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Identification and Genetic Analysis of *wunen*, a Gene Guiding *Drosophila melanogaster* Germ Cell Migration

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ABSTRACT

We describe a novel genetic locus, *wunen* (*wun*), required for guidance of germ cell migration in early Drosophila development. Loss of *wun* function does not abolish movement but disrupts the orientation of the motion causing the germ cells to disperse even though their normal target, the somatic gonad, is well formed. We demonstrate that the product of this gene enables a signal to pass from the soma to the germ line and propose that the function of this signal is to selectively stabilize certain cytoplasmic extensions resulting in oriented movement. To characterize this guidance factor, we have mapped *wun* to within 100 kb of cloned DNA.

GERM cell migration and gonad formation in Drosophila (Rabinowitz 1941; Sonnenblick 1941; Counce 1963) have received renewed attention using reagents that identify the germ cells (Hay *et al.* 1988; Lasko and Ashburner 1988) and the somatic gonad (Brookman *et al.* 1992) and techniques to examine the migration both *in vivo* and in culture (Jaglarz and Howard 1994, 1995). Pole cells, the precursors of the germ line, form at the posterior pole outside the somatic tissue at stage 4 of Campos-Ortega and Hartenstein (1985) and move into the invaginating gut primordium as gastrulation begins. The germ cells then enter the developing body by passing across the epithelium of the posterior midgut primordium (PMGP) at stage 10. After this they enter the mesoderm and associate with the primordium of the somatic gonad before the beginning of germ band retraction at stage 13.

Genes required for this migration will affect at least three interrelated processes: (1) determination of the germ cells and the somatic tissues through which they migrate, (2) generation of motility itself, and (3) the orientation of this activity. To date all the described mutations in the first class are thought to act in the soma. These interfere with transepithelial migration (Jaglarz and Howard 1994; Warrior 1994) and gonad condensation (Cumberledge *et al.* 1992; Warrior 1994; Boyle and DiNardo 1995; Grieg and Akam 1995). Mutations in the second class of genes will result in immotile cells, while the third class of mutations will result in cells that, though motile, will be defective in guidance.

In an attempt to locate such genes, we screened a set of deficiency chromosomes from the Bloomington Stock Center by staining collections of embryos from these stocks with anti-vasa antibody and looking for both dominant and recessive effects of the deficiency on migration (N. Zhang and K. Howard, unpublished data). The deficiency chromosomes *Df(2R)44CE*, *Df(2R)cn9*, and *Df(2R)GR15*^{L}*L2*^{R} [an inversion chromosome that throughout this manuscript we will describe as *In(2LR)GR15*^{L}*L2*^{R}] all showed a characteristic failure in germ cell guidance and identify *wunen* (*wun*), a gene belonging to the guidance class. The available data on this material suggested that *wun* lay in a small region in 44D on the polytene map.

Here we show that in each of these chromosomes lesions in *wun* lie outside of the described deletions and that *wun* is located at 45D. We use these lesions, a deficiency and two cytologically invisible lesions, to describe the *wun* loss of function migration phenotype and use cell transplantation to show that *wun* acts in the somatic tissue. We also show that both cytologically invisible alleles display a temperature-sensitive semi-lethal phenotype. Finally, we use the genetic material described here and cloned DNA from the genome projects described in FlyBase (Ashburner and Drysdale 1994) to map *wun* to within 100 kb of DNA.

MATERIALS AND METHODS

Fly stocks: Unless otherwise noted all stocks used in this study are described in Lindsley and Zimm (1992). Flies were reared at 25° on a standard medium of yeast extract, cornmeal, and sugar seeded with live bakers' yeast.

X-ray screen: Four- to five-day-old males homozygous for *ptc*^{H13}, a viable *P*[*lac*, *w*$^{+}$] insert at *ptc* (J. Hooper, personal

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communication), were irradiated in a Torex 120D machine running at 1.2 kV 4 mA delivering an unfiltered dose of 4–5 krad. About 50 irradiated males were crossed en masse with 200 *w;Sp/CyO* virgin females. These flies were raised in bottles and transferred four times at 2-day intervals. w^- F_1 flies were crossed singly to *w;Sp/CyO* virgins to establish stocks. These w^- stocks were tested for lethality over *Df(2R)44CE,* and those that were lethal were examined cytologically.

Complementation tests: Crosses for complementation tests were set up using 10 2- to 5-day-old virgin females and 10 males. The cultures were maintained at a constant temperature and transferred to fresh vials every 3 days. All the emerging progeny were scored for Cy (crosses using *CyO*) or Roi (crosses using *SM6, Roi eve-lazZ*). In all cases, all the progeny from individual vials were examined and at least 100 flies scored in total. Noncomplementation was scored if there were no transheterozygous progeny in the sample. Normally the crosses were maintained at 25°. Other temperatures were used, as indicated in the text, with transfer times at 27° reduced to 2 days and at 18° extended to 4 days.

Histology: Embryos were collected on nylon mesh using a 12-place Millipore Filtration manifold, dechorionted in Clorox, fixed in phase partition (ZALOKAR and ERK 1977) essentially as described by MITCHISON and SEDAT (1983). Embryos were then incubated in 1:10,000 dilution of an anti-vasa antiserum raised in chicken, and the signal visualized using secondary reagents from Jackson Immuno Research. In some cases mutant embryos were identified using the lacZ balancer chromosome *SM6, Roi eve-lazZ.* To visualize the somatic gonad, stained embryos were further processed using digoxigenin-labeled *in situ* probes to transcripts of the 412 transposon (BROOKMAN *et al.* 1992). For the developing gut we used either the enhancer trap line 4850 (HOWARD *et al.* 1993) or *forkhead* (WEIGEL *et al.* 1989) nucleic acid probes prepared by PCR.

Mosaic analysis: Pole cells were transplanted from a *faf-lacZ* homozygous stock where the germ cells are labeled by a transgenic lacZ fusion protein construct (FISCHER-VIZE *et al.* 1992) into embryos from the *Df(2R) wun* ^{GL}*/SM5,Roi eve lacZ* stock using standard techniques. The embryos were maintained at 18° and fixed 24–30 hr later using 4% paraformaldehyde; the vitelline membranes were removed using a combination of agitation in methanol and manual manipulation before staining with anti beta gal antiserum to reveal the transplanted germ cells and the genotype of the recipient. If more than five transplanted germ cells had entered the gonad, development was scored as normal. If fewer than five had entered the gonad and at least that many cells could be identified, the specimen was scored disperse. If the morphology of the embryo was too disturbed to reliably identify both gonads or fewer than five germ cells could be identified, the sample scored N.D.

Molecular biology: Standard molecular techniques were used for clone isolation and mapping. We used the λ fix genomic library from Stratagene and grew YAC DNA according to CAI *et al.* (1994) and *P* clone DNA according to the protocols of Genome Systems. Plasmid rescue was performed by homogenizing 10 adult flies in a microfuge tube in 100 μl of 0.3 M TrisHCl pH 8.8, 0.1 M EDTA, 1.25% SDS, 5% sucrose, 0.5% DEPC. The homogenate was incubated at 60° for 30 min, cooled to room temperature, 15 μl of 8 M KOAc added, and the mixture incubated on ice for 30 min. After centrifugation for 30 min at 13 krpm at 4°, 80 μl of the supernatant was mixed with 600 μl of Qiagen buffer PB and purified on Qiagen PCR spin column. The eluate was digested with appropriate restriction enzymes, and the reaction was terminated by addition of 600 μl Qiagen buffer PB followed by further purification on a Qiagen PCR spin column. One-fifth of the digested DNA was ligated in a 100-μl reaction incubated on melting ice overnight before another Qiagen PCR spin column purification and transformation of Nova Blue competent cells.

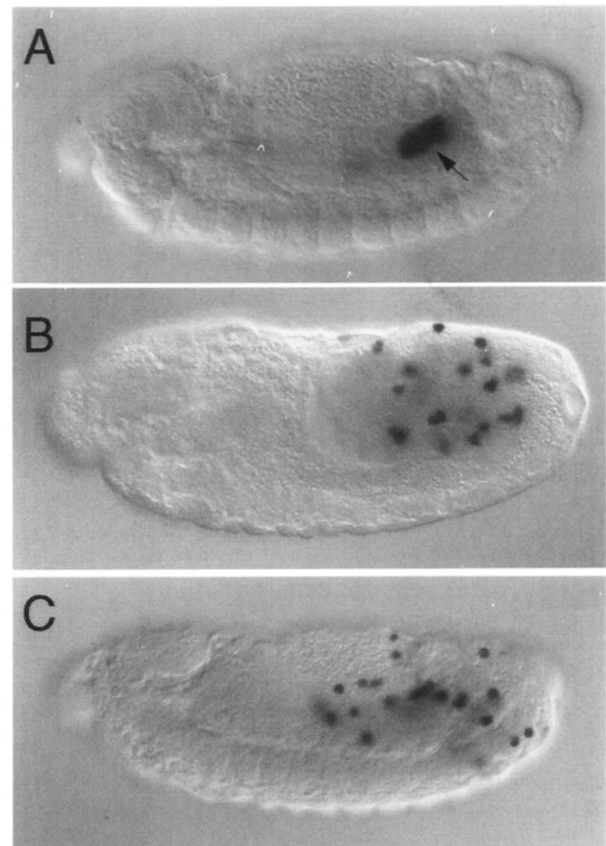


FIGURE 1.—The *wun* phenotype revealed by deficiency chromosomes. (A) Germ cells in a normal gonad (arrow) in a stage-14 wild-type embryo. (B) Dispersed germ cells in a homozygous *Df(2R)44CE* embryo at a comparable stage showing the *ptc* segment polarity phenotype and dispersed germ cells. (C) A homozygous *In(2R)GR15*^{L}*L2*^{R} embryo showing dispersed germ cells but no segment polarity phenotype.

DNA sequencing was performed by the Roche Institute Core Sequence Group using a *P*-element primer and an IBI sequencing system. Sequence analysis was performed using Kodak Mac Vector and Assembly Lign Software.

PCR was performed on a MJ Research PTC 100 machine, using standard 3-STEP [94° for 1 min; 30 × (92° for 40 sec, 60° for 40 sec, 75° for 90 sec); 5 min at 75°), Perkin Elmer Taq polymerase and Boehringer PCR buffer.

RESULTS

The phenotype: As is shown in Figure 1B (compare with the wild type shown in Figure 1A), embryos homozygous for *Df(2R)44CE* show unusually disperse germ cells. These embryos also show the segment polarity phenotype of the *patched* (*ptc*) gene that is removed by this deficiency (HOOPER and SCOTT 1989). Examination of *ptc* point mutants showed that *ptc* is not responsible for the migration phenotype (data not shown).

Mapping *wun*: To localize this gene, we examined heteroallelic combinations of *Df(2R)44CE* with the

TABLE 1

Genetic material used in this study

Genotype	Cytology	wun	Reference	Note
In(2LR) GTE146(Z)GR15/CyO	035C;044D	+	*a*	*f*
Df(2R)NCX11/CyO	043C1-2;044C1-2	+		*f*
Df(2R)NCX10/CyO	043F1-2;044C4-5	+		*f*
Df(2R)P14TE/CyO	044D1;044E	+		*f*
In(2R)GR15^L*L2*^{R} + *Df(2R)wun*^{GL}*/CyO*	042;044D,045C;045E	−	*b*	*f, g*
Df(2R)cn9 + *wun*^{CN}*/CyO*	042E;044C,045C-E	−		*f*
Df(2R)44CE + *wun*^{CE}*/CyO*	044C;044E1-4,045C-E	−		*f*
P[ry$^+$*; hs-neo; FRT] 42D, P[w*$^+$*; hs pM] 45F*	042D;045F	+	*c*	*h*
P[ry$^+$*; hs-neo; FRT] 42D, P[w*$^+$*; hs NM] 46F*	042D;046F	+	*c*	*h*
Df(2R) Np 3 CyO	044D2-E1;045B8-B1	+	*d*	*h*
Df(2R)Np1/CyO	044F02-04;045B05-06	+	*d*	*h*
Df(2R)Np4/CyO	044F11-045A1;045B08-C01	+	*d*	*h*
Df(2R)Np5/CyO	044F09-10;045D09-E01	−	*d*	*h*
Df(2R)45-30n/CyO	045A06-07;045E02-03	−	*d*	*h*
Df(2R)w73-1/CyO	045A09-10;045D05-08	−	*d*	*h*
Df(2R)G63-73/CyO	045A13-B02;045D05-08	−	*d*	*h*
Df(2R)H3C1/CyO	43F;44D	+	*e*	*i*
Df(2R)H3D3/CyO	44D;44F04-05	+	*e*	*i*
Df(2R)H3E1/CyO	44D;44F12	+	*e*	*i*
wun^{CE}*,b pr cn/SM5,Roi eve lacZ*	045C8;045D8	−	*e*	*i*
wun^{CE}*,b pr cn/CyO*	045C8;045D8	−	*e*	*i*
42NM wun^{CE}*,vg c a px bw mr sp/CyO*	045C8;045D8	−	*e*	*i*
42πM wun^{CN}*/CyO*	045C8;045D8	−	*e*	*i*
Df(2R) wun^{GL}*/CyO*	045C8;045D8	−	*e*	*i*
Df(2R) wun^{GL}*/SM5,Roi eve lacZ*	045C8;045D8	−	*e*	*i*

References: *a*, GUBB *et al.* 1988; *b*, HOOPER and SCOTT, 1989; *c*, XU and RUBIN 1993; *d*, KONEV *et al.* 1991a,b; *e*, this work; otherwise see LINDSLEY and ZIMM (1992) or FlyBase. Notes: *f*, chromosomes used in the initial deficiency screen; *g*, HOOPER and SCOTT (1989) did not describe the deficiency on this chromosome, which is referred to in this manuscript as *In(2R)GR15*^{L}*L2*^{R}; *h*, deficiency chromosomes used later to localize *wun;* *i*, chromosomes generated and described in this study.

other deficiency chromosomes identified with note *f* in Table 1. These studies revealed that *Df(2R)cn9* showed aberrant migration and failed to complement the migration phenotype of *Df(2R)44CE,* whereas *Df(2R)P14, Df(2R)NCX10* and *Df(2R)NCX11,* the other deletions in this region, did not. These data suggested that *wun* lay in region A in Figure 2.

We then examined the putative synthetic deficiency *In(2R)GR15*^{L}*L2*^{R} (HOOPER and SCOTT 1989). This chromosome was made using autosynaptic techniques and, based on its failure to complement the lethality of *Df(2R)44CE,* was thought to be a deficiency extending distally from 44D. To our delight it showed the wun migration phenotype with disperse germ cells (Figure 1C, this phenotype is shown in more detail in Figures 7 and 8, and discussed further later in this manuscript). These data localized *wun* in a small region between the 44D breakpoint of *In(2R)GR15*^{L}*L2*^{R} and the 44D breakpoint of *Df(2R)P14.* Furthermore, since *Df(2R)44CE* and *Df(2R)P14* uncover *ptc* and molecular data (HOOPER and SCOTT 1989) show that *Df(2R)P14* extends further distally than *Df(2R)44CE, wun* should have been proximal to *ptc.*

We pursued analysis of this region both molecularly (not shown) and by generating new deficiencies. To do this we irradiated a viable w^+ *P* insert at *ptc* and recovered three new w^- deficiency chromosomes (see MATERIALS AND METHODS). These chromosomes are shown in Figure 3, A–C and diagrammed in Figure 2. As expected these deficiencies fail to complement *Df(2R)44CE* for lethality and show the ptc phenotype. However, they are wun$^+$ for migration and viable in trans to *In(2R)GR15*^{L}*L2*^{R}.

These surprising data showed that *wun* did not lie in 44D at all, and reexamination of the cytology of the putative synthetic deficiency chromosome *In(2R)GR15*^{L}*L2*^{R} showed it to be a synthetic duplication for a portion of proximal 2R (Figure 3D), not a deficiency. To the left of 44D this chromosome, which contains one or more lesions that fail to complement *Df(2R)44CE* both for the *wun* phenotype and for viability, derives from *In(2LR)-TE146(Z)GR15* (GUBB *et al.* 1988) via an autosynaptic intermediate. We examined this inversion and determined that it did not show the wun migration phenotype (data not shown). This suggested that *wun* lies distal to 44D on 2R.

Following this line of reasoning, we extracted the right arm of *In(2LR)GR15*^{L}*L2*^{R} distal to the inversion breakpoint in a normal sequence configuration. This was achieved exploiting the TE-derived intact copy of *white* at the left inversion breakpoint (35B; 44D1) of *In(2R)GR15*^{L}*L2*^{R} and *ptc*^{H89}, a lethal *P*[w^+] insert at *ptc.*

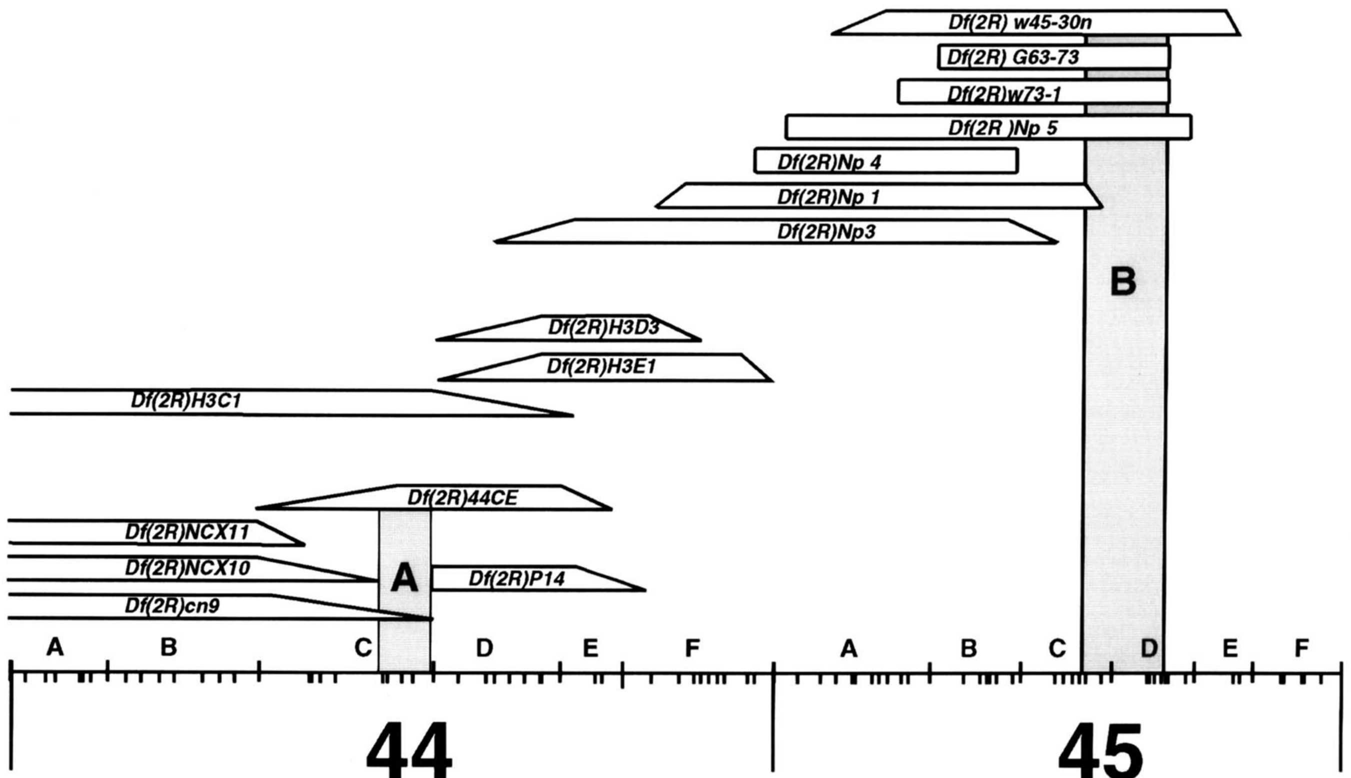


FIGURE 2.—A cartoon map of the deficiencies used in this study. Deleted regions are shown by the open boxes that are labeled with the names of the lesions. *In(2R)GR15*^{L}*L2*^{R} and *Df(2R)wun*^{GL} are not shown here. Region A was originally thought to contain *wun*. Region B is the area determined in this study to contain *wun*.

Recombination events between the right inversion breakpoint and the *P* element were selected by recovering white^{-} flies that lose both the inversion and the *P* element (Figure 4A). Some of these chromosomes showed the wun migration phenotype and contain an allele that we call *wun*^{GL}.

Similarly, we reasoned that there must be lesions in *wun* on both *Df(2R)44CE* and *Df(2R)cn9* distal to the cytologically visible deficiencies, and we separated these alleles from the deficiencies using the scheme shown in Figure 4B. We call these *wun* alleles *wun*^{CN} (derived from *Df(2R)cn9*) and *wun*^{CE} (derived from *Df(2R)44CE*).

To map the lesion in *wun*^{CE}, we used the multiply marked chromosome *al dp*^{ov} *b pr cn vg c a px bw mr sp*. In a preliminary experiment, males with exchanges between each marker on the right arm were back crossed to *wun*^{CE} and scored for the migration phenotype localizing *wun* between *cn* and *vg*. To localize *wun* in more detail, we selected more *cn*$^{+}$ *vg*$^{-}$ flies from the above cross scoring 9/95 *cn*$^{+}$ *wun*$^{-}$ *vg*$^{-}$ and 86/95 *cn*$^{+}$ *wun*$^{+}$ *vg*$^{-}$ chromosomes. These data place *wun*^{CE} distal to *cn* at about 1/10 of the distance between *cn* and *vg*.

Deficiencies localize *wun* to 45C08 to 45D08: The deficiencies identified as class *h* in Table 1 (KONEV *et al.* 1991a,b) map *wun*. Of these, *Df(2R)G63-73, Df(2R)45-30n, Df(2R)w73-1* and *Df(2R)Np5* showed the wun phenotype, while *Df(2R)Np4, Df(2R)Np1, Df(2R)Np3* and *Df(2R)G75* did not. These data show that *wun* lies between 45C08 (the distal breakpoint of *Df(2R)Np1*) and 45D08 (the distal breakpoint of both *Df(2R)G63-73* and *Df(2R)w73-1*). These data are summarized in Figure 2, with the shaded box B being the location of *wun*.

We then obtained the single *P*-element inserts identified in Table 2 from the Drosophila genome projects and performed complementation analysis. The relevant results of these tests are diagrammed in Figure 5. These data show that *wun*^{GL} is a small deficiency since it fails to complement the two *P* element-induced lethals *l(2)06736* and *l(2)03659* that complement each other and do not show migration phenotypes (data not shown). This conclusion was confirmed by further cytological analysis (Figure 6A). From now on we will call this lesion *Df(2R)wun*^{GL}. In contrast, *wun*^{CE} complements all the *P* lethals but is itself semi-lethal over deficiencies for the region. These data suggest that *wun*^{CE} is a smaller lesion. While unsurprisingly *Df(2R)wun*^{GL} is a lethal, both *wun*^{CE} and *wun*^{CN} are temperature-sensitive semi-lethal lesions (Table 3). The *wun*^{CN}/*Df(2R)wun*^{GL} and *wun*^{CE}/*Df(2R)wun*^{GL} adult hemizygotes were relatively vigorous and survived for >3 weeks at 22° (room temperature).

We wondered if these flies would show some effect of the early errors in germ cell migration and assayed both *wun*^{CN}/*Df(2R)wun*^{GL} and *wun*^{CE}/*Df(2R)wun*^{GL} females raised at room temperature for ovarian development. Interestingly, we found that the female files were often agametic. In a sample of 82 *wun*^{CE}/*Df(2R)wun*^{GL}

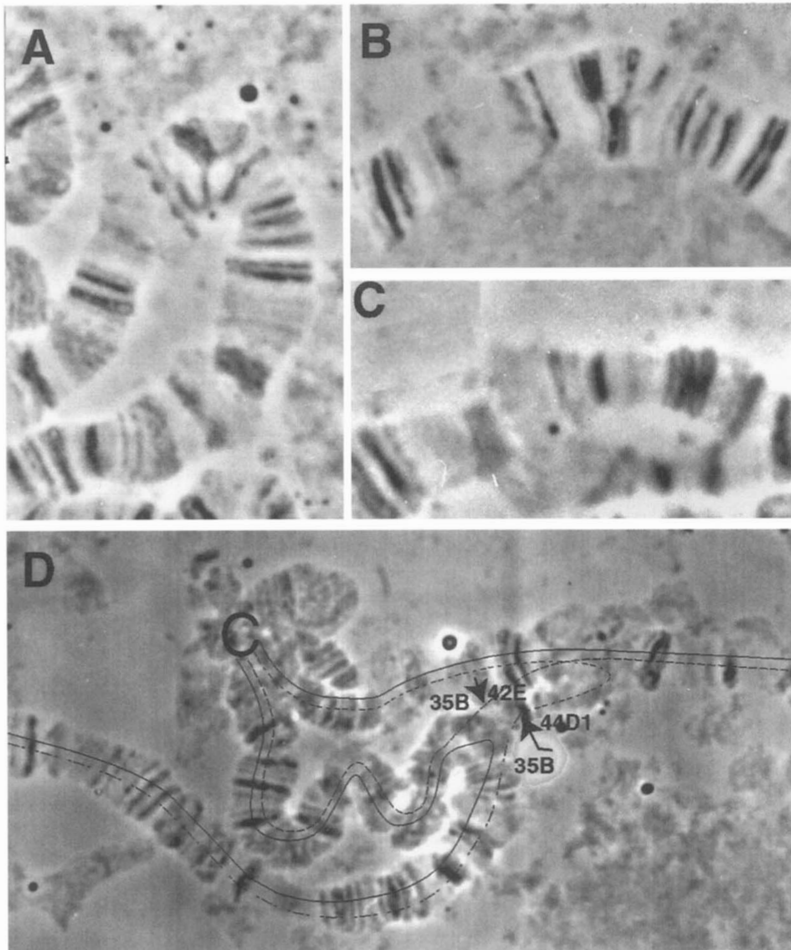


FIGURE 3.—Cytology of some chromosomes used in this study: *Df(2R)H3D3* (A), *Df(2R)H3E1* (B), *Df(2R)H3C1* (C), *In(2R)GR15^{L}L2^R/ptc^{H89}* (D) with a superimposed interpretive drawing (C, centromere).

females, 14 were completely agametic, 29 showed apparently normal germ line development in only one ovary, while 39 developed bilaterally. In contrast the males showed sperm bundles in all cases examined. These flies were largely sterile and we could not establish stocks nor recover progeny in significant numbers from either males or females of either genotype.

The migration phenotype: In wild type as gastrulation proceeds (from 5:00 to 5:30 hr) most germ cells are found at the end of the PMGP. After this they pass across the gut epithelium and enter the embryo. Any difference between the wild-type and wun mutant embryos before exit are too subtle for us to see and just as in wild type, wun embryos show germ cells clustered

$\frac{w}{w}\ \frac{P[w^+]ptc^{H89}}{CyO}$ x $\frac{w}{?}\ \frac{In(2LR)GR15L2}{CyO}$

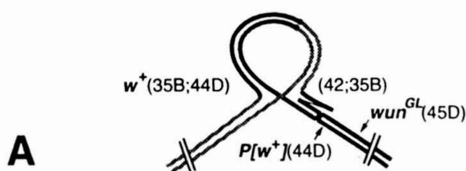
$\frac{w}{w}\ \frac{In(2LR)GR15^L L2^R}{P[w^+]ptc^{H89}}$ x $\frac{w}{?}\ \frac{Sp}{CyO}$

Select white flies, make stocks and test for wun:

$\frac{w}{w}\ \frac{P[ry^+;\ hs\text{-}neo;\ FRT]42D\ P[w^+;\ hs\text{-}NM]46F}{P[ry^+;\ hs\text{-}neo;\ FRT]42D\ P[w^+;\ hs\text{-}NM]46F}$ x $\frac{w}{?}\ \frac{Df(2R)44CE}{CyO}$

$\frac{w}{w}\ \frac{Df(2R)44CE}{P[ry^+;\ hs\text{-}neo;\ FRT]42D\ P[w^+;\ hs\text{-}NM]46F}$ x $\frac{w}{?}\ \frac{Sp}{CyO}$

Select white neoR flies, make stocks and test for wun and loss of the deficiency:



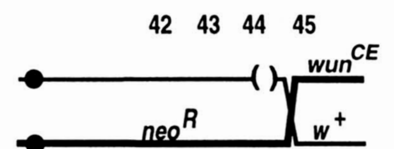


FIGURE 4.—Recombination schemes. (A) The lesion in *In(2R)GR15^{L}L2^R* was recovered in this scheme where the inversion chromosome was placed in trans to the white$^+$ transposon insertion at *ptc*. (B) Recovery *wun^{CN}* from *Df(2R)cn9*. *wun^{CE}* was recovered from *Df(2R)44CE* in a similar scheme using *P[ry$^+$; hs-neo; FRT] 42D P[w$^+$; hs pM]45F*. Both of the *neo* chromosomes are described in XU and RUBIN (1993).

TABLE 2

Rescue DNA clones

P-induced lethal	Cytological location	Rescue clone	Sequence accession number	PCR primer pair
P{PZ}l(2)rG232	45C1-2	—	—	—
P{PZ}l(2)06736	45C1-2	11B2	U40156	TTCGACTCATAGACGGGCGTAG TGGATGACTGGCACTTCACAGATAG (146)
P{PZ}l(2)03659	45D1-2	1A1	U40155	GCGTAACTAAGTCTGGCGATGTG TGTTTCCACTGTACTGCGTCGTCGTG (248)
l(2)k16806	45D4-5	6.1[a]	—	—

Value in parentheses is product size/base pairs.

[a] This clone shows repetitive signal in *in situ* hybridization to polytene chromosomes and was not pursued further.

inside the distal part of the PMGP in a 5:00–5:30 hour collection.

After exit the wild-type and *wun* mutants begin to show differences. Normally the germ cells cluster on the dorsal posterior surface of the PMGP proximal to the mesoderm and then separate into two groups in the mesoderm. In *wun* mutants these processes fail, and germ cells do not cluster dorsally (compare Figure 7A with B and C) and can be found as far as the primordia of the malpighian tubules (compare Figure 7D with E and F). Examination of the embryonic gut primordium using an enhancer trap and *forkhead* expression did not reveal significant molecular or morphological changes in the PMGP (Figure 7).

Examination of the development of the somatic gonad in these mutants using expression of the 412 transposon (Brookman *et al.* 1992) did not show detectable changes in this tissue. Even at this relatively late stage in development, the germ cells continue to display ameboid morphologies both as individual cells and in clusters (Figure 8).

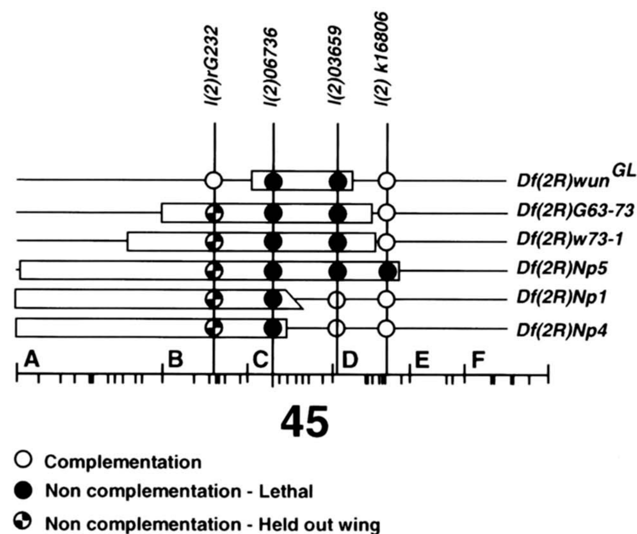


Figure 5.—The complementation map produced in this study. *l(2)RG2322* is a viable lesion that shows a held-out wing phenotype. □, deleted regions; —, intact sequences.

As far as our assays allowed us to determine, all three *wun* alleles show identical and temperature-independent migration phenotypes. Furthermore the phenotype of *Df(2R)Np5,* which removes the flanking lethals *l(2)06736* and *l(2)k16806,* is very similar to that of these alleles. We conclude that the migration phenotype of these alleles show the consequences of loss of *wun* function.

***wun* functions in the soma:** To address the question of the site of action of *wun,* we generated mosaic animals where the germ cells were wild type and the soma either *wun* mutant (experimental samples) or wild type (control samples). The results of this experiment, tabulated in Table 4, show that *wun* function is required in the somatic tissue of the embryo. The reciprocal experiment of placing *wun* mutant germ cells in a wild-type somatic environment and scoring for migration in the embryo is technically challenging since individual

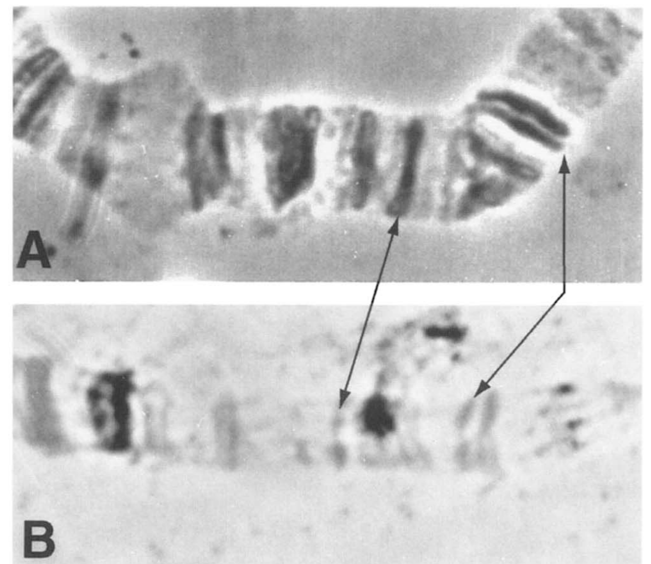


Figure 6.—Cytological characterization of wun lesions. (A) *Df(2R)wun*^{GL}. (B) The *P* element in the region of *wun*^{CN} in *Df(2R)cn9*. Corresponding regions in 45D on both chromosomes are indicated by the arrows. The *P* element in *Df(2R)cn9* lies within the region deleted in *Df(2R)wun*^{GL}.

TABLE 3

The results of complementation analysis of the three *wun* alleles

Cross	33/333 (0)	33/333 (0)	33/333 (0)
$Df(2R)wun^{GL}/SM6,Roi \times Df(2R)wun^{GL}/SM6,Roi$	0/144 (0)	0/153 (0)	0/79 (0)
$wun^{CE}/SM5,Roi \times Df(2R)wun^{GL}/SM6,Roi$	42/138 (30)	16/207 (8)	7/138 (5)
$wun^{CN}/CyO \times Df(2R)wun^{GL}/SM6,Roi$	ND[a]	17/95 (18)	22/197 (11)

The data were collected at three different temperatures and are shown as [hemizygotes]/[total progeny]. Numbers in parentheses are percentages.

[a] Hemizygotes in this cross could not be reliably scored using the Cy marker at 18°. The hemizygous flies emerged late and in the crosses at higher temperatures many pupae failed to eclose.

donor embryos need to be genotyped and the results of transplantation from each donor to be followed. We did not attempt this experiment.

The nature of the alleles wun^{CN} and wun^{CE}: Both the parental chromosomes *Df(2R)cn9* and *Df(2R)44CE* were derived during dysgenic crosses of MRF chromosomes (YANNOPOULOUS *et al.* 1981). This dysgenesis results in the mobilization of hobo and *P* elements and presumably other mobile elements. This raised two possibilities: either these two alleles are caused by two different mutagenic events or they are independent isolates of one allele that was present in one of the parental stocks. To investigate this, polytene squashes of these two chromosomes were hybridized with hobo and *P*-element probes. These experiments did not show any evidence for hobo sequences at 45D on either chromosome. However, the *P*-element probe detected a signal at 45D on *Df(2R)cn9* (Figure 6B), while there was no such signal on the *Df(2R)44CE* chromosome. These data suggest that these two chromosomes are independent of each other and show that there is a *P* element in wun^{CN} that might be the mutagenic lesion in this chromosome.

Molecular studies: To begin molecular studies, we obtained clones mapping to this region. These was a YAC clone from Dr. IAN DUNCAN (CAI *et al.* 1994) and cosmids maintained by the European Drosophila genome project (SIDEN *et al.* 1990). We also used plasmid rescue to recover flanking DNA from some of the single insert *P*-element lethal stocks from the genome project. For selected rescue plasmids we confirmed cytological

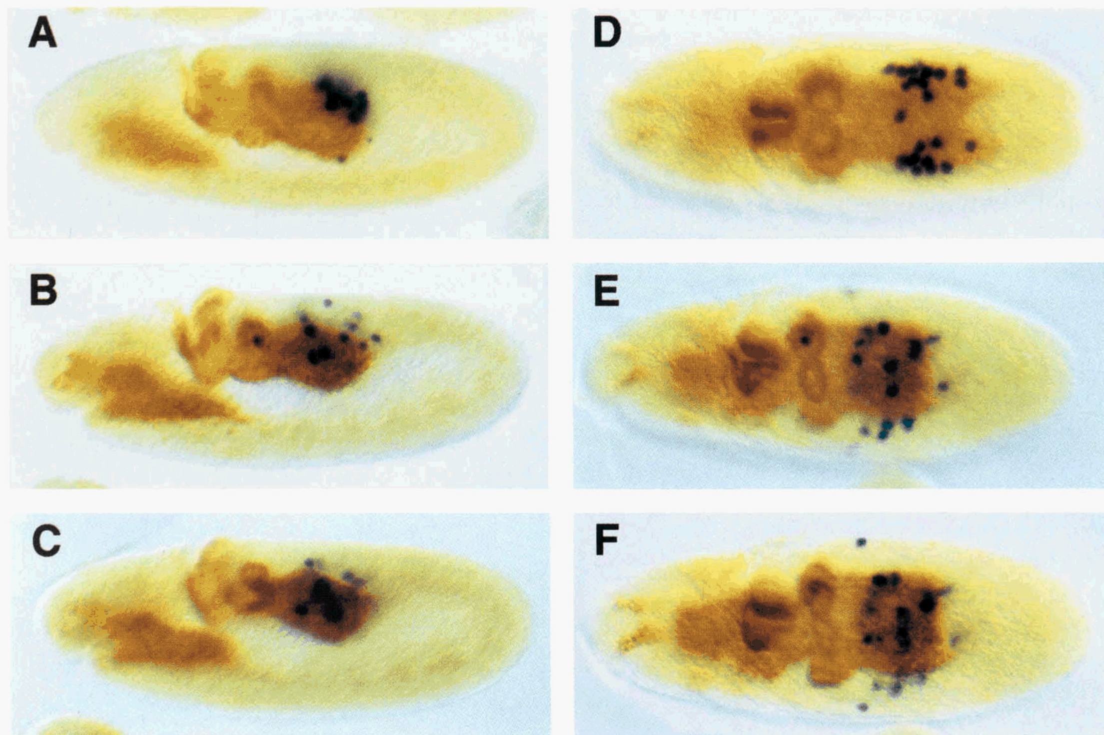


FIGURE 7.—The wun phenotype in $Df(2R)wun^{GL}/Df(2R)wun^{GL}$ embryos during the extended germ band stage. The PMGP is stained with the 4850 enhancer trap (brown) and the germ cells visualized with anti vasa antibody (blue). (A) Lateral view showing the normal situation. (B and C) The wun phenotype at two different focal planes in one specimen. (D) A wild-type embryo from a dorsal aspect. (E and F) The wun phenotype from a dorsal aspect at two different focal planes in one specimen showing the failure of the germ cells to align bilaterally in wun mutants.

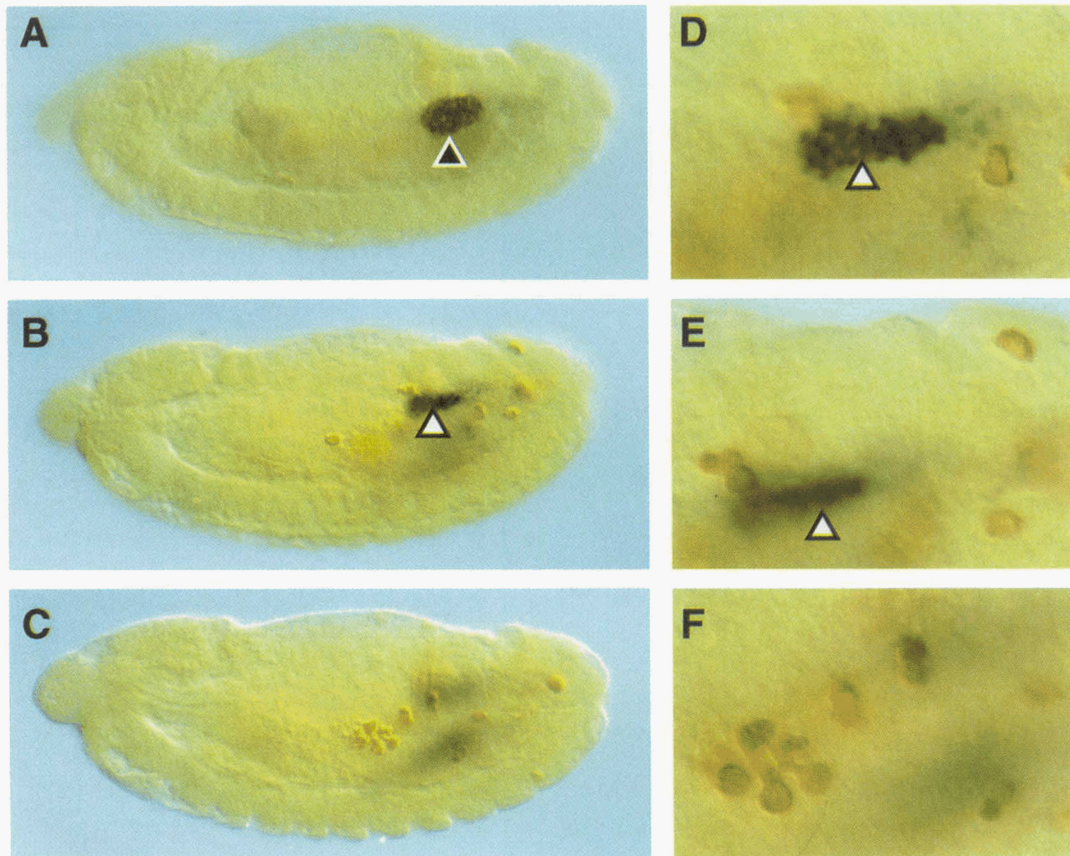


FIGURE 8.—The wun phenotype in $Df(2R)wun^{GL}/Df(2R)wun^{GL}$ embryos after germ band shortening. The somatic gonad is stained with 412 probe (blue) and the germ cells visualized with anti-vasa antibody (brown). (A) Wild-type embryo showing a normal gonad (black triangle with white border). (B) and (C) Different focal planes of a wun mutant embryo showing an empty somatic gonad (white triangle with black border) and dispersed germ cells. E and F are higher power views of B and C. D is another focal plane of the same specimen.

localization, performed sequence analysis and in chosen cases generated PCR primer pairs to use as Sequence Tag Site markers. We also used one primer pair to recover P1 clones from the library of Dr. DAVE SMOLLER (see MATERIALS AND METHODS). The data on the rescue clones are summarized in Table 3, and all the cloned material generously provided and used in this study is listed in Table 5.

We used the YAC clone N20-67 to isolate a contig in lambda phage, created a restriction map, and used *in situ* hybridization to map the ends of *Df(2R)Np1* and *Df(2R)w73-1* in this contig so defining the region where we expect *wun* function to reside. One of these clones, λY26, lies proximal to the wun region and inside the wun^{+} deletion *Df(2R)Np1,* while λLR4 maps to the breakpoint of the wun^{-} deletion *Df(2R)w73-1.* Analysis of the region between these two points confirmed that the rescue sequences from *l(2) 03659* come from this region and therefore that this *P* element-induced lethal falls in the deficiency region by molecular as well as genetic criteria. Similarly, *in situ* analysis of $Df(2R)wun^{GL}$ showed that it was a deficiency removing all of this DNA. These data are summarized in Figure 7.

DISCUSSION

Drosophila germ cell migration and somatic tissue: The wun mutant phenotype in which germ cells migrate but do not locate their final target, the somatic gonad, is novel and identifies a guidance factor not required for the initiation of motility. This situation where cells migrate but guidance fails is reminiscent of the *unc-6* gene of *Caenorhabditis elegans* where the loss-of-function phenotype involves loss of orientation and not simply lack of movement of the affected neuronal grow cones and mesodermal cells (HEDGECOCK *et al.* 1987; CULOTTI 1994). The product of the *unc-6* gene is the first identified member of the Netrin family of guidance factors that displays both attractive and repulsive activities in different contexts (reviewed in KEYNES and COOKE 1995). We suggest that *wun* encodes a signaling component of an analogous germ cell pathway.

This situation can be contrasted with that of the Drosophila FGF receptor *breathless* and its role in tracheal cell migration. The loss of function phenotype of this gene approximates to a simple lack of motile activity in the affected cells (GLAZER and SHILO 1991). Furthermore, it has been shown that ubiquitous and unregu-

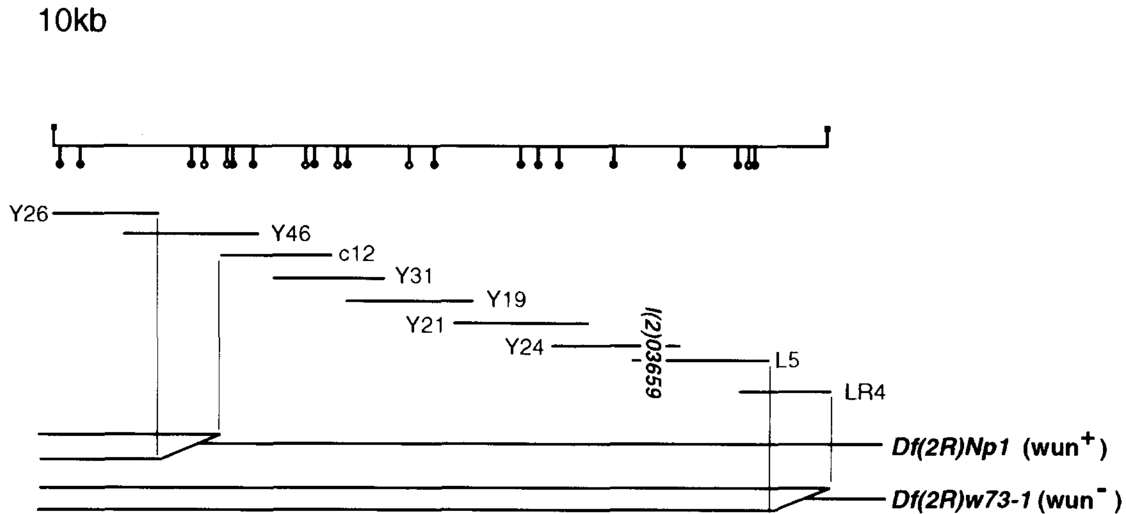


FIGURE 9.—A map of the λ phage recovered in this study. *NotI* sites at the ends of the phage contig are indicated by filled boxes, *EcoRI* sites in the map are indicated by circles that are open only when the order of the flanking fragments is uncertain, otherwise they are filled. The diagrammed relationship of this map to the breakpoints of *Df(2R)Np1* and *Df(2R)w73-1* that are shown with the deleted region represented by the open box was determined by *in situ* hybridization.

lated activity in this pathway rescues the mutations in this gene showing that the ligand for this system is not providing spatial guidance (REICHMAN *et al.* 1994).

In the case of germ cell migration there may be *wun*-independent signals analogous to the *breathless* signal that initiate motility. However, an independent guidance pathway involves the activity of *wun*. The fact that *wun* acts in the somatic tissue indicates that it encodes either a ligand, a matrix component or a specific transport molecule required to present this signal to the germ cells.

How does guidance work?: Studies of the germ cells morphology both *in vivo* and in primary culture *in vitro* led us to suggest that guidance is achieved by selection of appropriate cellular extensions from a population of alternatives produced by the germ cells (JAGLARZ and HOWARD 1995). The important parameter is the relative stability of different projections with guidance being provided by a gradient of stability across a cell with extensions in the direction of motion being more stable than those on the other side of the cell. We favor the interpretation that the wun phenotype is the result of failure in this selection mechanism.

This change in the relative stability could be mediated with either positive (reinforcing) or negative (collapsing) changes from baseline. At one extreme, loss of reinforcement would result in reduced overall motility, while loss of collapsing activity could promote movement. Because germ cells disperse further in wun mutants than in wild type, we favor the alternative that *wun* has a collapsing activity.

The distinction between attractive and repulsive activities of the *wun* pathway can be addressed by experiments that present germ cells with regulated quantities and patterns of the ligand that affect the cellular behavior. Although the *wun* product itself may not be the appropriate component of this system for use in such assays, these questions may be addressed in experiments where somatic mosaics of wun activity are generated.

The origin of the two alleles, *wun*^{CN} and *wun*^{CE}: These two alleles may be isolates of the same original lesion present in the MRF stock. In this case, differences in the *P*-element content of these stocks at 45D would have occurred during establishment of the stocks. Both alleles produce temperature-sensitive lethality and, at the permissive temperature, an adult hypogametic and

TABLE 4

The results of the mosaic analysis of *wun* function

wun	Germ cell class	*n*
+	Disperse	0
+	Condensed	16
+	ND	14
−	Disperse	6
−	Condensed	1
−	ND	3

See MATERIALS AND METHODS for a description of the germ cell classes. ND, not determined.

TABLE 5

Cloned material used in this work

			STS PCR	
Clone	Vector	Source	1A1	11B
71C11	Cosmid	Siden-Kiamos	+	+
7140	P1 clone	Genome Sys.	+	−
7141	P1 clone	Genome Sys.	+	−
N20-67	YAC	Duncan	+	ND

STS PCR shows the results of PCR with these clones using the two primer pairs shown in Table 3. ND, not determined.

sterile phenotype. This could be the consequence of a persistent and unusual form of dysgenesis. We do not favor this alternative since the MRF-derived chromosomes have been outcrossed many times. Instead, we suggest that the hypogametic phenotype is a consequence of the early failure of the majority of the germ cells to enter the gonad. We do not understand the basis of the temperature-sensitive lethality nor the sterility. It is possible that this reflects another function of *wun*, perhaps mediating signaling between cells in different contexts. In any case, these alleles raise the possibility of pursuing analysis of *wun* using enhancer and suppresser screens.

Is *wun* unique?: The wun phenotype was only encountered once in our survey of the deficiency set maintained at Bloomington. A previous attempt to isolate migration genes by examining enhancer trap mutations reporting expression in the developing gut identified only mutants with gross morphological defects (HOWARD *et al.* 1993). Therefore, we believe that this is one of a relatively small class of genes with this specific embryonic phenotype. This question will be answered when systematic genetic screens now underway (BROIHIER *et al.* 1994; MOORE *et al.* 1995) are finished.

Evolutionary considerations: Migration is a very general phenomenon and was undoubtedly manifest early in cellular life when it helped cells feed and engage in sexual activity. Migration is also a vital part of the development and homeostasis of metazoan animals, and we expect that aspects of the regulation of this primordial migration will have been conserved in germ cells and reused in somewhat divergent forms to allow interactions in development. This reasoning leads us to suggest that a phylogenetic analysis of *wun* may be rewarding.

Molecular analysis: The prospects for molecular analysis of this gene are encouraging. We have demonstrated that it is very likely to lie within the 100 kb defined in this manuscript. Efforts are underway to locate the gene using a combination of transformation assays, transcriptional analysis and further mutatgenesis.

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