Specification, migration and assembly of the somatic cells of the *Drosophila* gonad

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**SUMMARY**

The adult ovaries and testes contain several specialized somatic cell types that support the differentiation of germ cells into mature gametes. Each of these cell types arise from mesodermal cells that constitute the embryonic gonad. To explore the mechanisms governing the development and differentiation of these cells, we focus on the formation of the gonad during *Drosophila* development. Using markers for the precursors of the somatic cells of the gonad, we identify discrete steps in the development of the gonad. Our results suggest the existence of different populations of gonadal precursors at early stages in gonadogenesis that represent precursors of cell types found within the adult gonad. The functions of the homeotic genes *abdominal A* and *Abdominal B* are required for the development of gonadal precursors, however, here we provide evidence that each plays a distinct role. *abd A* activity alone specifies anterior gonadal precursor fates, whereas *abd A* and *Abd B* act together to specify a posterior subpopulation of gonadal precursors. Once specified, gonadal precursors born within posterior parasegments must move to the site of gonad formation. Here, we show that the proper regional identities, as established by homeotic gene function, are required for the arrest of migration at the correct position. Finally, our analysis of late stages of gonadogenesis suggests that *abd A* is required in a population of cells within parasegments 10 and 11 that partially ensheath the coalescing gonad. Mutations in *iab-4* abolish expression of *abd A* within these cells, and as a result block the coalescence of the gonad.

Key words: *Drosophila*, gonad formation, abdominal A, Abdominal B, organogenesis

**INTRODUCTION**

The formation of a gonad involves the coordinated development of cells derived from two distinct lineages, the germ line and soma. In a variety of organisms, germ cells are segregated away from the rest of the embryo early in development. It is not until a later stage that germ cells reenter the embryo and migrate to somatic cells, derived from the mesoderm, with which they will form the gonad (Sonnenblick, 1941; Chiquoine, 1954; Blackler, 1962; Eyal-Giladi et al., 1981). The somatic cells of the embryonic gonad give rise to the various cell types in the adult ovary and testis that support the differentiation of the germ cells into mature gametes (reviewed by Lindsley and Tokuyasu, 1980; Mahowald and Kambysellis, 1980; De Felici and Dolci, 1987). Although the somatic cells are essential to the function of the gonad, their origin and role in gonadogenesis is not understood.

The origin of the germ cells (called pole cells) and their incorporation into the gonad in *Drosophila*, have been described by Sonnenblick, (1941); Mahowald (1962); Hay et al. (1988), and Lasko and Ashburner (1990). Soon after fertilization, the pole cells bud off from the posterior pole of the embryo where they remain until later stages in embryogenesis when pole cells squeeze through the gut epithelium and reenter the embryo. Pole cells then migrate to lateral mesoderm of the posterior abdominal parasegments 10 through 13 (Jaglarz and Howard, 1994; Howard et al., 1993; Warrior, 1994). Subsequently pole cells move anteriorly and are incorporated into the gonad at parasegment 10.

Less is known about the somatic component of the gonad. There are 25 to 35 mesodermal cells present in the embryonic gonad (Sonnenblick, 1941). The precursors of these mesodermal cells have been identified by the expression of the retrotransposon 412 (Brookman et al., 1992). This marker suggests that gonadal mesoderm arises from a primordium spanning parasegments 10 through 11, consistent with previous mosaic analysis (Gehring et al., 1976; Lawrence and Johnston, 1986; Szabad and Nöthiger, 1992). Here, we provide further evidence for this extended origin of gonadal mesoderm.

The finding that gonadal mesoderm derives from such a large primordium has several implications for gonadogenesis. For example, there appear to be different populations of gonadal precursors within the primordium. Some gonadal precursors reside within PS10 where the gonad forms, whereas other precursors, derived from more posterior parasegments, must move to the site of gonad formation. It is not known what factors specify the different populations of gonadal precursors within the primordium, nor whether these populations serve different functions in gonad formation. In addition, it is not understood how gonadal precursors from posterior parasegments are directed to the final position of gonad formation. Finally, whether the different populations represent precursors...
for the various cell types found in the adult ovary and testis is not known.

Previous genetic studies have suggested that there are two distinct steps in gonad formation (Lewis, 1978; Karch et al., 1985; Brookman et al., 1992; Cumberledge et al., 1992). First, function of the homeotic gene abdominal A (abdominal A) is required for the specification of all gonadal precursors. In the absence of abdominal A, few, if any, gonadal precursors develop and pole cells, after initially contacting mesoderm, scatter throughout the embryo (Brookman et al., 1992). Abdominal B (Abdominal B) function is also required early in gonadogenesis, since fewer gonadal precursors and pole cells are found in the gonad in abdominal B mutants compared to wild type (Brookman et al., 1992). However, in this case it is not known which gonadal precursors are affected, and whether this results from a defect in the initial specification of gonadal precursors or a later step in their development.

A second step in gonadogenesis was defined through the analysis of embryos carrying mutations in an abdominal A cis-regulatory region, called iab-4 (Lewis, 1978; Karch et al., 1985; Cumberledge et al., 1992; Warrior, 1994). Unlike abdominal A null mutants, pole cells in iab-4 mutants do not scatter until a late stage in gonad formation. Mosaic experiments showed that iab-4 function is not required in the germline, but rather in the soma (Cumberledge et al., 1992). However, it is unclear which somatic cells require abdominal A function at this later stage in gonad formation.

To begin an analysis of the mechanisms controlling gonad morphogenesis, we have used several markers for the precursors of the somatic cells of the gonad. By following the gonadal precursors, we describe discrete steps in gonadogenesis. Our analysis provides strong support for the existence of a migratory population deriving from PS11 and PS12 that may serve to guide pole cells to the final position of gonad formation. In addition, we describe evidence for the existence of subpopulations of gonadal precursors at early stages of gonadogenesis. abdominal A is required in anterior parasegments to specify anterior gonadal cell fates, whereas in more posterior parasegments, abdominal A and abdominal B act together to specify posterior gonadal cell fates. These subpopulations of gonadal precursors give rise to subpopulations within the somatic cells of the formed gonad. Finally, our analysis of late stages of gonadogenesis suggests that abdominal A is also required in a population of cells within PS10 and PS11 that partially ensheath the coalescing gonad. Mutations in iab-4 abolish the expression of abdominal A in these cells, and as a result block the coalescence of the gonad.

MATERIAL AND METHODS

Fly stocks

Welcome Bender provided us with three transgenic fly lines that he and colleagues had characterized as expressing lacZ in gonads. These lines were recovered in transformation experiments designed to test the regulatory capacity of various pieces of the Bithorax Complex (BX-C). The gonadal expression appears to be due to position effects at the sites of insertion. The P element carries a 6.8 kb fragment from the abx regulatory region fused to lacZ just inside the Ubx reading frame. The construction of these lines and the analysis of the expression attributable to the abx sequences are reported by Simon et al. (1990). We used one of these three lines, 68-77, routinely and it is featured in Figs 1A-F, and 4A,B. The two other markers show essentially the same lacZ expression pattern in gonadal precursors.

A second marker line, bluetail, was isolated by Francois Karch, and consists of 1 kb of the iab-7 regulatory region fused to a minimal promoter regulating lacZ. This construct has fortuitously inserted within the BX-C, about 20 kb away from the resident iab-7 region (Galloni et al., 1993). This line is used in Fig. 1G-J.

The hspt70-abdA transgenic line was obtained from Gines Morata through Richard Mann, and is kept over a balancer that carries a wg-lacZ transgene. For ectopic expression of abdominal A, hs-abdA abdominal A transgenic embryos were collected on agar plates and aged at 25°C in a humidified chamber. Heat shock treatments were administered at 4 hours and 6 hours of development for 30 minutes each (Lamka et al., 1992), by floating agar plates in a 37°C water bath. Embryos were returned to 25°C and aged until the times noted in the text, when they were prepared for staining (see below). Only heat shocks administered between these hours produced the described phenotype. However, in our experiments, 1 hs-abdA embryo out of approximately 40, showed aberrant migration of pole cells from the midgut pocket to PS11-13 mesoderm. Pole cells in all other embryos exhibited wild-type behavior at stage 12. Gonads in both heat shock treated wild-type embryos and non-heat shock treated hs-abdA abdominal A embryos were examined and found to be indistinguishable from wild type. In addition, heat shock treated hs-abdA abdominal A embryos allowed to age until 24 hours show segmental transformations within the larval cuticle consistent with phenotypic consequences of ectopic homeotic gene expression (Gonzalez-Reyes and Morata, 1990).

The Abd BM1 stock was also obtained from Gines Morata through Richard Mann. Abd BM1 abdominal M1 mutant embryos were identified using engrailed expression (Delorenzi and Bienz, 1990). Phenotypic analysis of the Abd BM1 mutation has been described by Casanova et al. (1986). Abd BM1 abdominal M1 mutant embryos exhibit phenotypes indistinguishable from that of deletion of the gene.

The iab-4 mutant stocks, iab-410b, iab-4302 and iab-450b were obtained from Ed Lewis through Shigeru Sakonju, and are kept over a balancer that harbors a ftz-lacZ transgene, facilitating the identification of homozygous, or heteroallelic mutant embryos. Cytological and molecular analysis of mutations of the iab-4 cis-regulatory region of the abdominal A gene have been described by Karch et al. (1985). The expression of abdominal A protein in these mutants has been previously examined by Karch et al. (1990), and by Cumberledge et al. (1992). For the analysis carried out in an iab-4 mutant background, we constructed stocks that were P[68-77] / P[68-77]; iab-4 TM3-ftz-lacZ. Heteroallelic and heteroallelic combinations over the iab-4, 50b allele were examined, showing only slight variations in the phenotype. In all mutant combinations, occasional coalesced gonads were identified in mutant embryos, as described in Cumberledge et al. (1992).

Agametic embryos were derived from mothers homozygous for the osk101 mutation (Lehmann and Nüsslein-Volhard, 1986). When mothers are raised at 18°C, the formation of pole cells is blocked in progeny. To monitor the gonadal precursors in agametic embryos, homozygous osk mothers were mated with males carrying the blue tail enhancer trap.

Immunohistochemistry and RNA in situ hybridization

Embryos were collected, aged for the appropriate time in a humidified chamber, and then fixed, devitellinized, and processed in a standard manner (Kellerman et al., 1990). Antibodies, and their very generous providers, were as follows: anti-vasa, used at a dilution of 1:400, Paul Lasko (Lasko and Ashburner, 1990); mAb DMabd B, a generous provider, was used at 1:1000, Cappel. Secondary antibodies were from Vector Labs, and streptavidin-conjugated HRP from Chemicon.

In situ hybridization to RNA in whole-mount embryos was as described by Tautz and Peifer (1989), with modifications for antibody-RNA double labeling as described by Dougan and DiNardo (1992). Probes were made following Boehringer Mannheim instructions. For
erg, a PCR-derived fragment of an *escargot* cDNA, kindly provided by Judy Kassis (Whiteley et al., 1992), was used. The 412 retrotransposon was obtained from Rob White through Ken Howard. Finally, the *eyes absent* probe was kindly provided by Nancy Bonini (Bonini et al., 1993).

Embryos were staged according to Campos-Ortega and Hartenstein, (1985). All photography was done using Kodak Ektar 160 color slide film. Color slide film was scanned and digitized, and figures generated using the Aldus Photostyler 2.5 and Pagemaker 5.0 programs.

**RESULTS**

The embryonic gonad is composed of 10-15 pole cells intermingled with approximately 25-35 mesodermal cells (Sonnenblick, 1941). To follow the development of the somatic cells of the gonad, we used the P-element enhancer trap line 68-77 (see Materials and Methods; see also Warrior, 1994). *lacZ* expression in this line occurs in the somatic cells of the newly formed gonad (see below). The origin of the somatic gonadal precursors and their incorporation into the gonad can be followed by tracing the expression of *lacZ* back to earlier stages of development. At approximately 5 hours after egg laying (AEL), pole cells migrate through the posterior midgut (PMG) and contact mesoderm in the posterior portion of the embryo, primarily within parasegments 11 through 13 (PS11-13). At this time, *lacZ* expression occurs in patches in the mesoderm in PS2 to PS14 (Fig. 1A). Higher levels of expression are found in the more posterior parasegments (Fig. 1A, bracket), in particular in PS10 (Fig. 1D, open arrowhead) and in cells in PS11 and PS12 (arrowheads) that contact pole cells (arrows). Although expression is also detected within PS13, these cells are not always associated with pole cells. As development proceeds, pole cells migrate anteriorly towards PS10, remaining in contact with strongly staining somatic cells (Fig. 1B,E). Finally, by 10 hours AEL, gonadal precursors and pole cells coalesce into a gonad (Fig. 1C,F). Therefore, line 68-77 suggests that gonadal precursors originate in PS10, PS11 and PS12, in accordance with previous results monitoring expression of the 412 retrotransposon (Brookman et al., 1992).

**Fig. 1.** Development of somatic gonadal mesoderm and assembly of the gonad. Anterior is to the left in all figures, except Fig. 1D. (A-F) Somatic gonadal precursors visualized by anti-β-galactosidase antibody staining; line 68-77; (A-C) lateral view, PS10-PS12 in brackets; (D-F) 100x magnified views. (A) Stage 11, approximately 6 hours after egg laying (AEL) *lacZ* is expressed in patches in the mesoderm from PS2-PS14 (and in the ectoderm, see Materials and Methods) with higher levels of expression in posterior regions PS10-PS12. (B) Stage 12, approximately 9 hours AEL. *lacZ* expression is maintained in cells that appear to move anteriorly, towards PS10. (C) Stage 14, 13 hours AEL. Gonadal precursors have coalesced into a gonad at PS10. (D) Embryo of stage shown in A, focusing on the mesoderm of PS10-13. β-gal-staining mesodermal cells in PS11-12 (solid arrowheads) can be seen surrounded by large, unstained pole cells (arrows). β-gal-staining mesodermal cells in PS10 (open arrowhead), slightly out of focus, are not associated with pole cells. (E) Embryo at the stage shown in B. Pole cells remain in contact with staining gonadal precursors during migration. (F) Formed gonad from an embryo dissected at the stage shown in C. The somatic gonadal cells (arrowhead) are found intermingled with pole cells (arrow). (G-J) Bluetail enhancer trap line, stained with anti-β-gal, magnified 100x. (G) Embryo stage equivalent to that shown in A. D. β-gal-staining cells in PS12 (arrowhead) can be seen contacting pole cells, while pole cells in PS11 (arrow) do not contact staining cells. (H) Embryo stage equivalent to that shown in B. E. Arrow shows anterior limit of migrating cluster. Note that only posterior-most somatic cells are stained (arrowhead). (I) Embryo stage equivalent to that shown in C. Dashed line marks the limit of the gonad, while arrowhead points to stained mesodermal cells, which populate only the posterior of the gonad. (J) Wild-type bluetail embryo. (I) *lacZ* expression in agametic bluetail embryo. Somatic gonadal cells have migrated and coalesced as the posterior portion of the agametic gonad (arrowhead).


**Abd B function is required for the specification of a posterior sub population of gonadal precursors**

The posterior somatic cells of the gonad, as judged by bluetail expression, derive from PS12, illustrating that distinct sub populations of gonadal precursors can be distinguished early in gonadogenesis. Indeed, gonadal precursors also differ in the expression of endogenous genes, as indicated by the expression of the gene eyes absent (eya, Bonini et al., 1993). During migration, eya is expressed in posterior gonadal precursors in a manner similar to bluetail (Fig. 2A, arrowhead, compare with Fig. 1H). eya expression is also maintained within the posterior somatic cells of the coalesced gonad (data not shown). Therefore, the differential expression of bluetail and eya among gonadal precursors illustrates the existence of a distinct sub population shortly after specification.

Posterior gonadal precursors derive from regions of the embryo that require Abd B function for proper segmental identity (Lewis, 1978; Karch et al., 1985; Sanchez-Herrero et al., 1985; Casanova et al., 1986; Celniker et al., 1990). Therefore, we asked whether Abd B function is required for their specification. No eya-expressing cells are observed surrounding the migrating pole cells in Abd B^{M1} mutants (Fig. 2B), while all other sites of eya expression are unaffected (data not shown). Furthermore, no eya expression was detected in coalesced gonads (data not shown). Thus, Abd B function is required within PS12 for the specification or maintenance of posterior gonadal fates, consistent with the finding that Abd B mutant gonads are composed of fewer gonadal precursors (Brookman et al., 1992).

**abd A function restricts gonadal precursor specification to posterior parasegments**

In contrast to the restricted requirement for Abd B, abd A is required for the specification of gonadal precursors within all three parasegments (Brookman et al., 1992). To test whether
*abd A* function is sufficient to determine gonadal precursor fates, we examined the consequence of ectopic *abd A* expression on the development of gonadal precursors, by monitoring the expression of the 412 retrotransposon. In wild-type embryos, 412 expression becomes restricted to gonadal precursors, and by the time of gonad formation, is visible only in somatic cells that have coalesced with pole cells at PS10 (Fig. 3A). In contrast, when *abd A* is ectopically expressed (see Materials and Methods), 412 expression marks the gonadal precursors of PS10 through PS12 and expands anteriorly, often as far as PS2 (Fig. 3B). To verify that ectopic 412 expression reflects the development of extra gonadal precursors and not simply increased 412 expression in other mesodermal cells such as the developing fat body (Brookman et al., 1992), we examined heat shock treated *hs-abd A* embryos for expression of the gene *escargot (esg)*, which marks the anterior somatic cells of the formed gonad (Fig. 3C, arrow; Gönczy et al., 1992). In *hs-abd A* embryos, *esg* expression also expands anteriorly, in a pattern reminiscent of 412 expression (Fig. 3D, arrow). We conclude that extra gonadal precursors are specified in anterior regions. However, not all mesodermal cells within anterior regions express the 412 or *esg* marker, showing that *abd A* is not sufficient to determine gonadal precursor fates within a particular parasegment. Instead, *abd A* acts to make parasegments competent for gonadal precursor development.

Gonadal precursors specified in anterior regions by ectopic *abd A* express markers specific for anterior gonadal fates, but not for posterior fates such as the gene *D-wnt-2* (data not shown, Russell et al., 1992). Therefore, *abd A* activity alone specifies anterior gonadal precursor fates, whereas *abd A* and *Abd B* together specify posterior gonadal precursors.

**Ectopic *abd A* expression affects the ability of pole cells to arrest migration at PS10**

In the above experiments, we observed that gonadal precursors present anterior to PS10 were associated with pole cells. This suggests that ectopic *abd A* expression also affects the ability of pole cells to arrest migration at PS10. To confirm this, we followed early steps of pole cell migration and coalescence using the vasa antibody (Hay et al., 1988; Lasko and Ashburner, 1990). In wild-type embryos, pole cells initially contact gonadal precursors in PS11 and PS12 (Fig. 3E), migrate anteriorly and eventually coalesce into a gonad at PS10 (Fig. 3G, bracket). In heat shock treated *hs-abd A* embryos, initial steps of pole cell migration are unaffected, since pole cells contact gonadal precursors in PS11 and PS12 (Fig. 3F) and migrate normally towards PS10. Some pole cells halted migration at PS10, coalescing into a gonad (Fig. 3H, bracket), normally intermingled with gonadal precursors (data not shown). However, many pole cells failed to arrest, continuing their migration, often as far anteriorly as PS7 (Fig. 3H). Pole cells in anterior regions remain tightly associated, but do not migrate anteriorly and eventually coalesce into a gonad at PS10 (bracket). (H) Stage 15 *hs-abd A* embryo. Some pole cells cluster into gonad at PS10 (bracket), others are found in anterior regions, as far as PS7.
not appear to fully coalesce into a gonad. Therefore, the transformation of anterior parasegments to more posterior identities by ectopic *abd A* expression affects the ability of pole cells to arrest migration at PS10. We infer that gonadal precursors also fail to arrest migration since pole cells were associated with migratory gonadal precursors at earlier steps. Unfortunately, because these pole cells migrate into regions of ectopic 412 expression, we cannot distinguish between gonadal precursors born in posterior regions that may migrate too far and gonadal precursors that are now specified in ectopic anterior regions.

***abd A* function is required for the coalescence of the gonad**

A later requirement for *abd A* in gonadogenesis has been suggested by the analysis of pole cell migration in embryos carrying mutations in a cis-regulatory region of *abd A*, called *iab-4* (Lewis, 1978; Karch et al., 1985; Cumberledge et al., 1992; Warrior, 1994). Pole cells in *iab-4* mutants contact mesoderm and begin migration anteriorly, but then disperse. Although *iab-4* function is required in the somatic cells and not the germline (Cumberledge et al., 1992), the consequence of *iab-4* mutations on the development of gonadal precursors has not been tested. Therefore, we followed the fate of the gonadal precursors in *iab-4* mutant embryos to define the point at which *abd A* executes this later, *iab-4*-dependent role.

Early stages in gonad formation appear unaffected in *iab-4* mutant embryos. Gonadal precursors are specified and associate with pole cells in PS11 and PS12 (data not shown). The cluster initiates migration towards PS10 just as in wild type (Fig. 4A, compare to Fig. 1E). However, these gonadal precursors do not coalesce into a gonad. Instead, they appear stalled, distributed throughout the PS10-PS11 region (Fig. 4B). In addition, pole cells are released from the stalled gonadal precursors, and scatter throughout the embryo (Cumberledge et al., 1992, data not shown). Lastly, the further differentiation of the precursors is prevented, since *esg* is not expressed in the anterior gonadal precursors (compare Fig. 4C, arrowhead, and 4D). Therefore, *iab-4*-dependent *abd A* function is required for the coalescence of gonadal precursors and pole cells into a gonad. Furthermore, the differentiation of gonadal precursors is either directly dependent upon *abd A* function or depends on the coalescence of the gonad which is regulated by *abd A*.

**PS10 and 11 cells express abd A in an iab-4 dependent manner**

Since *iab-4* function is required for coalescence of the gonad, we asked whether cells within the PS10 region express *abd A* in an *iab-4*-dependent manner. In wild type, *abd A* is expressed in the ectoderm in PS7-12 (Fig. 5A), in the visceral mesoderm of PS8-12 flanking the developing gut, and in developing tracheal cells (Fig. 5A, arrowhead; Karch et al., 1990; Macias et al., 1990). As the migrating precursors approach PS10, a population of mesodermal cells located in PS10 and PS11 expresses high levels of *abd A* (Fig. 5A, arrow). The relationship of these *abd A*-expressing cells to the arriving cluster of gonadal precursors and pole cells is shown in a series of optical sections (Fig. 5B-C). Focusing first on the arriving cluster, *abd A*-expressing cells are present on the ventral and lateral surfaces of the incoming gonadal precursors (Fig. 5B-C). Focusing progressively towards the body wall mesoderm, more *abd A*-expressing cells can be detected (Fig. 5D). Therefore, the gonadal precursors appear to migrate into a region of *abd A*-expressing cells which partly envelope the arriving cluster. When the gonad is fully formed, *abd A* expression decreases dramatically, but can be detected in cells that partially ensheathe the gonad (Fig. 5E, arrows), as reported earlier (Karch et al., 1990; Cumberledge et al., 1992). This suggests that the high level *abd A*-expressing cells in PS10 and PS11 contribute to the formation of the gonadal sheath.

In contrast to wild type, only low levels of *abd A* expression could be detected in the mesoderm of PS10 and 11 in *iab-4* mutant embryos (Fig. 5F, arrow), while *abd A* expression in the visceral mesoderm and tracheal cells was not affected (data not shown). *abd A* expression in PS10 and 11 cells was affected.

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**Fig. 4.** Late requirement for *iab-4* dependent *abd A* function in gonad formation. (A,B) *iab4* mutant embryos carrying the 68-77 enhancer trap, stained with anti-β-gal to visualize gonadal precursors. (A) Late stage 12 embryo, PS10 region (bracket). Gonadal precursors (arrowhead) migrate to PS10 with pole cells as in wild type (compare with Fig. 1F). (B) Stage 14 embryo, dissected PS10 region (bracket). The gonadal precursors never coalesce, remaining stalled within the PS10-11 region, slightly extending into PS9. (C,D) *esg* expression. (C) Magnified view of coalescing gonad, dissected from early stage 13 wild-type embryo. Note the expression of *esg* in the anterior gonadal precursors (arrowhead). (D) *iab4*-mutant, same stage as in C. Arrowhead marks pole cell in PS10 region (bracket). No *esg* expression is visible in the vicinity of PS10, where the stalled gonadal mesoderm persists.
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in iab-4 mutants but not in embryos carrying mutations in another cis-regulatory region of abd A, called iab-5 (Fig. 5G, Karch et al., 1985). In iab-5 mutants, gonads form and are ensheathed in abd A-expressing cells. Therefore, iab-4 controls the expression of abd A in a population of mesodermal cells within PS10 and 11. These cells appear to require abd A function to allow for or induce the coalescence of the gonad at PS10.

DISCUSSION

By following the gonadal precursors in wild type and both abd A and Abd B mutant embryos, we have identified the following steps in gonad formation: (A) Specification of gonadal precursors among the mesodermal cells of PS10 through PS12 (stippled cells, Fig. 6A). (B) Pole cells (circles) recognize and adhere to gonadal precursors in PS11 and PS12 (Fig. 6B). (C) Anterior migration of precursors from PS11 and PS12, with associated pole cells, into a region expressing high levels of abd A (crosshatched cells, Fig. 6C). (D) Arrest of migration at PS 10 (Fig. 6D). (E) Coalescence of somatic precursors and pole cells into a gonad, ensheathed by abd A-expressing cells.

We discuss each of these steps, describing the roles of abd A and Abd B in gonad assembly.

Specification of gonadal precursors

A small number of gonadal precursors are specified within the mesoderm of PS10 through PS12. These cells are first observed when contacting pole cells (Fig. 1D), however this contact does not specify gonadal fate, because gonadal precursors develop normally in the absence of pole cells (Aboim, 1945; Brookman et al., 1992; Fig 1I). Although the mechanism by which gonadal precursors are specified within a particular parasegment remains unknown, posterior parasegments are made competent for gonadal precursor development by the action of the homeotic gene abd A (Lewis, 1978; Karch et al., 1985; Sanchez-Herrero et al., 1985; Brookman et al., 1992; this work). abd A function is required within all three posterior parasegments for gonadal precursor development (Brookman et al., 1992). Furthermore, we have shown that when anterior parasegments are transformed into more posterior identities by ectopic expression of abd A, these anterior parasegments are now capable of developing gonadal precursors (Fig. 2C).
Abd B is required, however, to specify posterior gonadal cell fates. A marker for the posterior most gonadal precursors,eya, is not expressed in Abd B mutants (Fig. 2B), and the resulting gonads are reduced in size (Brookman et al., 1992). Because the posterior-most gonadal mesoderm derives largely from PS12, these results suggest that Abd B is required within PS12 for gonadal specification. Since abd A function is required within all three parasegments, Abd B must act along with abd A to specify posterior gonadal cell fates. Indeed, in regions of the embryo where Abd B alone is present, such as PS13 and PS14, no gonadal precursors develop. Furthermore, transformation of anterior parasegments to these more posterior identities through the ectopic expression of Abd B abolishes gonadal precursor specification. Therefore, Abd B alone does not specify gonadal precursor fates, but together with abd A, posterior gonadal precursors are specified. In contrast, anterior gonadal precursors do not require Abd B function for specification, as markers specific for anterior gonadal precursors such as esg, are still expressed in Abd B mutant gonads (M. B. and S. D., unpublished observations). This suggests that abd A alone is sufficient to promote anterior gonadal precursor development. Indeed, when abd A is ectopically expressed, gonadal precursors induced in anterior regions express esg (Fig. 3D), but not markers for posterior gonadal fates such as D-wnt-2 (M.B. and S.D., unpublished observations).

**Association of gonadal precursors and pole cells**

Once pole cells migrate through the PMG, they contact posterior mesoderm and adhere to gonadal precursors specified within PS11 and PS12. Subsequently, these gonadal precursors delaminate from the mesodermal layer and migrate towards PS10. Throughout migration, gonadal precursors are tightly associated with pole cells. This suggests that pole cells do not migrate independently, but are guided to PS10 by migratory gonadal precursors. If so, the recognition and binding of each pole cell with migratory gonadal precursors is crucial in ensuring that pole cells are incorporated into the gonad. Indeed, in Abd B mutants, posterior gonadal precursor development is affected and a large number of pole cells are not incorporated into the gonad (Brookman et al., 1992; this work). Perhaps in the absence of posterior gonadal precursors, pole cells initially contact PS12 but then dissociate from the mesoderm and scatter throughout the embryo. An alternative interpretation is that Abd B mutations affect the migratory cues normally read by pole cells. This seems unlikely however, since pole cells associated with more anterior gonadal precursors migrate to PS10 normally in Abd B mutants.

**Migration of gonadal precursors and pole cells**

One possible mechanism that directs migration of gonadal precursors anteriorly is the production of attractive signals by cells within PS10. Although a likely candidate for the source of such a signal is the patch of high level abd A-expressing cells in PS10, our data argue against this possibility. The iab-4 mutation has a dramatic effect on the expression of abd A in mesodermal cells within PS10 and PS11, suggesting that their identity has been shifted towards a more anterior character as has been shown in the adult epidermis (Karch et al., 1985). Yet, these changes do not dramatically affect the ability of gonadal precursors and associated pole cells to initiate anterior

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**Fig. 6.** The stages in gonad formation. In each panel the lower boxes represent the ectoderm of abdominal parasegments 9-13 (PS9-PS13). The solid area overlying the ectoderm is the mesoderm. (A) Stage 11. Gonadal precursors are specified in PS10 through PS12 and possibly PS13. (B) After pole cells (white circles) exit the midgut they recognize and associate primarily with specific cells laterally positioned in the mesoderm of PS11 through PS12. These are the migratory gonadal precursors (stippled cells), which delaminate from the mesodermal cell sheet. (C) Gonadal precursors and pole cells migrate anteriorly, where they contact cells in PS10 that express high levels of abdA in an iab-4-dependent manner (crosshatched cells). (D) Gonadal precursors and pole cells arrest migration at PS10. (E) Finally, high level abdA-expressing cells partially ensheath the arriving cells, and the cluster coalesces into a gonad.
migration. Therefore, the source of an anterior migratory signal remains unknown.

Similarly, the iab-4 dependent abd A expression within PS10 is not required for the arrest of gonadal precursors at PS10, since in the iab-4 mutant, migratory gonadal precursors halt migration within the PS10 and PS11 region. Therefore, the identity of PS10, as established by the iab-4 dependent abd A expression is not required for the production of signals to arrest migration. Our results do show however, that proper regional identities as established by homeotic gene function are required for arrest at the correct position. Pole cells and presumably gonadal precursors no longer arrest migration at PS10 when abd A is misexpressed. Failure to arrest migration occurs only when abd A is ectopically expressed at early stages, between 4 and 6 hours of development, prior to the onset of migration. Presumably only at this earlier stage can abd A expression effectively transform anterior parasegments toward posterior identities. This suggests that in wild-type embryos, gonadal precursors and pole cells arrest when they encounter a region anterior to PS10 that is not conducive to further migration. Ectopic abd A expression disrupts this boundary by transforming anterior parasegments toward posterior identities. Perhaps substrates required for migration are now produced in anterior regions thereby allowing further migration. Alternatively, inhibitory signals normally present in anterior regions might not be produced when these regions are transformed. Interestingly, homeotic genes have been shown to control cell migration in other systems. For instance, in Caenorhabditis elegans, both the mab-5 and lin-39 homeotic genes act autonomously within neuroblasts to control their migratory paths through specific domains of the embryo (Salser and Kenyon, 1992; Clark et al., 1993; Wang et al., 1993). While in this example the migratory paths selected depend on Hox gene function in the migrating cells, during gonadogenesis in the fly, it may be the environment through which migration occurs that is controlled by homeotic gene function. We have not tested whether abd A function is also required within gonadal precursors to control their migratory path.

Coalescence of gonadal precursors and pole cells
The iab-4 mutant phenotype suggests a specific role for abd A in the coalescence of the gonad (Figs 4, 5). Cells within PS10 and PS11 express high levels of abd A in an iab-4 dependent manner, as the migratory gonadal precursors enter the region. Cells of PS10-11 partially envelope the arriving cluster, and contribute to the mesodermal sheath surrounding the gonad. These observations suggest that the coalescence of the gonad is dependent upon abd A function in specific cells within PS10.

Although high level abd A expression is required for coalescence of the gonad, it is not sufficient. High levels of abd A expression induced during migration does not cause premature clustering in regions posterior to the normal position of gonad formation. Perhaps in PS10, abd A acts in conjunction with another as yet unidentified protein to induce the coalescence of the gonad. This unidentified protein may not be present in posterior segments, explaining why premature coalescence cannot occur there. Alternatively, it is possible that overexpressing abd A has no phenotypic consequence in the posterior mesoderm. This could be due to the presence of the homeotic gene Abd B. Such posterior dominance of homeotic gene function has been described for patterning in the ectoderm (Gonzalez-Reyes and Morata, 1990; Gonzalez-Reyes et al., 1990; but see Lamka et al., 1992; Michelson, 1994).

Differentiation of gonadal precursors
All of the differentiated somatic cell types found in the adult testes and ovaries derive from the population of gonadal precursors found in the embryonic gonad. Our results illustrate that the differentiation of the somatic cells of the gonad begins quite early in gonadogenesis. For example, the enhancer trap line blueadult identifies a posterior sub population of Abd B-dependent gonadal precursors which make up the posterior mesodermal cells of the gonad. In the adult testis, blueadult marks the terminal epithelial cells of the testis that form the connection between the testis and the seminal vesicle (Gönczy et al., 1992; M.B. and S.D., unpublished observations), suggesting that the posterior gonadal precursors begin differentiating at very early stages in gonadogenesis. Supporting this, we find that the expression of eya, a marker restricted to the posterior-most gonadal precursors, is expressed during early stages of migration (Fig. 2).

Similarly, esg expression is initiated in the anterior gonadal precursor cells prior to coalescence (Fig. 4C). In the adult testes, esg is expressed in a cluster of somatic cells, called the apical cells, around which the germ line stem cells anchor (Gönczy et al., 1992; C. Bromleigh and P. Gönczy, unpublished observations). Taken together, these results demonstrate that the precursors of the distinct cells types found in the adult ovaries and testes can be identified and begin differentiating early in gonadogenesis. This suggests that mutations in genes whose activities are required for the development of particular cell types could result in defects at specific stages in gonad formation. Consistent with this, we find that gonadogenesis is affected in embryos with null mutations in eya (M. Boyle, N. Bonini and S. DiNardo, unpublished data).

Some aspects of gonad formation in Drosophila are conserved in other species. For example, in both Drosophila and mammals the formation of a gonad does not depend upon the presence of germ cells (Mintz and Russel, 1957). In addition, recent work suggests that, like Drosophila, part of the somatic component of the mammalian gonad derives from a migratory population of gonadal precursors originating in the developing kidney that invades the genital ridge (Rodemer et al., 1986; Rodemer-Lenz, 1989; Buehr et al., 1993). Unlike Drosophila though, this migratory population is not associated with the migratory germ cells. Therefore, these migratory gonadal precursors do not guide germ cells to the position of gonad assembly. The mechanism whereby germ cells are guided to the genital ridge in mammals is not known. However, recent results suggest that the first germ cells to migrate through the gut epithelium do so directly into the region of the prospective genital ridge. These ‘pioneer germ cells’ contact the remaining germ cells through long cytoplasmic processes which may assist in their accumulation within the genital ridge (Gomperts et al., 1994). A further link between Drosophila and vertebrate gonad formation may be found in the roles for the homeotic genes abd A and Abd B. The vertebrate homologues of abd A and Abd B within the Hoxd complex are expressed in the somatic gonadal cells as the germ cells populate the gonad (Dollé and Duboule, 1989; Izipisúa-Belmonte et al., 1990; Dollé et al., 1991). It will be interesting to determine whether
the functions of these homeotic genes in gonad formation are also conserved.

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REFERENCES


Brookman, J. J., Toosy, A. T., Shashidhara, L. S. and White, R. A. H. (1992). The enhancer trap lines isolated by him and his colleagues Mike O’Conner is partially funded by NRSA Training Grant no. GM08485-02. S.D. is partially funded by NRSA Training Grant no. GM08485-02. S.D. was a Lucille P. Markey Scholar, and work in the lab is supported by the Markey Charitable Trust.


genes are required for spatial control of the \( abd\ A \) and \( Abd\ B \) homeotic products. *Development* **114**, 493-505.


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