Development 1993 Supplement, 213-218 (1993)
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Migration of *Drosophila* germ cells: analysis using enhancer trap lines

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SUMMARY

Cell migration is a common feature of development. In order to understand more about the factors that control these movements we have embarked on further analysis of the migration of *Drosophila* germ cells. This process involves passage of the germ cells across the gut primordium and migration toward the mesoderm where the somatic gonad forms. We are particularly interested in the early phase of this migration when the germ cells interact with the amnioproctodeal invagination, the developing gut, before entering into association with the mesoderm. We will summarize the results of our and other studies of these events before describing a number of enhancer trap lines which show expression in the amnioproctodeal invagination during the early phase of germ cell migration. These reveal more about the complexity of this tissue and suggest this tissue is capable of guiding the early phase of germ cell migration.

Key words: germ cell migration, *Drosophila*, enhancer trap lines

INTRODUCTION

Drosophila melanogaseter germ cell migration was first studied in some detail by Sonnenblick (1941) and Rabinowitz (1941). These studies and those of later workers, particularly Poulson, Mahowald, and Technau (Illmensee and Mahowald, 1974; Poulson and Waterhouse, 1960; Technau and Campos-Ortega, 1986) described the migratory route and behavior of the germ cells as they arrive at the gonad. The understanding of the nature of the processes around the mid 1980s is well summarized in (Campos-Ortega and Hartenstein, 1985). We are interested in further analysis of the events of early migration because this is a situation in which a directed movement of cells during development can be analyzed using both experimental manipulation and genetic approaches.

A related matter, the determination and fate of the pole cells has been the subject of considerable study. The formation of the pole cells is directed by the germ plasm, a determinant localized at the posterior pole of the embryo. A number of components of this plasm have been identified (for review see Lasko, 1992). This factor directs these cells to enter the germ line. This seems to be the only fate available to these cells (Technau and Campos-Ortega, 1986). The mode of action of the determinant, both in the formation of morphological pole cells and in the determination of the germ line per se is not well understood. Only recently has Kobayashi produced data suggesting that a particular splice is made productively only in those pole cells that enter the germ line (Kobayashi et al., 1993).

The early events of germ cell formation and migration are cartooned in Fig. 1. The germ cells form at the posterior pole of the egg before cellularization of the blastoderm in response to the posteriorly localized determinant which performs two functions: specifying germ cells and setting up the pattern of the posterior abdominal region of the embryo (reviewed in St-Johnston and Nüsslein-Volhard, 1992). As the blastoderm layer forms, the cells that lie under the germ cells are assigned an endodermal fate and will eventually go on the form the midgut. These fates are determined by the torso signaling system and are independent of the posteriorly localized determinant. At the same time cells along the ventral mid-line are assigned a mesodermal fate by the dorsal signaling system (reviewed in Rushlow and Arora, 1990). Some of these cells will eventually go on to form the somatic tissues of the gonad.

The geometry of the cellular blastoderm is transformed by the events of gastrulation and germ band extension. The mesodermal cells form the ventral furrow as they enter the embryo, before coming to lie laterally in the interior. At about the same time the cells at the posterior pole begin to change shape and form the polar plate. This invagination moves forward and deepens during the fast phase of germ band elongation becoming the amnioproctodeal invagination, the primordium of the gut. This structure then elongates both by growth of the cells and by recruitment of ectodermal cells into the hindgut primordium (see Skaer, 1993 for a review). During these processes the germ cells enter the amnioproctodeal invagination and come to lie at the blind posterior end of this pocket.

During late stage 10 of development the germ cells pass across the endoderm and begin to migrate around on the surface of this tissue, moving between it and the ensheathing yolk sac. By the end of this phase the majority of germ cells lie on the ventral side of the germ band close to the mesoderm. As the germ band begins to retract the germ cells

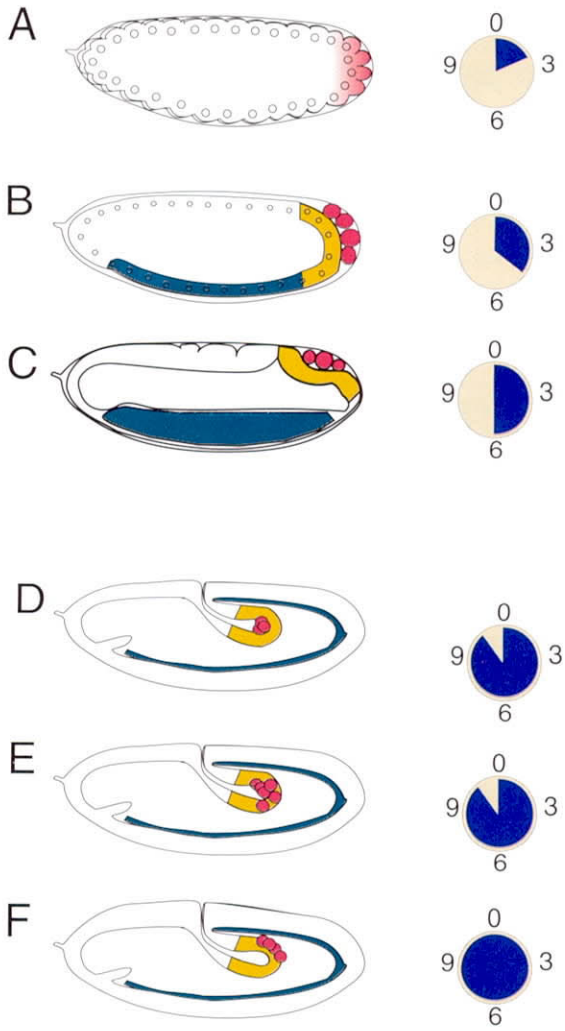

Fig. 1. A summary of the early events in germ cell and gonad formation and migration. The figures show cartoons of the very early stages of germ cell and mesoderm formation and of the subsequent migration of the germ cells from the posterior mid-gut primordium to the mesoderm. Anterior is to the left, dorsal surface uppermost. The clock to the right of each figure show the developmental time in hours at 18°C (this is approximately half the speed of development at the standard 25°C). (A) Early in blastoderm formation (approx. 2 hours and 20 minutes) the posterior determinant (pink) is localised toward the posterior pole and clevage nuclei coming to lie under this pole are associated with polar buds. (B) A little later (approx. 4 hours and 40 minutes) the pole cells have formed (pink) and blastoderm nuclei are being specified. Those lying under the pole cells assume endodermal fates (yellow) under the influence of the terminal information system. Those lying ventrally become mesodermal (blue) as directed by the dorsal system. (C) As specification becomes complete (a little later than 6 hours) gastrulation begins and the endodermal cells form the polar plate and move anteriorly in germ band extension. Mesoderm enters the embryo forming the ventral furrow. The germ cells are pushed along with the endoderm. (D) The movements are complete and the germ band is fully extended after 10 hours of development. At this time most of the germ cells lie inside the amnioproctodeal invagination (pocket) in association with the endoderm. (E) Soon thereafter at around 11 hours the germ cells pass through the walls of the pocket and appear dispersed on its surface. (F) In the following 90 minutes the germ cells migrate on the surface of the pocket eventually coming to lie juxtaposed to the mesoderm on the ventral surface of the pocket.

migrate from the endoderm into the mesoderm where they associate with the gonadal mesoderm. The cells that form this mesoderm are distinguished by their expression of the transposon 412 (Brookman et al., 1992). It is particularly interesting to note that this structure assembles normally even in the absence of germ cells.

During this migration the behavior of the germ cells is regulated in two generally important ways. First, the time of onset of migration is controlled so that cell movement begins at a particular stage in development. Second, this movement is directed so that the cells move from inside the gut primordium to their eventual site of differentiation (the somatic gonad). In order to understand more about these behaviors of the germ cells we have taken three distinct approaches: (1) real time visualization of germ cells during migration, (2) analysis of germ cell migration in well characterized mutants altering the pattern of the embryo and (3) screening the genome for mutants specifically altering the behavior of germ cells during their migration.

The first of these sets of experiments has involved labeling of host germ cells with nuclear-tagged rhodamine dextran and transplantation of fluorescein-labeled cells into the amnioproctodeal invagination of these labeled embryos (Jaglarz and Howard, 1994). In this way we can visualize both host and transplanted cells using an automated low light video microscope. We have transplanted blastoderm cells and find that they do not move from the amnioproctodeal invagination with the germ cells of the host embryo. Instead they remain in the gut whilst transplanted germ cells migrate along with the host cells (Jaglarz and Howard, 1994). This experiment shows that the initial migration is not simply the consequence of the physical displacement of the contents of the gut. Instead it seems that there are specific interactions between the germ cells and their environment, the developing gut, which initiate the migration.

In keeping with this suggestion is the fact that even in the absence of the mesoderm, which will eventually form the somatic gonad, the migration of the germ cells begins. Remarkably not only is the migration initiated, but it is

Table 1. Enhancer trap lines

Name	Source
l(3)2093	Spradling
l(2)0255	Spradling
l(2)5315	Spradling
l(2)6430	Spradling
l(3)2670	Spradling
4850	Mathies/Scott
6208	Mathies/Scott
l(2)4230	Spradling
l(3)2624	Spradling
l(3)3980	Spradling

These stocks were provided by Alan Spradling or Laura Mathies. The expression pattern in each of these lines had been provisionally assigned by Laura Mathies to the gut . These genes might therefore to be required more or less specifically for the proper migration of the germ cells. However, in all but one case the migration is relatively unaffected and a substantially normal gonad formed with relatively few scattered germ cells. A similar analysis of another 12 lines kindly provided by Steve Beckendorph has revealed no phenotype affecting gonad formation.

correctly oriented toward that side of the gut which would normally face the mesoderm (Jaglarz and Howard, 1994). Although not demonstrating conclusively that signals in the developing gut both initiate the migration of germ cells from the gut and guide these cells toward the mesoderm, these data are provocative and suggest that this explanation of the early events of germ cell migration is plausible . Consequently we are particularly interested in the developing amnioproctodeal invagination.

In particular we are searching for genes that are expressed here at the correct stage of development. Mutations in these genes could conceivably produce several different phenotypes. First, they may result in no defects in either gut development or germ cell migration. This could occur either if the gene's function is served by other complementary genes expressed in the same way, or if the expression is unrelated to any function, and is in some sense frivolous. Secondly, mutations in these genes may result in severe disruption of the development of the gut. For instance all the cells of the gut may die. In this case it will not be surprising if the migration of the germ cells is perturbed. A third sort of outcome may be that the development of the gut itself is perturbed but that the migration of the germ cells is unaffected. This could be for a number of reasons, for instance the gene may act in gut development after the germ cells have left it and entered the mesoderm. A fourth outcome, and the one that we are most interested in, would be disturbance of the migration of the germ cells without any substantial change in the structure of the gut. These might identify signals emanating from the gut and guiding germ cells toward the mesoderm.

If such a mutation were to exist it would be relatively difficult to detect at the stage when germ cells are moving toward the mesoderm on the amnioproctodeal invagination since even in wild type embryos there is a stage in the migration when the germ cells are disposed relatively chaotically on the surface of the amnioproctodeal invagination. The expected phenotype would be persistence of this chaotic form beyond the appropriate stage in development in an embryo which was otherwise morphologically normal. Unfortunately the exit and migration toward mesoderm occur over a relatively short period of development, at the

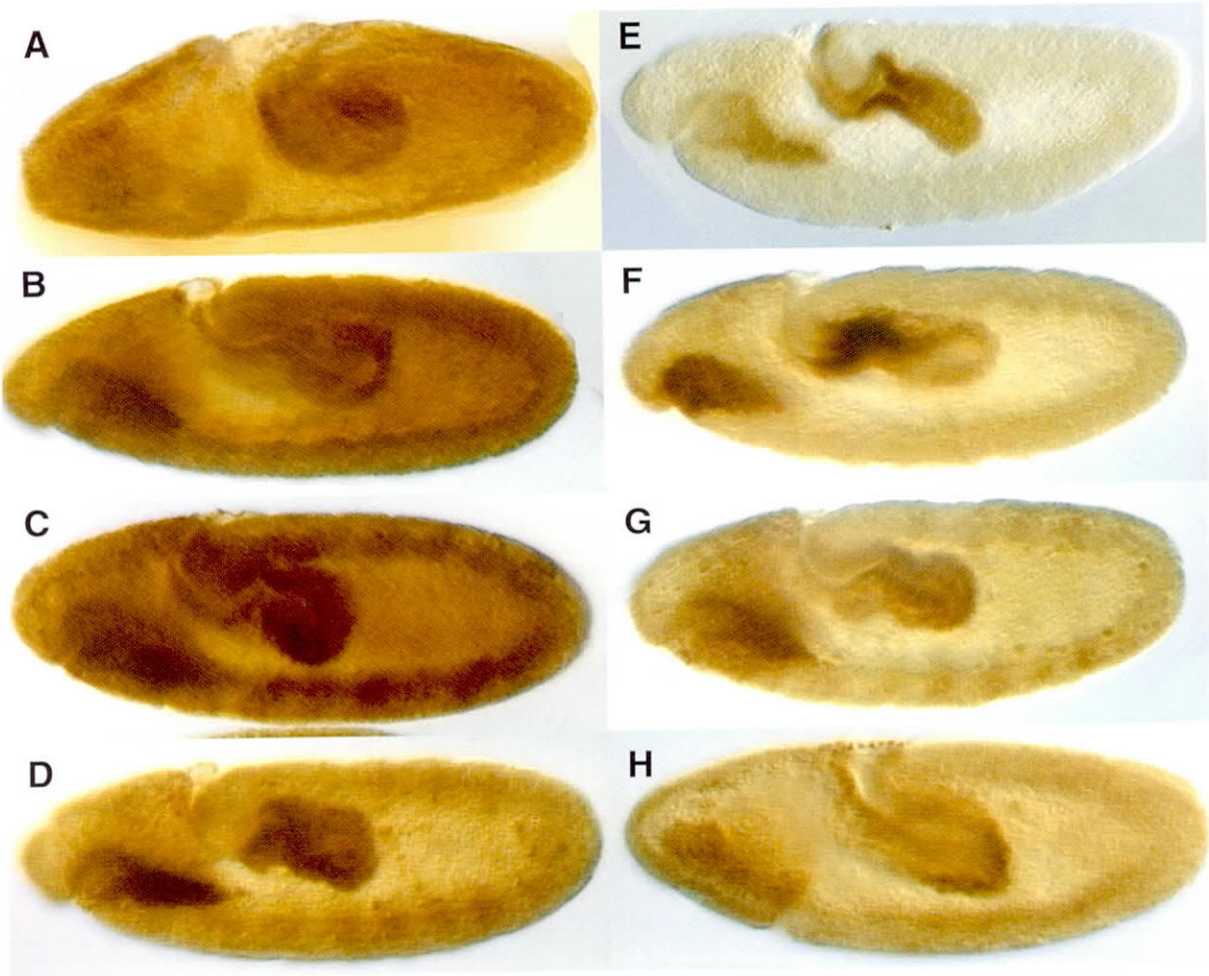

Fig. 2. Enhancer trap expression patterns: 1. Beta-galactosidase expression patterns visualized using an antibody to the bacterial protein in seven of the enhancer trap lines studied. All embryos are oriented with their anterior to the left and are seen from the side, except (A) which is an oblique dorsal view. (A) *l(3)2093*, (B) *l(2)5315*, (C) *l(3)2670*, (D) *l(2)0255*, (E) *4850*, (F) *l(2)6430*, (G) *6208*, (H) *l(2)0255*. Note that 6208 was a viable insert. We generated a lethal derivative using *Δ2-3* excision and found no phenotype of this lethal chromosome affecting gonad assembly.

extended germ band stage, and there are no facile morphological markers which enable us to assign a particular specimen to a particular stage within this period. Consequently our initial analysis has focused on a much later stage of development (shortened germ band) when any errors in germ cell migration are fairly clear since by this time most of the germ cells are normally found clustered in the gonad at the level of the fifth abdominal segment. A mutation that randomized the early migration on the gut because it interfered with the putative signals from gut to germ cells but did not disturb migration in the mesoderm or assembly of the somatic gonad would result in an embryo which would still form a gonad but which would have an unusually large number of scattered germ cells.

In order to find genes involved in producing signals in the gut we have chosen to look at enhancer trap lines which are expressed there. A number of labs have done large enhancer trap screens and are making their material available. We have been fortunate enough to have the help of Laura Mathies and Matthew Scott who are studying gut development and had identified a number of enhancer trap lines expressed in the gut. They were kind enough to pass on information about these lines to us. These lines were generated both in screens done in the Scott lab and in the Spradling lab.

RESULTS AND DISCUSSION

Here we report results from 10 of these lines which are listed in Table 1. We documented the expression pattern of beta-galactosidase in a collection of embryos staged between 5 and 9 hours and on the distribution of germ cells in later embryos in a staged collection from 12-15 hours.

As expected all of these lines show expression of beta-galactosidase in the amnioproctodeal invagination (Figs 2 and 3). The intensity of this staining is very variable. The weakest signal is seen in the line *l(3)2093* (Fig. 2A) where it is only barely detectable as the amnioproctodeal invagination forms. With the possible exception of *l(3)2093* all the lines show expression of beta-gal in the stomodeal invagination as well as the amnioproctodeal invagination. This is entirely consistent with the homology between the terminal regions of the embryo demonstrated genetically by the existence of the terminal class of mutants (St-Johnston and Nüsslein-Volhard, 1992). Interestingly, each of these lines show spatially modulated expression within the amnioproctodeal invagination revealing differences along both the proximodistal and dorsoventral axes. For example *l(2)5315* is expressed preferentially in the dorsal side of distal part of the amnioproctodeal invagination (Fig. 2B) whereas *l(2)6430* is expresses in the more anterior part of the amnio-

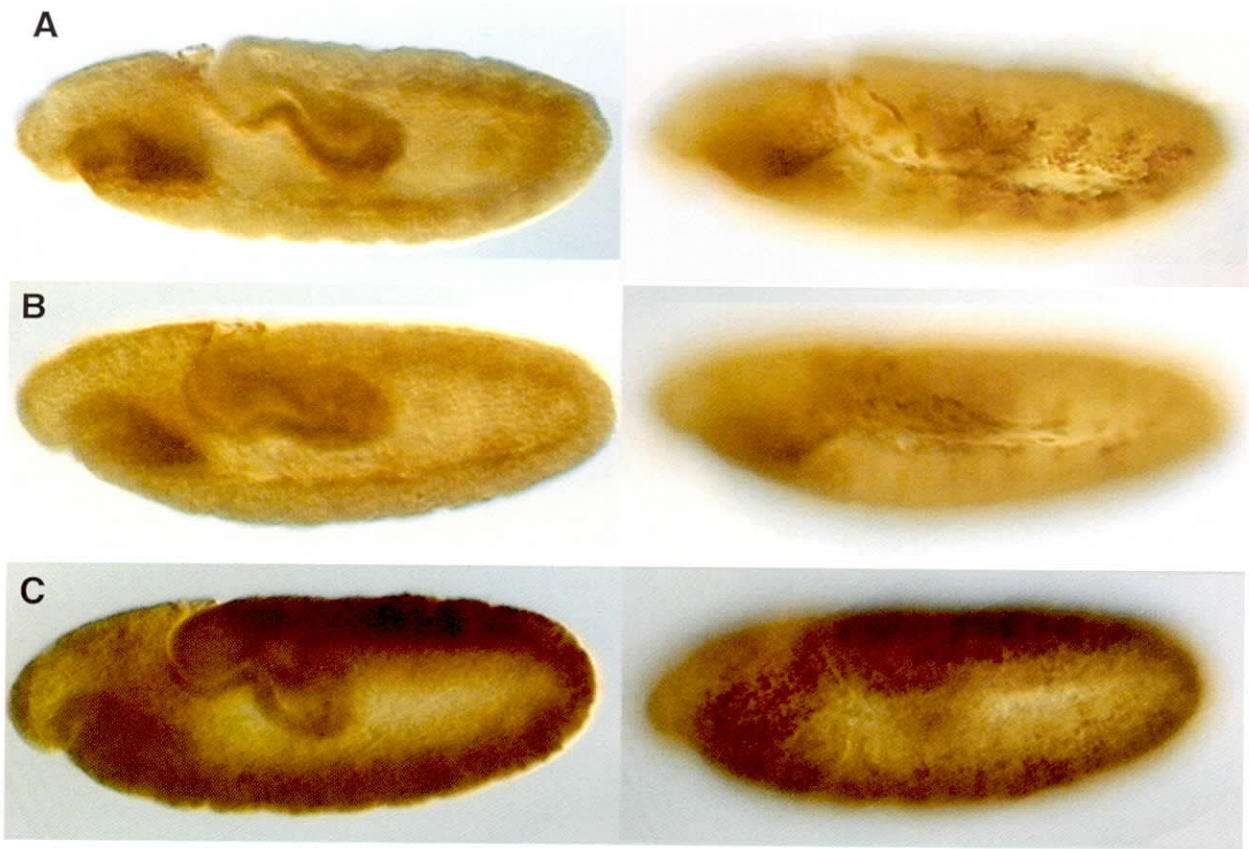

Fig. 3. Enhancer trap expression patterns: 2. Beta-galactosidase expression patterns visualized using an antibody to the bacterial protein in the remaining three enhancer trap lines studied. All embryos are oriented with their anterior to the left and are seen from the side. Two views of each embryo are shown: in the left column the focus is on the amnioproctodeal invagination, in the right column focus is on the surface of the embryo. (A) *l(3)2624*, (B) *l(2)4230*, (C) *l(3)3980*.

proctodeal invagination around the primordia for the Malpighian tubules (Fig. 2F).

We have not attempted a complete analysis of the dynamics of each of these expression patterns. However, we note that each pattern develops in a characteristic way and show two stages in the development of the expression of *l(2)0255* (Fig. 2D,H). This expression begins at the distal end of the amnioproctodeal invagination (Fig. 2H) before developing into a more uniform pattern (Fig. 2D). In addition to showing expression in the amnioproctodeal invagination and the stomodeal invagination three of these lines show marked expression in other parts of the embryo (Fig. 3) suggesting either that these enhancer traps are inserted near regulatory elements for two different tissue-specific functions or that the function being regulated by the enhancer(s) is not specific to the terminal tissues.

These data indicate that at the time that the germ cells leave the amnioproctodeal invagination it is well enough differentiated to be capable of providing signals both to initiate and guide the migration of the germ cells.

It was of great interest to determine what, if any, consequence for the behavior of germ cells these enhancer trap inserts had. The stocks were not marked so that we could distinguish homozygous enhancer insert embryos from their sibs. We therefore examined >100 embryos from each later collection looking for evident defect in gonad assembly. Apart from rare defects in development which are probably due to random factors such as aneuploidy due to the balancer chromosomes, we saw only relatively normal embryos at this late stage in development (12-15 hours) in 9 of the 10 lines examined. An example of this morphology is shown in Fig. 4A. We conclude that these inserts have not disrupted functions necessary for normal germ cell migration. In contrast, another line *l(3)2093* showed defects in gonad assembly Fig. 4B. In these mutant embryos clusters of germ cells do form at about the normal site of gonad assembly (arrow in Fig. 4B). However, most of the germ cells are dispersed in the posterior 1/3 of the interior of the embryo. These embryos are relatively normal in appearance and form segments, secrete cuticle and assemble a nerve chord.

This phenotype can be seen as the result of some early disturbance of germ cell migration with only some of those cells which fortuitously enter the appropriate part of the mesoderm thereafter finding the somatic gonad. There are two extreme ways in which this disturbance in germ cell migration could be generated: the developing gut may have degenerated relatively early in its development thereby randomizing the germ cell movements, or alternatively, the gut may develop perfectly normally except that it lacks the ability to signal position to the germ cells causing them to migrate randomly at this stage. We favor the first sort of interpretation since we have never observed a properly formed gut in our collections. The beta-galactosidase staining of these embryos is relatively weak, just barely above background (Fig. 2A) and is consequently not very provocative. In addition the chromosome is lethal, consistent with an extreme failure in gut formation. This phenotype is more extreme than that seen in the well characterized mutant *fork head* which prevents proper development of the midgut (Weigel, 1989). In this case the majority of the germ cells leave the amnioproctodeal invagination and enter the developing gonad undisturbed (Warrior and Howard, unpublished data). This suggests that the gene disrupted in this enhancer trap line may act early in the differentiation of the gut. It is interesting to note that in early (3-5 hour) collections from this stock a number of embryos were observed in which the majority of the germ cells have not yet migrated to the mesoderm and appeared randomly distributed on the surface of the amnioproctodeal invagination. This observation is provocative. However, more work is necessary characterizing the development of these mutants using molecular markers for gut development and stocks where the mutant embryos can be unambiguously distinguished, to define further its role both in gut development and to understand the reasons for the dispersion of germ cells seen in the mutant embryos.

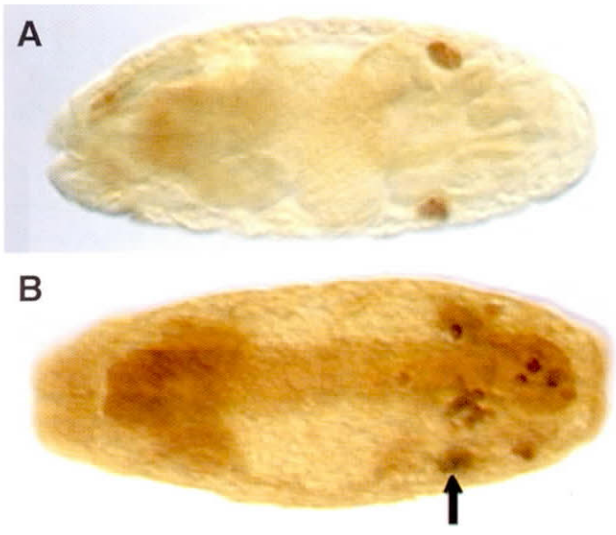

Fig. 4. *vasa* staining reveals the state of assembly of the gonad. Immunostaining of embryos with anti-*vasa* antibody. The formation of a normal gonad with relatively few dispersed cells, which is characteristic of all the embryos in the later collections for all but one of the lines described here is shown in A. B shows an example of the pattern seen in line l(3)2093. In this case although a reduced gonad seems to form (arrow) many germ cells are scattered.

We would like to thank Laura Mathies, Matt Scott, Steve Beckendorph and Alan Spradling for information and fly stocks. Tony Brown and Phil Ingham for their patience and Chris Rushlow, Tom Gridley, Colin Stewart and an anonymous referee for helpful comments on the manuscript.

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