as four short pockets of tissue evaginating from the anterior midgut, adjacent to the proventriculus; the appearance of the caeca is preceded by small invaginations that form at the base of the developing caeca (REUTER and SCOTT 1990). For the purposes of this article, we refer to these invaginations as the gastric caeca constrictions. During all of these shape changes, no cell division occurs (BATE 1993).

Deficiency stocks that produce embryos lacking a single constriction were identified, as well as deficiency stocks lacking multiple or all constrictions. In addition, several deficiency stocks produce embryos in which the position of a constriction, but not its formation, is aberrant.

Deficiencies causing the absence of a single constriction: Deletion of any of three genomic intervals produces embryos lacking a single constriction. In collec-

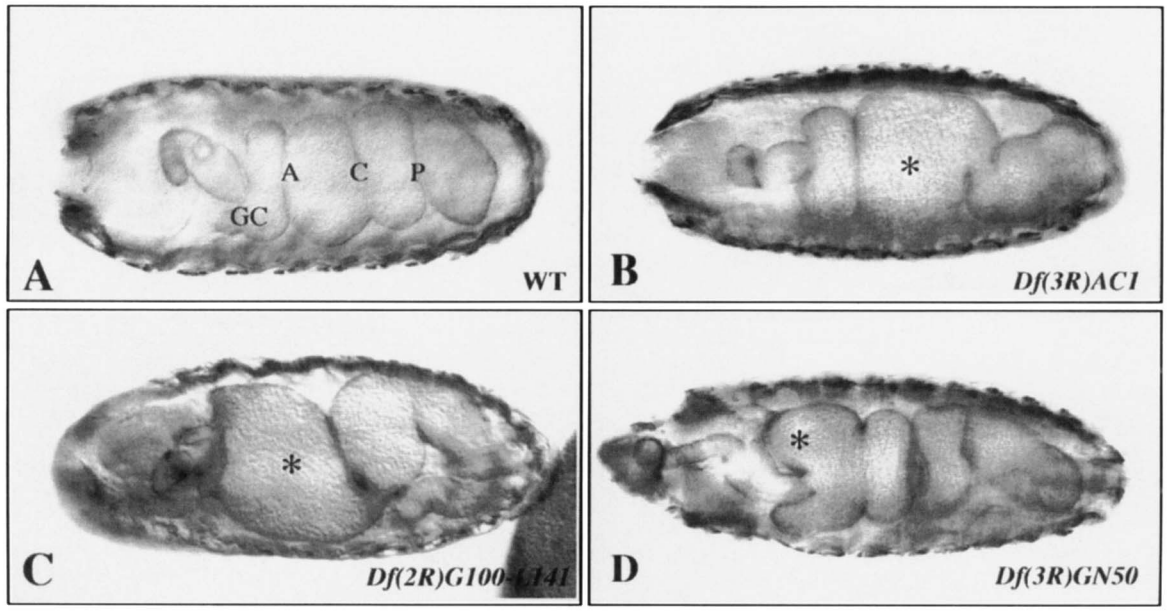

FIGURE 3.—Deficiency stocks that lack a single constriction. Constriction absence is denoted by an asterisk (*). (A) Wild-type stage 16 embryo, showing complete formation of the anterior (A), central (C), and posterior (P) midgut constrictions, which delineate the four equally sized midgut compartments. The budding gastric caeca (GC) are indicated. (B) Stage 16 *Df(3L)AC1* embryo. The central constriction is absent. (C) Late stage 16 *Df(2R)G100-L141* embryo. The anterior constriction is absent. (D) Late stage 16 *Df(3L)GN50* embryo. The anterior of the midgut extends anterior to the proventriculus; the narrow tubes of gastric caeca have not formed.

tions from the stock *Df(3L)AC1,* stage 16 embryos lack the central constriction, whereas the anterior, posterior, and gastric caeca constrictions form normally (Figure 3B). This phenotype is similar to that seen in *Ubx* and *wg* mutants (TREMML and BIENZ 1989; IMMERGLÜCK *et al.* 1990; REUTER *et al.* 1990). *Df(2R)G100-L141,* a stock assembled from the Y-autosome translocations *G100* and *L141* (LINDSLEY *et al.* 1972; K. MATTHEWS, personal communication), produces embryos deficient for the 56D;56F region. Embryos collected from this stock lack the anterior constriction but have normal central and posterior constrictions, like *Antp* mutants (Figure 3C). Embryos homozygous for *Df(3L)GN50* have drastic defects in head involution and aberrant somatic muscle development. In embryos with these defects, the anterior, central, and posterior midgut constrictions form normally but the constrictions underlying the gastric caeca fail to form (Figure 3D). Two large pouches are seen in place of the normal four narrow tubes of gastric caeca, and the midgut extends significantly anterior to its wild-type location. This phenotype is similar to that described for mutations in the PS integrin subunit encoded by the *inflated* gene (BROWN 1994).

Deficiencies blocking formation of multiple constrictions: The absence of either of two genomic intervals prevents the formation of more than one constriction while allowing other constrictions to develop normally. In embryos homozygous for *Df(2L)cn88b,* the anterior and central midgut constrictions fail to form (Figure 4A), as in *tsh* mutants (MATHIES *et al.* 1994). In late stage 16 embryos, small invaginations are seen in the proper locations for the anterior and central constrictions, but the invaginations pinch in only slightly below the surface of the endoderm and never make the deep divisions in the yolk made by wild-type constrictions.

Df(3R)B81 homozygotes have defects in multiple tissues and do not develop far enough to evaluate constriction morphogenesis. This early death is prevented in a *Df(3R)B81* stock that carries a duplication, *Dp(3;1)67A,* that replaces most chromosomal material lost in the deficiency. With this genetic makeup, embryos lack only the 99D region; such embryos develop normally except their midguts form only a central constriction (Figure 4B). No evidence of the anterior, posterior, and gastric caeca constrictions is ever seen. In addition, the central constriction is often broader than in wild-type, spanning several cell diameters (see, *e.g.,* Figure 5D).

Deficiencies that block formation of all constrictions: Embryos homozygous for *Df(3L)vin7* reach stage 16 without forming significant midgut constrictions (Figure 4C). The visceral mesoderm appears normal at this stage, but the endoderm cells are clearly abnormal. The epithelium is significantly thinner, and individual cells are wider than the narrow columnar array seen in the wild-type gut. In addition to this midgut phenotype, the hindgut is greatly reduced and the pharyngeal muscles, which normally form an ordered bilaterally symmetric array, are disorganized (data not shown).

A number of other deficiencies exist that overlap the breakpoints of *Df(3L)vin7. Df(3L)vin5* embryos show midgut, hindgut, and pharyngeal muscle phenotypes identical to *Df(3L)vin7,* narrowing the region required

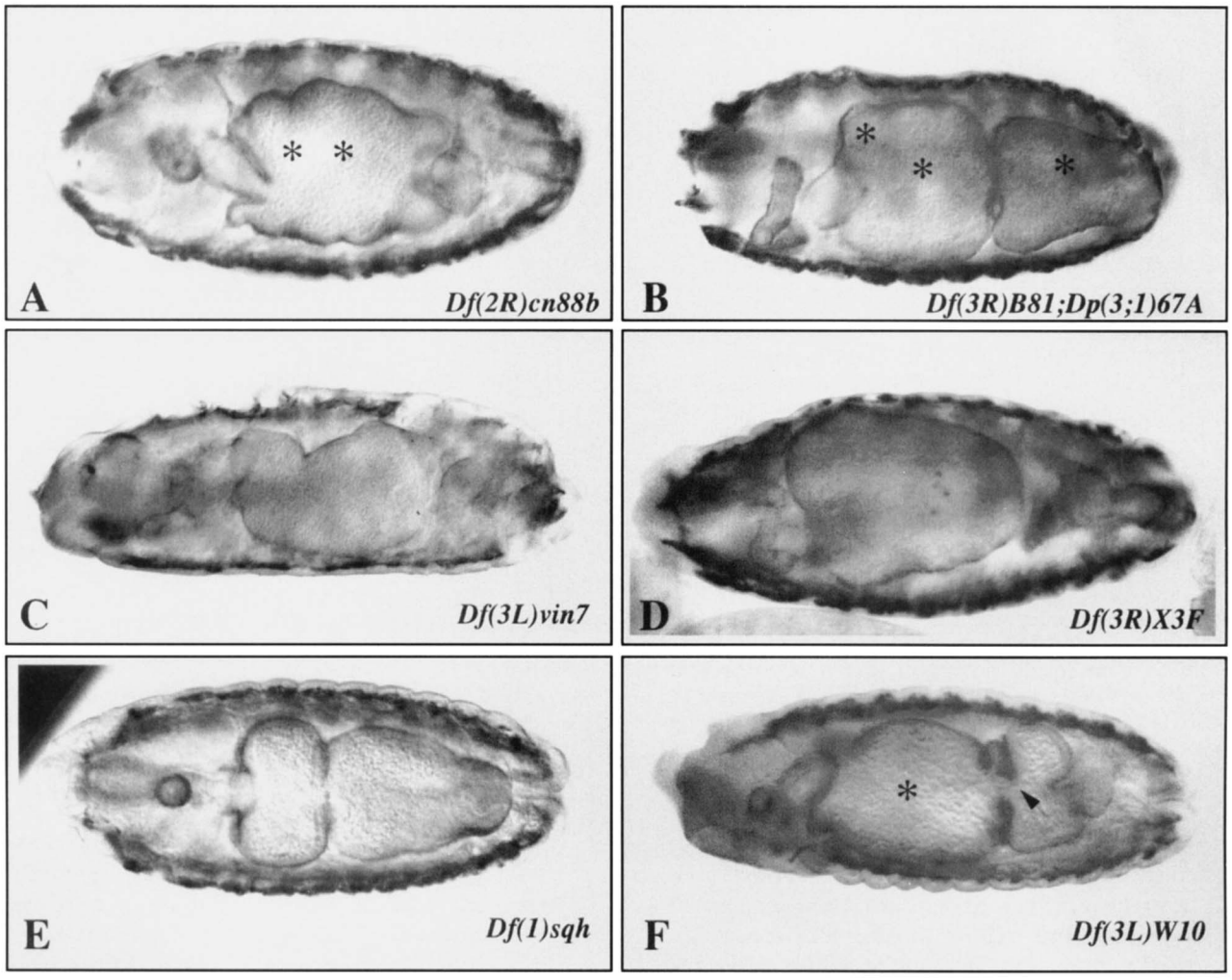

FIGURE 4.—Deficiency stocks that lack multiple constrictions or show misplaced constrictions. (A) Late stage 16 *Df(2R)cn88b* embryo. Small invaginations at the sites of the anterior and central constrictions are seen (*) but no constrictions form at these sites. (B) Late stage 16 *Df(3R)B81;Dp(3;1)67A* embryo. The anterior and posterior constrictions are absent and the gastric caeca do not form. (C) Stage 16 *Df(3L)vin5* embryo. No constrictions form. (D) Late stage 16 *Df(3R)X3F* embryo. No constrictions form. (E) Stage 15 *Df(1)sqh* embryo. Deep invaginations appear between the sites of the wild-type anterior and central constrictions. (F) Stage 16 *Df(3L)W10* embryo. The anterior constriction is absent and an ectopic constriction (arrowhead) can be seen forming immediately posterior to the normally placed central constriction.

for wild-type development of these structures to 68C8-11;68F3-5. KISPERT *et al.* (1994) found that the hindgut phenotype of such embryos can be rescued by a transgene carrying 20 kb of genomic DNA from the 68D region. The transgene contains a single transcription unit, the *Drosophila T-related gene* (*Trg*). The transgene does not rescue the midgut or pharyngeal muscle phenotypes nor is *Trg* product detected in midgut or pharyngeal muscle tissues. It is possible that the *Trg* transgene does not contain regulatory information capable of providing fuctional rescue of the midgut and pharyngeal muscles. It is also possible that additional genes required for the development of these structures lie within the 68C;68F region.

Df(3R)X3F embryos also complete embryogenesis without forming the midgut constrictions or gastric caeca. Although homozygous embryos have variable defects in multiple tissues, even embryos that have wild-type somatic muscle and nerve cord morphology at stage 17 fail to form constrictions (Figure 4D). The midgut appears as an inflated balloon-like structure, significantly broader in the anterior. Endodermal cells are irregularly sized and spaced in such embryos.

Deficiencies that cause misplaced constrictions: In embryos hemizygous for *Df(1)sqh,* a deep invagination appears in the stage 15 midgut, in a position midway between the appropriate sites for the anterior and central constrictions (Figure 4E). This invagination causes a division in the yolk less extreme than that caused by proper midgut constrictions. This aberrantly placed "constriction" persists, and anterior and central constrictions do not form.

A misplaced constriction is also seen in embryos homozygous for *Df(3L)W10.* In these embryos, immedi-

ately after the appearance of the central constriction, a constriction forms just posterior to it, giving rise to a greatly reduced third midgut compartment (Figure 4F). No constriction forms posterior to this new constriction, suggesting that it may be a posterior constriction that forms in a more anterior location. In addition to the misplaced posterior constriction, *Df(3L)W10* embryos never form anterior constrictions and make only stunted gastric caeca.

Novel regulator of homeotic genes in 99D: Several of the midgut phenotypes seen in deficiency homozygotes are novel and reveal new genetic functions required in the midgut. We analyzed in greater detail two deficiencies with intriguing phenotypes and accessible genetics. Embryos produced by the stock *Df(3R)B81;Dp(3;1)67A,* which lacks chromosomal bands 99D3;99D9-E1, have a unique phenotype. The embryonic midgut of homozygotes develops with an "hourglass" phenotype: only the central constriction remains. This phenotype is also seen in embryos that carry *Df(3R)L127* (99C;99E) in *trans* to *Df(3R)B81;Dp(3;1)-67A,* indicating that the phenotype is due to removal of genetic functions residing in the 99D region. Mutations in *sry δ*, the only gene in the region for which mutations are available, do not cause midgut phenotypes (data not shown).

The *Df(3R)B81;Dp(3;1)67A* phenotype is due at least in part to changes in the regulation of homeotic genes. In wild-type embryos, *Scr* is expressed just posterior to the gastric caeca (Figure 5A), whereas *Antp* is expressed in the anterior constriction (Figure 5C), and *abd-A* is expressed posterior to the central constriction thoroughout the third and fourth compartments (Figure 5E). In *Df(3R)B81;Dp(3;1)67A* homozygotes, *Scr* and *Antp* proteins are absent from their normal regions of the visceral mesoderm (Figures 5, B and D), although the visceral mesoderm cells are clearly present and produce myosin. Repression of *Scr* and *Antp* is not due to ectopic expression of *Ubx*, since *Ubx* expression is wild-type in these embryos (data not shown). Expression of *Scr* and *Antp* in the nervous system, epidermis, and somatic muscle is unchanged in *Df(3R)B81;Dp(3;1)67A* embryos. *Scr* and *Antp* are required for formation of the gastric caeca and anterior constriction, respectively, so these aspects of the *Df(3R)B81;Dp(3;1)67A* midgut phenotype are explained by the absence of the two homeotic proteins. However, *abdA* expression is unchanged in *Df(3R)B81;Dp(3;1)67A* embryos (Figure 5F), so the absence of the posterior constriction cannot be accounted for by a change in homeotic gene expression.

Involvement of the *E75* ecdysone-responsive gene in midgut morphogenesis: Deletion of chromosomal bands 75B3-75C1 in *Df(3L)W10* embryos causes loss of the anterior constriction and formation of an ectopic constriction in the posterior of the midgut. To precisely define the genetic region responsible for these defects, the midgut phenotypes of several deficiencies with breakpoints in 75B were examined (Figure 6A). *Df(3L)W4* embryos have no defects in midgut morphogenesis, limiting the region responsible for the *Df(3L)W10* midgut phenotype to 75B3-75B10 (75B10 is the proximal breakpoint of *Df(3L)W4*). *Df(3L)x48* is a 105-kb deficiency that removes the prominent 75B puff (SEGRAVES and HOGNESS 1990). Homozygous *Df(3L)x48* embryos have a midgut phenotype similar to that of *Df(3L)W10* embryos (Figure 6B, compare with Figure 4F), as do embryos transheterozygous for the two deficiencies (data not shown).

Df(3L)x48 removes a single lethal complementation group, *E75*. The *E75* gene encodes a steroid receptor superfamily member that is induced in response to ecdysone, the molting hormone (SEGRAVES and HOGNESS 1990). Embryos homozygous for the EMS-induced allele $E75^{e213}$ (SEGRAVES 1988) display the small third midgut compartment and the absent first constriction seen in *Df(3L)W10* and *Df(3L)x48* embryos (Figure 6C). The midguts of embryos transheterozygous for $E75^{e213}$ and *Df(3L)x48* are indistinguishable from *Df(3L)W10* homozygotes, demonstrating that the midgut phenotype seen in *Df(3L)W10* embryos is due to loss of the *E75* gene. The absent anterior constriction in *E75* embryos is the same phenotype seen in *Antp* and *tsh* embryos. To determine how *E75* might fit into the Hox-regulated hierarchy directing constriction formation, *E75* homozygous embryos were stained with antibodies to *Antp* and *tsh* proteins. No changes in *Antp* (data not shown) or *tsh* expression are seen (Figure 6, D and E), suggesting that *E75* functions downstream of *tsh,* or in a parallel pathway, to form the anterior constriction.

DISCUSSION

Interpreting the results of the deficiency screen for midgut defects: We screened chromosomal deficiencies covering 58% of the genome to look for genomic regions necessary for midgut morphogenesis. Twenty-one percent of the genome does not contain strictly zygotically active genes required for midgut morphogenesis. Fifteen genomic regions are required for specific aspects of midgut morphogenesis. Twelve of these regions do not contain genes previously known to be involved in midgut development and thus provide a map of genomic regions in which unknown genes required for midgut morphogenesis lie.

Screening deficiency homozygotes for embryonic phenotypes is an old idea, dating back to POULSON (1937). The principal advantage of a deficiency screen is that it allows one to rapidly survey, using existing stocks, a large proportion of the genome for zygotic genes involved in a process of interest. For genes lacking a maternal contribution, the phenotype will reflect complete loss-of-function. A deficiency screen will miss genes whose mutant phenotype can be maternally rescued or whose functions are redundant.

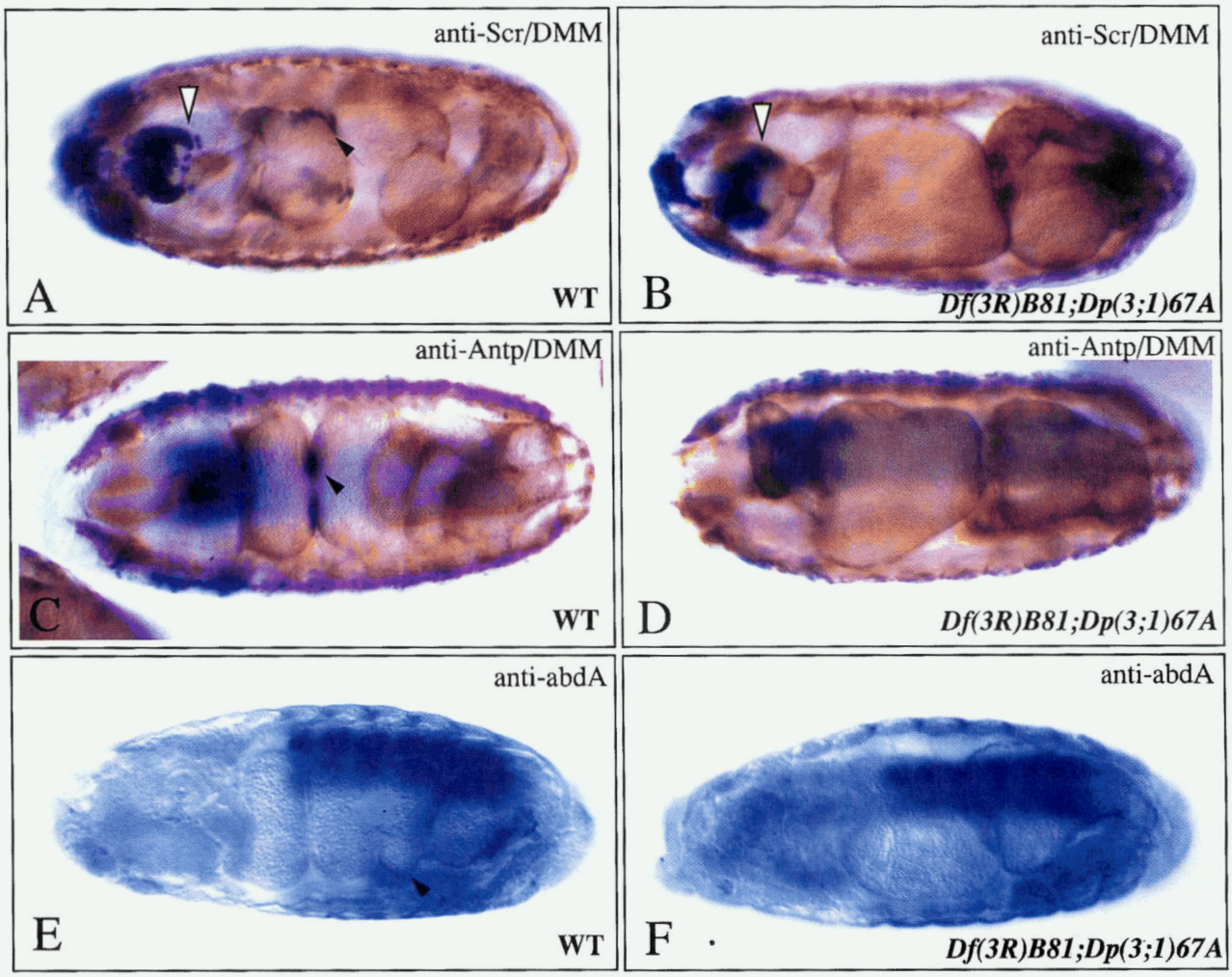

FIGURE 5.—Homeotic gene expression in wild-type and *Df(3R)B81;Dp(3;1)67A* embryos. *Scr* (arrow, black nuclei in A) is expressed in wild-type embryos posterior to the gastric caeca, as well as in the brain (open arrowhead). In *Df(3R)B81;Dp(3;1)67A* embryos (B), all midgut expression is absent, although the expression in the nervous sytem is unaffected (open arrowhead). Muscle myosin staining (brown in A–D) proves that the visceral mesoderm is present in *Df(3R)B81;Dp(3;1)67A* embryos. *Antp* protein is affected in a similar fashion to *Scr*: *Antp*, normally present surrounding the anterior constriction (black nuclei, C) is absent in *Df(3R)B81;Dp(3;1)67A* embryos. Expression of *abdA* protein throughout the third and fourth compartments of wild-type embyros (blue nucei, E) is unchanged in *Df(3R)B81;Dp(3;1)67A* embryos.

A significant proportion of the deficiencies examined are uninterpretable with respect to midgut constriction formation, often due to removal of genes known to be zygotically required for patterning the early embryo (Table 3). Nevertheless, a fair number of deficiencies display highly expressive defects in midgut morphogenesis while leaving gross development of other internal tissues largely intact. In a recently published paper, HARBECKE and LENGYEL (1995) stained embryos from a similar deficiency collection with an anti-crumbs antibody that labels ectodermal derivatives such as hindgut and malpighian tubules. They identified several deficiencies that caused specific defects in these structures, as well as deficiencies that showed midgut defects. Most of these midgut-defective deficiencies are in agreement with this report; the differences may be the consequence of examination of the midgut with DIC optics alone instead of with a histochemical stain. HARBECKE and LENGYEL's results concerning deficiency embryos that are interpretable in middle to late embryogenesis are also largely in agreement with our findings. These two studies, in combination with others examining different tissues at different stages (JAN *et al.* 1987; DRYSDALE *et al.* 1993; SMITH *et al.* 1994), should prove useful as a guide for future deficiency screens.

Caution must be used in concluding that the phenotype observed in embryos homozygous for the deficiency chromosome is due to deletion of a single gene within the interval. The phenotype might be caused by lethal mutations harbored on the chromosome outside the cytologically visible deficiency or the phenotype could be due to the combined effects of multiple missing genes. However, in a number of cases (*Df(3L)vin7*, *Df(3L)W10*, *Df(3R)B81;Dp(3;1)67A*), we found overlap-

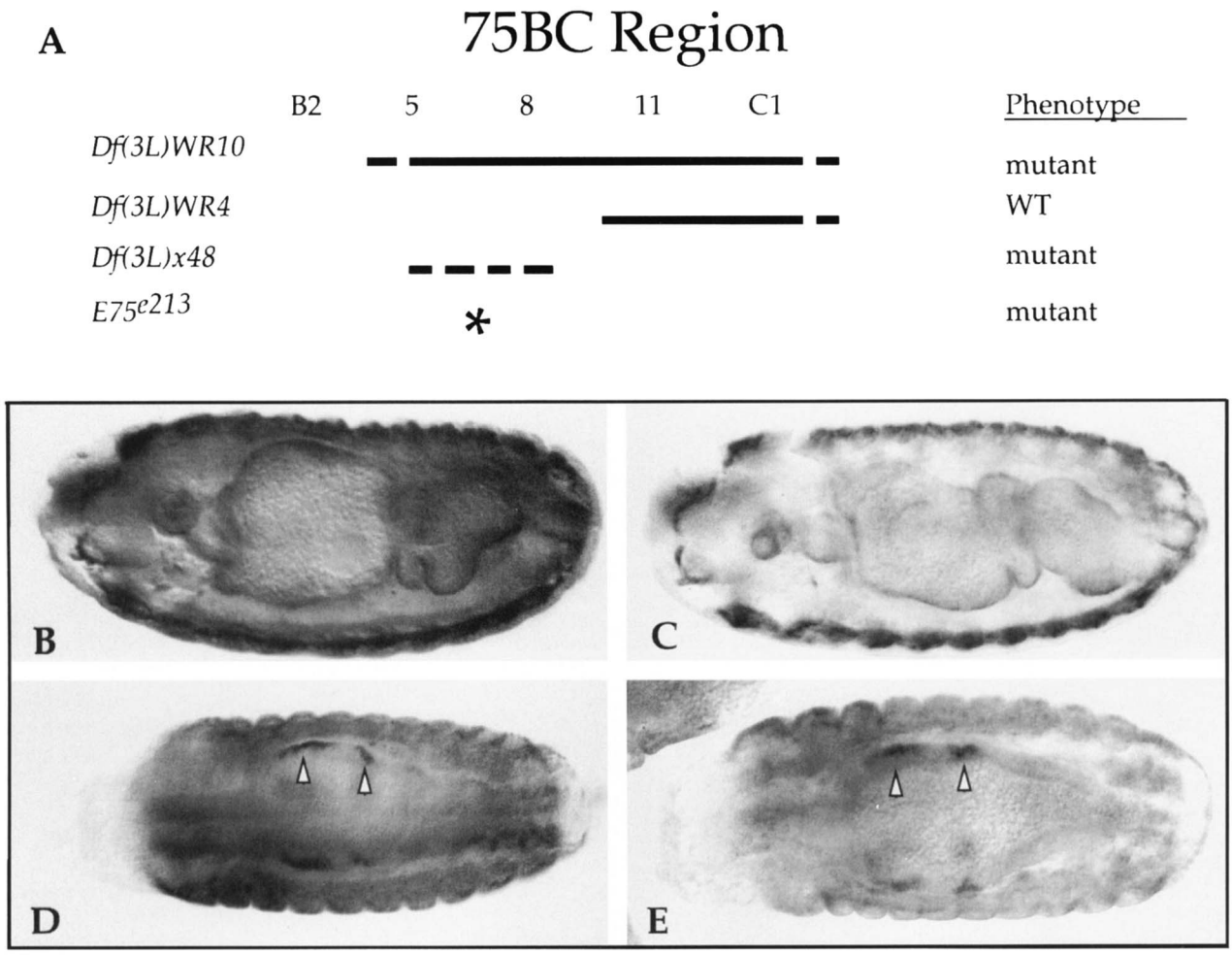

FIGURE 6.—Futher characterization within the 75BC region. (A) Embryos from a collection of deficiencies that overlap *Df(3L)W10* were examined for midgut phenotypes. Solid lines denote the chromosomal bands removed in each deficiency, with dotted lines indicating uncertainty. Homozygous *Df(3L)x48* embryos (B) lack the anterior constriction and form a small third compartment identical to that seen in *Df(3L)W10* embryos (see Figure 3F). $E75^{c213}$ homozygotes (C) display the same phenotype, demonstrating that the defect seen in *Df(3L)W10* homozygotes is due to removal of the *E75* gene. Although *E75* embryos lack the first constriction, the expression of *tsh*, a transcription factor required for formation of the anterior and central constrictions, is unchanged; *tsh* protein (arrowheads) is present in the anterior and central constrictions of both wild-type (D) and *E75* (E) embryos (identified by lack of β-galactosidase staining, see MATERIALS AND METHODS) at stage 12.

ping deficiencies that produce midgut phenotypes identical to that of the original deficiency when homozygous or transheterozygous. Such results confirm that the deficiency is the source of the phenotype and further refine the location of the gene(s) responsible for the phenotype. We provide one example in which a mutation in a single gene with a previously unidentified function in midgut development (*E75*) lying within a midgut-defective deficiency (*Df(3L)W10*) can account for the phenotype observed in the deficiency. For other deficiencies, such as *Df(3R)B81;Dp(3;1)67A*, screening for new mutations that lie within the deficiency may be necessary to identify the responsible gene(s).

Assembly of midgut components: We identified deficiencies in which the early development of either component of the midgut—the endoderm or visceral mesoderm—is aberrant. Two deficiencies specifically disrupt formation of the visceral mesoderm. The midgut phenotype seen in *Df(2L)dp79b* and *Df(3R)VW3* embryos—absence of the visceral mesoderm and aberrant migration of endodermal primordia—is indistinguishable from that seen in mutants for *tinman (tin)*, which encodes a trancription factor expressed in the progenitors of visceral and cardiac muscle (AZPIAZU and FRASCH 1993; BODMER 1993). *tin* mutants also fail to generate the cells that will become the myocardium of the dorsal vessel (the embryonic heart), leading to the proposal that the *tin* gene product is required in the dorsal mesoderm prior to the segregation of cardiac mesoderm from visceral mesoderm (AZPIAZU and FRASCH 1993; BODMER 1993). Because *Df(2L)dp79b* and *Df(3R)VW3* embryos form a wild-type dorsal vessel, the genetic functions removed in these two deficiencies seem to be required for proper development of the midgut visceral mesoderm after it segregates from the myocardium and thus may lie genetically downstream of *tin*.

TABLE 3

Deficiencies whose midgut phenotypes are uninterpretable

Deficiency stock	Cytology	Gene(s) removed[a]	Deficiency stock	Cytology	Gene(s) removed[a]
Df(1)sc[J4]	1B; 3A3	*giant*	*Df(3L)emc5*	61C3-4; 62A8	
Df(1)N-8	3C2-3; 3E3-4	*Notch*	*Df(3L)R-G5*	62A10-B1; 62C4-D1	
Df(1)A113	3D6-E1; 4F5	*hindsight*	*Df(3L)R-G7*	62B8-9; 62F2-5	
Df(1)HA32	6E4-5; 7A6		*Df(3L)HR119*	63C6; 63E	
Df(1)RA2	7D10; 8A4-5	*stardust*	*Df(3L)GN24*	63F4-7; 64C13-15	
Df(1)v-N48	9F; 10C3-5		*Df(3L)ZN47*	64C; 65C	
Df(1)N71	10B2-8; 10D3-8		*Df(3L)pbl-X1*	65F3; 66B10	
Df(1)N105	10F7; 11D1	*twisted gastrulation*	*Df(3L)h-i22*	66D10-11; 66E1-2	*hairy*
Df(1)JA26	11A1; 11D-E		*Df(3L)29A6*	66F5; 67B1	
Df(1)N12	11D1-2; 11F1-2		*Df(3L)Ly*	70A2-3; 70A5-6	
Df(1)C246	11D-E; 12A1-2	*twisted gastrulation*	*Df(3L)fzGF3b*	70C1-2; 70D4-5	*shadow*
Df(1)g-1	12A; 12E		*Df(3L)fzM21*	70D2-3; 71E4-5	*shadow*
Df(1)r-D17	14F6; 15A6		*Df(3L)rdgC*	77A1; 77D1	
Df(1)N19	17A1; 18A2		*Df(3L)ri79C*	77B-C; 77F-78A	*knirps*
Df(1)JA27	18A5; 20A	*runt, folded gastrulation*	*Df(3L)Pc-MK*	78A3; 79E1-2	*Polycomb*
Df(1)mal3	19A1-2; 20E-F	*runt, folded gastrulation*	*Df(3R)Tp110*	83C1-2; 83D04-05	
Df(1)DCB1-35b	19F1-2; 20E-F	*folded gastrulation*	*Df(3R)Antp17*	84B1-2; 84D11-12	*fushi tarazu*
Df(1)JC4	20A1; 20E-F	*folded gastrulation*	*Df(3R)p712*	84D4-6; 85B6	*hunchback*
Df(2L)J136-H52	27C2-9; 28B3-4	*wingless*	*Df(3R)pXT103*	84F14; 85C-D	*hunchback*
Df(2L)spdX4	27E; 28C	*wingless*	*Df(3R)p819*	85A03; 085B06	*hunchback*
Df(2L)MdhA	30D-30F; 31F		*Df(3R)by10*	85D8-12; 85E7-F1	*knipkopf*
Df(2L)J39	31A; 32C-E	*pimples?*	*Df(3R)by62*	85D11-14; 85F6	*knipkopf*
Df(2L)prd1.7	33B2-3; 34A1-2	*paired, extra sex combs*	*Df(3R)M-Kx1*	86C1; 87B1-5	
Df(2L)b87e25	34B12-C1; 35B10-C1		*Df(3R)T-32*	86E2-4; 87C6-7	
Df(2L)osp29	35B1-3; 35E6	*snail*	*Df(3R)pb Antp*	87B1-2; 87C	*fushi tarazu*
Df(2L)TW50	36E4-F1; 38A6-7	*screw?*	*Df(3R)ry615*	87B11-13; 87E8-11	
Df(2L)pr76	37D; 38E	*caudal, screw*	*Df(3R)ry[506-85C]*	87D1-2; 88E5-6	
Df(2L)TW84	37F5-38A1; 39D3-E1	*caudal, screw*	*Df(3R)P115*	89B07-08; 89E07-08	
Df(2R)cn9	43E; 44C		*Df(3R)C4*	89E; 90A	
Df(2R)eve1.27	46C3-4; 46C9-11	*even-skipped*	*Df(3R)P14*	90C2-D1; 91A1-2	*stripe?*
Df(2R)en-A	47D3; 48A5-6	*engrailed*	*Df(3R)ChaM7*	91A; 91F5	
Df(2R)en30	48A3-4; 48C6-8	*engrailed*	*Df(3R)D1BX12*	91F1-2; 92D3-6	*Delta*
Df(2R)Pc111B	54F6-55A1; 55C1-3	*Polycomblike*	*Df(3R)e-N19*	93B; 94	*tinman, bagpipe*
Df(2R)PuD17	57B4; 58B	*faint little ball*	*Df(3R)crbS87-4*	95E8-F1; 95F15	*crumbs*
Df(2R)M-c33a	60E2-3; 60E11-12		*Df(3R)crbS87-5*	95F7; 96A1	*crumbs*
Df(2R)kr10	60E10; 60F5	*Kruppel*	*Df(3R)awd-KRB*	100C; 100D	

[a] LINDSLEY and ZIMM (1992); DRYSDALE *et al.* (1993); SMITH *et al.* (1994).

We found a single deficiency in which migration and fusion of the midgut endoderm primordia were prevented. *Df(2L)30C* embryos have morphologically normal midgut primordia, suggesting that the terminal system that specifies the primordia and activates *fkh* within them is intact. The endodermal cells arrest shortly after beginning their migration along the visceral mesoderm, indicating that the 30A;30C interval contains a genetic function required for migration of midgut primordia. Although detailed ultrastructural analyses have been made of this process (TEPASS and HARTENSTEIN 1994), no genes have yet been implicated in endoderm cell movement.

Formation of midgut constrictions: Deficiencies were found that block formation of a single constriction, several constrictions, or all constrictions. We did not observe midgut phenotypes in which the midgut formed its constrictions in a different temporal order nor were stocks found in which embryos make extra constrictions. Two deficiencies, *Df(2R)G100-L141* and *Df(3L)AC1,* form some constrictions but not others. Such a phenotype is similar to mutations in the homeotic genes as well as *tsh, dpp,* and *wg.* The genes deleted may be new components of the homeotic regulated pathway for constriction formation. In *Df(2R)cn88b* embryos, the correct spatial information for formation of the anterior and central constrictions is evidently present, as two small invaginations are seen in the positions of the wild-type anterior and central constrictions. However, these are never elaborated into full constrictions. The genetic functions missing from this deficiency stock might link the positional information provided by the homeotic genes to the cytoskeletal events that create the constrictions.

Tissue-specific regulation of homeotic genes: Phenotypes of some deficiency homozygotes reveal novel regulatory relationships in the midgut. A genetic function in 99D removed by *Df(3R)B81;Dp(3;1)67A* acts as a tissue-specific regulator of homeotic gene expression. *Df(3R)-B81;Dp(3;1)67A* embryos have a striking loss of all but the central midgut constriction, a loss correlated with absence of visceral mesoderm expression of the homeotic genes *Scr* and *Antp.* Absence of homeotic gene ex-

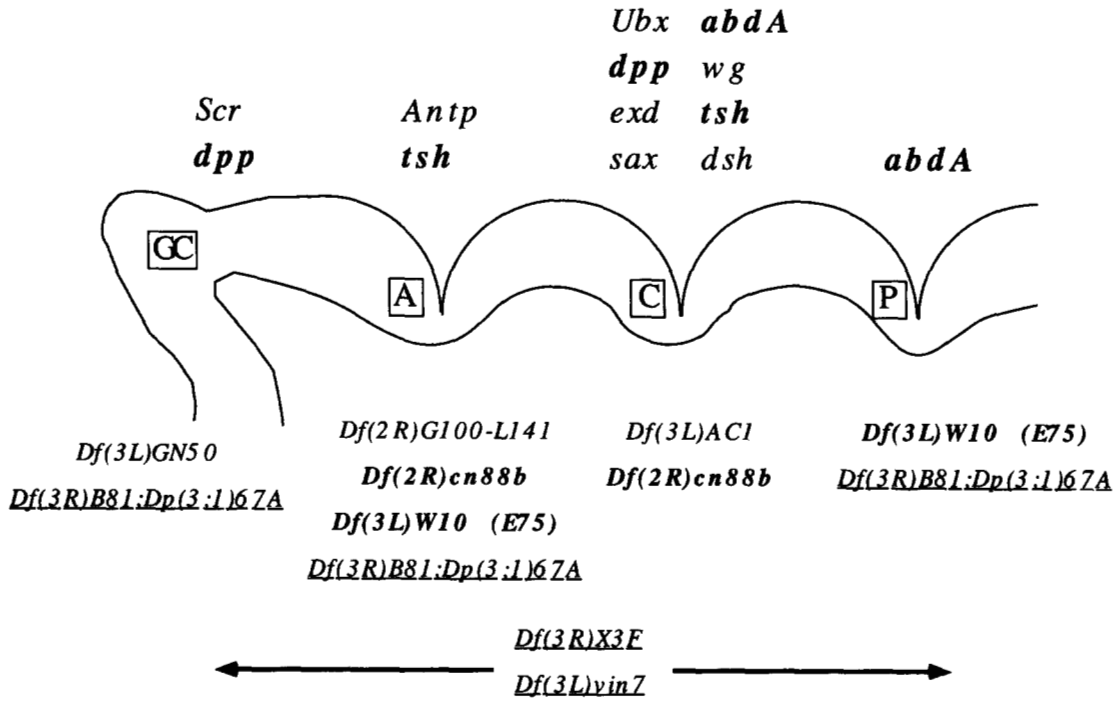

FIGURE 7.—Summary of the effects of novel constriction-defective deficiencies. A diagram of the midgut showing the anterior (A), central (C), and posterior (P) constrictions, along with the gastric caeca (GC) is shown. Above each constriction is a list of the genes known to be required for its formation (see the introduction). Below each constriction, the deficiencies found in this study to affect development of that constriction are listed. Genes and deficiency regions implicated in development of two constrictions are in bold font, and genes and deficiency regions implicated in development of three or four constrictions are underlined.

pression in a single tissue, while expression in other tissues remains unchanged, is unprecedented in mutant phenotypes and distinguishes the *Df(3R)B81;Dp(3;1)-67A* phenotype from Polycomb (MCKEON and BROCK 1991) and Trithorax (BREEN and HARTE 1993) group mutants that misregulate homeotic genes throughout the embryo. Interestingly, the midgut phenotype and loss of expression of *Scr* and *Antp* in the visceral mesoderm seen in *Df(3R)B81;Dp(3;1)67A* embryos is strikingly similar to the effect seen when *dpp* is activated throughout the visceral mesoderm using the GAL4 ectopic expression system (STAEHLING-HAMPTON and HOFFMAN 1994). However, no change in *dpp* expression is seen in the *Df(3R)B81;Dp(3;1)67A* embryos (data not shown). Ectopic expression of *dpp* may block the action of a 99D gene required for activation or maintenance of homeotic gene expression in the anterior midgut.

Positioning constrictions with *E75*: Homeotic gene expression is not the only source of anterior-posterior patterning information in the midgut. For example, expression of *dpp* in PS7 is strongly activated by *Ubx* but occurs in the correct position in the absence of *Ubx* function (REUTER *et al.* 1990). The regulators revealed by some of the midgut phenotypes we describe may be involved in anterior-posterior patterning independent of the homeotic genes. The ectopic constriction seen in *E75* mutants implies a role for *E75* in restricting where constrictions can form, a function that may be important in determining the exact sites of constrictions within the broad domains of *Antp* and *abdA* midgut expression. The ectopic constriction also suggests that a variety of cells in the midgut are competent to form constrictions and that the invariant locations of the wild-type constrictions are due to active and specific patterning.

Further studies are needed to elucidate how absence of the ecdysone-inducible steroid hormone receptor-like E75 protein causes loss of the anterior constriction and appearance of an ectopic constriction. *E75* has not previously been implicated in midgut morphogenesis but is involved in other embryonic developmental events (W. SEGRAVES, C. HUGHES and P. JENIK, unpublished data). The ligand for *E75*, if any, is unknown. Other "orphan" receptors implicated in pattern formation include those encoded by *tailless*, a gap gene required for development of the posterior terminalia, and *seven-up*, which is involved in generating photoreceptor diversity in the adult eye (reviewed in SEGRAVES 1994).

E75 is one of the first ecdysone-regulated genes for which a specific role in embryonic development has been described. In larvae, *E75* is transcribed soon after ecdysone exposure and therefore is probably regulated by the ecdysone-receptor complex itself (SEGRAVES and HOGNESS 1990). The products of the *E75* gene, together with the products of other "early" genes in the ecdysone-induced hierarchy, induce the expression of over

100 "late" genes that act as effector molecules for larval and imaginal development. In embryos, an ecdysone pulse that peaks at stage 10 is approximately coincident with initation of *E75* embryonic expression (SEGRAVES 1988) and with the appearance of homeotic proteins in the visceral mesoderm. The function of this embryonic ecdysone pulse remains unknown. In larvae and pupae, ecdysone triggers developmental programs in tissues throughout the animal. One effect of *E75* in embryos may be temporal coordination of midgut development with other developmental events, although *E75* must be more than a trigger since it affects spatial positioning, not just timing, of constrictions. Perhaps a change in the coordination of midgut formation with other developmental events affects sensitivity to signals that produce properly positioned constrictions.

Implications for genetics of constriction formation: independence and convergence: A surprising property of midgut development is the diversity of regulatory pathways (Figure 7) by which the homeotic genes specify the formation of the the ultrastructurally similar (REUTER and SCOTT 1990) constrictions. Although the genetic hierarchies are likely to ultimately converge on a common set of cytoskeletal regulators, each constriction is regulated by a unique combination of factors. The phenotypes seen in deficiency homozygotes such as *Df(3R)B81;Dp(3:1)67A* emphasize and extend these elaborate regulatory overlaps. Such independent regulatory pathways convey the ability for very fine patterning. Although the constrictions are morphologically similar, the midgut compartments are not. Compartment-specific expression of genes such as *labial* (IMMERGLÜCK *et al.* 1990) and *pdm-1* (AFFOLTER *et al.* 1993) and numerous enhancer traps (HARTENSTEIN and JAN 1992) reveal that the midgut compartments contain a great deal of regional specification. In insects (WIGGLESWORTH 1972) as well as vertebrates, ingested food meets with chemically distinct environments as it passes along the digestive tract. The homeotic-regulated midgut constrictions can be seen as an embryonic manifestation of the anterior-posterior differentiation of the alimentary tract necessary for its eventual function in digestion.

We thank KATHY MATTHEWS and the Bloomington Drosophila Stock Center for providing numerous stocks and for assembling the deficiency "kit." We are grateful to Dr. DAN KIEHART for anti-myosin antibody, to Dr. BILL SEGRAVES for helpful discussions, and to BILL CHIU and BARBARA HILL for assistance with staining. Critical reading of the manuscript was provided by Dr. BRUCE BAKER, DAVE EISENMANN, LAURA MATHIES and JOHN SISSON. D.B. was supported by a National Science Foundation predoctoral grant. This research was supported by NIH grant 18163. M.P.S. is an investigator of the Howard Hughes Medical Institute.

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Communicating editor: R. S. HAWLEY