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# Slow as Molasses is required for polarized membrane growth and germ cell migration in *Drosophila*

**Jennifer A. Stein[1], Heather Tarczy Broihier[1,2], Lisa A. Moore[1,3] and Ruth Lehmann[1,*]**

[1]Howard Hughes Medical Institute, Developmental Genetics Program, Skirball Institute at NYU School of Medicine, 540 First Avenue, New York, NY 10016, USA
[2]Department of Genetics, Washington University School of Medicine, 4566 Scott Avenue, St. Louis, MO 63110, USA
[3]Incyte Genomics, 3160 Porter Drive, Palo Alto, CA 94304, USA
*Author for correspondence (e-mail: lehmann@saturn.med.nyu.edu)

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## SUMMARY

***Drosophila* germ cell migration is directed by attractive and repulsive guidance cues. We have identified a novel gene, *slow as molasses* (*slam*), which is required for germ cell migration. In *slam* zygotic mutants, germ cells fail to transit off the midgut into the mesoderm. We show that *slam* is required at this stage in parallel to *HMG Coenzyme A reductase*, a previously identified germ cell migration gene. Removal of both zygotic and maternal *slam* results in an earlier defect: a failure to form a cellular blastoderm. Consistent with this phenotype, we found that *slam* is one of the earliest genes to be transcribed in the embryo, and Slam protein localizes to the growing basal-lateral membrane during blastoderm formation, but Slam is not detected during later stages of embryogenesis. Because *slam* RNA and protein are expressed earlier than the time when we observe defects in germ cell migration, we propose that Slam is required for the localization of a signal to the basal side of blastoderm cells that is needed later in the posterior midgut to guide germ cells.**

Key words: *Drosophila*, Germ cells, Cell migration, Cellularization, Cell polarity

## INTRODUCTION

Cell migration is an essential element of metastasis, inflammation, infection and embryonic development. The study of germ cell migration provides an excellent opportunity to gain insights into these processes, as germ cell migration shares with them key features such as invasion through an epithelium, cell-cell adhesion and directed migration to a target tissue. Germ cell migration is a well-conserved process in species such as human, mouse, fish and fly. *Drosophila* is an ideal animal in which to study the genetics of germ cell migration because it is easy to visualize migrating germ cells and to identify genes that control their migration.

*Drosophila* germ cells are the first cells to form in the embryo. During the first three hours of embryogenesis, nuclei divide synchronously without cytokinesis to form a syncytium. During the eighth and ninth cell cycle, nuclei migrate to the periphery of the embryo. Those nuclei that reach the posterior pole are surrounded by cell membrane as they bud off of the single-celled embryo and become primordial germ cells (for a review, see Williamson and Lehmann, 1996). The somatic cells of the embryo form later, when an apical to basal cell membrane growth encapsulates each nucleus. Owing to the two modes of cell formation in the early embryo, two cell layers are formed at the posterior pole: the germ cells, which form by budding at nuclear cycle 10, and the posterior somatic blastoderm cells, which form by polarized cellularization at nuclear cycle 14 (for reviews, see Müller, 2000; Schejter et al., 1992). The posterior somatic blastoderm cells give rise to the posterior midgut. The germ cells adhere to the posterior midgut primordium as it moves dorsally and involutes during gastrulation to form a pocket inside the embryo. By the end of gastrulation, the primordial germ cells are therefore located inside the pocket of the posterior midgut primordium. Subsequently, germ cells migrate through the epithelium of the posterior midgut, crawl dorsally along the midgut, and move off the midgut, into the mesoderm, to contact somatic gonadal precursor cells (SGPs), which form in parasegments 10-12 (Fig. 1A,B) (Boyle and DiNardo, 1995). Germ cells maintain contact with SGPs as they coalesce and form the embryonic gonad at stage 14 (Fig. 1C) (for a review, see Starz-Gaiano and Lehmann, 2001).

Several molecules have been identified that are known to guide germ cells. *wunen* and *wunen 2* encode phospholipid phosphatases and are expressed in the posterior midgut. Wunen phosphatase activity was shown to repel germ cells as they move on the midgut epithelium (Starz-Gaiano et al., 2001; Zhang et al., 1997). HMG-CoA reductase (Hmgcr) is highly expressed within the developing SGPs and is required to attract germ cells into the gonadal mesoderm. In *Hmgcr* mutants, most germ cells remain on the surface of the midgut and fail to enter the mesoderm. Hmgcr has also been shown to be sufficient to attract germ cells at a distance. When *Hmgcr* is misexpressed in the epidermis or in the nervous system, germ cells are

attracted towards the areas of high HMG-CoA reductase (Van Doren et al., 1998b). Although *Hmgcr* mutants have consistent and fully penetrant germ cell migration defects, some germ cells do enter the mesoderm, associate with SGPs and migrate correctly to the gonad. This suggests that additional germ cell guidance factors direct germ cells from the midgut to the mesoderm.

We report the identification of *slow as molasses* (*slam*), a new gene involved in germ cell migration. Embryos homozygous for point mutations in *slam* have a strong and penetrant germ cell migration defect, which resembles that of *Hmgcr* mutants. Our genetic analysis suggests that Slam acts independently of Hmgcr and we propose that Slam acts in the midgut and is required for germ cells to transit from the midgut to the mesoderm. In addition to the germ cell migration defect, we observed cellularization defects in mutant embryos after removal of the maternal and zygotic contribution of *slam*. Molecular characterization of *slam* reveals that *slam* is one of the first genes transcribed in the embryo. Consistent with a role in polarized cell growth during blastoderm formation, Slam protein localizes to the growing basal-lateral membrane during nuclear cycle 14. *slam* RNA and protein are no longer expressed at the time when we observe a defect in germ cell migration. Based on the mutant phenotype and the analysis of the protein distribution, we propose that Slam mediates the localization of a germ cell guidance signal to the basal side of posterior midgut cells.

## MATERIALS AND METHODS

### Fly stocks

*waldo*$^{1}$ and *waldo*$^{2}$ alleles of *slam* were identified in a zygotic mutagenesis screen for germ cell migration genes on the second chromosome (Broihier, 1998). The two alleles have similar germ cell migration defects. *slam*$^{waldo}$; *Hmgcr* double mutants were made with *slam*$^{waldo1}$ and *Hmgcr*$^{clb1}$ (Van Doren et al., 1998b). To test for dominant suppression of *Hmgcr* ectopic germ cell attraction by *slam* loss of function, *elav-gal4, UAS-Hmgcr* males were crossed to virgins with germline clones of *slam*$^{waldo}$; the progeny would therefore be completely lacking maternal *slam* and zygotically heterozygous for *slam* ($M^{-}Z^{-/+}$ *slam*$^{waldo}$). Germline clones of *slam*$^{waldo}$ were made by crossing *slam*$^{waldo1}$ *FRT 40A* / *CyO* virgins to *hs flp; ovo*$^{D}$ *FRT 40A* / *CyO* males and heat shocking the progeny at 37ºC for 2 hours on 2 successive days during first and second larval instar. To test the phenotype of $M^{-}Z^{-}$ *slam*$^{waldo}$ mutants, *hs flp*; *slam*$^{waldo1}$ *FRT 40A* / *ovo*$^{D}$ *FRT 40A* virgins that contained *slam*$^{waldo1}$ germline clones were crossed to *slam*$^{waldo2}$*/CyO, P(ftz-lacZ)* males.

### Mapping and molecular identification of *slam*

Meiotic recombination mapping placed *slam* between *dp* and *b*. The following P elements were used for male mitotic recombination: l(2)k10004 (25B), k13720 (26C), l(2)k07502b (26D1-2), k04917(26D6-8), k03201(26D6-9), k14206 (26F), l(2)k00605 (27A), EP2369 (27F), EP2625 (28B), l(2)rL220 (28C), 02496 (28D1-2), P 1478 (28D3-4), l(2)k11101(28D10-11), l(2)05836 (28E1-2) and l(2)k13638 (28E3-4). *slam* mapped between k13720 and l(2)k07502b. The mapped region included 16 candidate genes, two of which were uncovered by the deficiency *Df(2L)tig*$^{X}$ (a gift from T. Bunch) (Bunch et al., 1998), which complements *slam*, leaving 14 candidate genes. In situ probes were constructed for each of the 14 candidate genes either from an EST (Research Genetics) or an amplified genomic fragment, subcloned into pCR 4Blunt-TOPO using the Zero Blunt TOPO PCR cloning kit (Invitrogen). The following probes were made from ESTs: Pez (LD 11612), CPR (LD 45615 and LD 46590), CG9498 (GH 06262), and CG9505 (GH 11680 and GH 10925). The following probes were made from subcloned genomic fragments: CG9491, CG9497, CG9499, CG9501, CG9500, CG9506, CG13981, CG9507, CG9508 and CG9511. To transcribe in situ hybridization probes from ESTs, the plasmids were linearized with *Eco*RI and transcribed with Sp6 polymerase for antisense RNA probes; sense RNA control probes were transcribed with T7 polymerase after linearization with *Xho*I. For genomic fragments, RNA probes were transcribed with T3 polymerase after linearization of the subcloned fragments with *Not*I. T7 polymerase was used to transcribe probes after linearization with *Pme*I or *Spe*I. Transcription reactions were carried out with the digoxigenin RNA labeling kit (Roche).

Pez, CPR, and CG9506 were sequenced by PCR amplification of genomic DNA from both *slam* mutant alleles. Genomic DNA from mutant progeny of *slam*$^{waldo1}$ / *CyO P(Kr-GFP)* or *slam*$^{waldo2}$ / *CyO P(Kr-GFP)* was isolated after selecting homozygous mutant embryos (those that did not contain GFP). The PCR products were gel-purified with the Qiaquick kit (Qiagen), sequenced at the Rockefeller University DNA sequencing lab and analyzed with the SeqManII program.

### In situ hybridization and immunohistochemistry

Rabbit α-Slam was raised against the peptide ETPKDDLQTGEFDFAKP in the second exon. The following antibodies were used for immunostaining of embryos: rabbit α-Vasa (1/2500), rabbit α-β-galactosidase (Cappel, 1/20,000), rabbit α-Slam (1/3000 – 1/5000), mouse α-Neurotactin (BP106 Hybridoma bank, 1/50) and rabbit α-Myosin (1/750, gift from C. Field). Antibody detection was performed using a biotinylated secondary antibody (Jackson ImmunoResearch) and the ABC Elite Kit (Vector Labs), or with a directly conjugated Alexa 488 (Molecular Probes), Cy3 or Cy5 secondary antibody (Jackson ImmunoResearch). For staining with α-Vasa and α-β-galactosidase, embryos were fixed and devitellinized according to the method described by Gavis and Lehmann (Gavis and Lehmann, 1992), with the modification that 1×PBS was used in place of PEMS during the fixation. For staining with α-Slam, α-Neurotactin and α-Myosin, embryos were heat fixed by immersion for 5 seconds into boiling 0.03% Triton-X, 68 mM NaCl, followed by devitellinization in methanol. Embryos were rehydrated and subjected to antibody staining as described by Eldon and Pirotta (Eldon and Pirotta, 1991). Fluorescently labeled embryos were mounted in Aqua Poly/Mount (Polysciences) and analyzed with a Leica TCS/NT confocal microscope. For whole-mount analysis of immunohistochemically labeled embryos, mountings were made in PolyBed812 (Polysciences) according to Ephrussi et al. (Ephrussi et al., 1991) and analyzed with a Zeiss Axiophot using Nomarski optics. Section analysis was performed as described by Broihier et al. (Broihier et al., 1998). Sections were 2 μm. For double labeling of embryos with an antibody and in situ hybridization probe, embryos were first antibody stained as described above (except washes were made in 1×PBS, 0.1% Tween-20, 50 μg/ml heparin and 250 μg/ml tRNA), and then subjected to in situ hybridization. In situ hybridization was performed as described by Lehmann and Tautz (Lehmann and Tautz, 1994). Embryos were incubated with DIG-labeled RNA probes at 55ºC overnight. Probe hybridization was visualized with an alkaline phosphatase-conjugated anti-DIG antibody, followed by treatment with NBT and BCIP.

## RESULTS

### Slam mutant embryos have a defect in germ cell migration

We identified two alleles of *slam* in an EMS mutagenesis screen for zygotically acting genes that affect germ cell

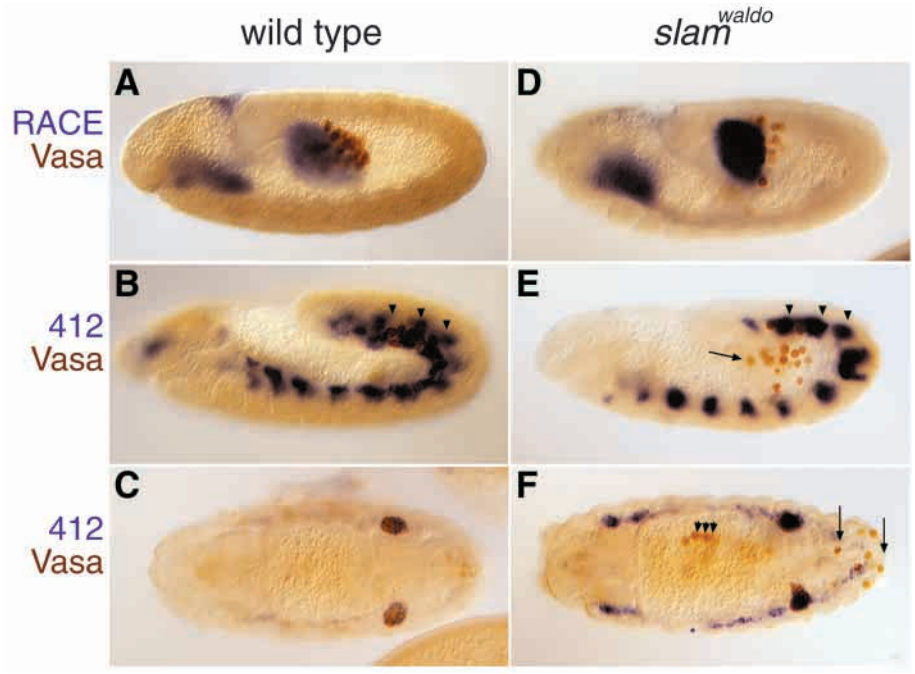


**Fig. 1.** *slam*$^{waldo}$ mutants have a strong and penetrant germ cell migration phenotype. Anterior is towards the left in all panels. (A,B,D,E) Lateral views. (C,F) Dorsal views. (A-C) Wild-type embryos. (D-F) *slam*$^{waldo}$ embryos. All embryos are labeled with α-Vasa to mark germ cells in brown. Embryos in A,D are labeled with RACE RNA to mark the midgut in blue. Embryos in B,C,E,F are labeled with the 412 retrotransposon RNA to mark lateral mesoderm and SGPs (arrowheads) in blue. (A,D) By stage 11, germ cells in *slam*$^{waldo}$ embryos have properly migrated through the gut epithelium; however, the midgut has not become mesenchymal and flattened out. (B,E) By stage 12, most of the germ cells in wild type (B) are in the mesoderm and in contact with three SGP clusters (arrowheads). Many germ cells in *slam*$^{waldo}$ embryos (E) are still on the midgut (arrow), though some are correctly located in the mesoderm next to the two posterior SGP clusters (left two arrowheads in E). (C,F) At stage 14, germ cells and SGPs coalesce to form two round gonads. Though some germ cells in *slam*$^{waldo}$ embryos are in the gonads (F), on average 50% are located at ectopic locations, either on the midgut (arrowheads) or in the posterior end of the embryo (arrows).

migration (Broihier, 1998). We refer to these two mutants as the *waldo* alleles of *slam*, or *slam*$^{waldo}$ alleles, to indicate their origin (see Materials and Methods). Both alleles have similar germ cell migration phenotypes. In *slam*$^{waldo}$ mutant embryos, germ cells migrate correctly through the midgut epithelium and along the midgut towards the dorsal side of the embryo (Fig. 1D), but fail to enter the mesoderm to contact the SGPs (Fig. 1E). Entering the mesoderm at this stage is crucial for germ cells to become incorporated into the embryonic gonad later. Thus, *slam*$^{waldo}$ mutant germ cells that fail to contact the SGPs at stage 11 will be located outside the gonads at stage 14 (Fig. 1F).

Detailed comparison between wild-type and mutant development suggests that germ cells in *slam*$^{waldo}$ mutants are delayed in their movement from the gut to the mesoderm during stage 11 (compare Fig. 1A,B with 1D,E). As a result, some germ cells remain on the midgut throughout embryogenesis and still are found on the midgut at stage 14 (Fig. 1F, arrowheads). Other germ cells move off the midgut but seemingly too late to reach the most posterior cluster of SGPs. Because the germ band has begun to retract, delayed germ cells move into a more posterior region of the mesoderm, which lacks SGPs. These germ cells are found outside and posterior to the embryonic gonad at stage 14 (Fig. 1F, arrows). The remaining germ cells are found associated with SGPs in the embryonic gonad at the end of embryogenesis. On average, 50% of germ cells are lost per embryo in *slam*$^{waldo1}$ and *slam*$^{waldo2}$, compared with fewer than 10% in the control (*slam*/+) embryos.

The germ cell migration phenotype in *slam* embryos could be due to a germ cell autonomous defect; however, Slam RNA and protein are not expressed in the germ cells (see Fig. 4D, inset). Alternatively, the phenotype could be due to a defect in either the specification or differentiation of the somatic tissues that contact germ cells during their migration, or more directly in the production or localization of a guidance cue. As the migration defect is first observed when the germ cells leave the midgut, we analyzed the development of the midgut and the gonadal mesoderm in mutant embryos. In situ hybridization with RACE, an enzyme expressed in the posterior midgut primordium, reveals a delay in the transition from an epithelium to a mesenchyme in mutant embryos. At stage 11, when in wild-type embryos the midgut primordium has already started to flatten out and extend along the mesoderm, the midgut remains more compact in the mutant. The length of the posterior midgut is visibly shorter in the mutant (Fig. 1D). This defect is transient, as later midgut markers, such as *dpp-lacZ*, reveal that a continuous digestive tract is formed; however, in some embryos the second midgut constriction fails to form (Broihier, 1998). FasIII staining reveals that the visceral mesoderm forms normally and surrounds the midgut (Broihier, 1998). Most mutant embryos become crawling larvae and are capable of passing colored yeast normally through their digestive tracts (data not shown). Even if a mild midgut defect exists, this cannot explain the germ cell migration defect of *slam*$^{waldo}$ mutants. The midguts of embryos that lack maternal and zygotic integrin function also fail to undergo an epithelial to mesenchymal change at stage 11 (Martin-Bermudo et al., 1999) and appear morphologically very similar to those of *slam*$^{waldo}$, but the integrin mutants have normal germ cell migration (data not shown). This implies that timing of the epithelial to mesenchymal transition in the posterior midgut is not crucial for germ cells to move from the midgut into the mesoderm, and suggests that the *slam*$^{waldo}$ mutant germ cell migration defect is not simply a result of the altered midgut morphology.

For the analysis of the mesoderm, we used two markers: RNA expression of the retrotransposon 412 and antibody

staining for Zfh-1 protein, which mark the lateral mesoderm at stage 10-11 as well as the gonadal mesoderm, later. Both markers are expressed in *slam*$^{waldo}$ mutants, and the number and distribution of lateral mesoderm cells in mutant embryos seems similar to that of wild type during stages 10-11 (Fig. 1B,C,E,F). However, later when gonadal mesoderm cells move towards each other to align and eventually coalesce to form the embryonic gonad, some mutant embryos show a reduction in the number of SGPs. In *abdA* mutants, lateral mesoderm forms correctly, but no SGPs are specified. Germ cells in these mutants still migrate off the midgut into the lateral mesoderm, but are lost later in embryogenesis (Moore et al., 1998). This suggests that lateral mesoderm, not later SGP formation, is necessary for germ cells to move off the midgut into the mesoderm. Therefore the reduction in SGP number in *slam*$^{waldo}$ mutants is not responsible for the *slam*$^{waldo}$ germ cell migration defect. Taken together, these data suggest that the defect in *slam*$^{waldo}$ mutants is more likely to be due to a defect in midgut signaling or guidance than to a failure in gonadal mesoderm specification or midgut development.

## Slam and HMGCR affect germ cell migration independently

The phenotype of *slam*$^{waldo}$ mutant embryos is strikingly similar to that of *Hmgcr* mutants. In both mutants, germ cells fail to move off the midgut and do not associate with SGPs in the mesoderm at stage 11-12. We also see some germ cells correctly migrating in both single mutants, even in null alleles of *Hmgcr* (Fig. 2). An instructive role as a germ cell attractant was demonstrated for *Hmgcr* by the finding that mis-expression of *Hmgcr* leads to attraction of germ cells to the ectopic site independent of SGP differentiation (Van Doren et al., 1998b).

To analyze a possible interaction between the two genes, we analyzed *Hmgcr* expression and function in *slam* mutant embryos. *Hmgcr* RNA is properly expressed in *slam*$^{waldo}$ mutants, indicating that *slam* is not required upstream of *Hmgcr* for its RNA expression (data not shown). To test whether *slam* acts downstream of *Hmgcr*, we reduced the levels of Slam activity after ectopic expression of *Hmgcr*. We found that ectopic *Hmgcr* expression is still capable of attracting germ cells in a *slam*$^{waldo}$ heterozygous mutant background (see Materials and Methods), suggesting that Slam is not required for Hmgcr-mediated germ cell attraction to the mesoderm. Together, these data make it less likely that Slam and Hmgcr act within the same pathway and favor the hypothesis that *Hmgcr* and *slam* act independently and provide separate guidance cues. This conclusion is further supported by the analysis of *slam*$^{waldo}$; *Hmgcr* double mutants. The germ cell migration phenotype in double mutant embryos was stronger than either single mutant, as no germ cells move off the midgut. However, the specificity of this defect is unclear as the double mutant embryos were poorly differentiated, a phenotype that is not found in collections from either single mutant (compare Fig. 2D with 2B and 2C). The fact that the double mutant has a novel phenotype suggests that, while the activity of *Hmgcr* and *slam* may not directly rely on each other's function, the two genes may act in parallel and may regulate common downstream pathways.

## *slam* is also required for cellularization of the early embryo

There are several explanations for why not all germ cells in *slam*$^{waldo}$ mutants are lost. One is that the function of *slam* is partially redundant with other germ cell guidance genes, such as *Hmgcr* (see above). Another possibility is that *slam*$^{waldo}$ mutants are not complete null alleles and/or that maternal contribution of *slam* partially rescues the germ cell migration phenotype. To address the second hypothesis, we generated germline clones homozygous mutant for *slam*$^{waldo}$ using the *flp-FRT-ovo*$^{D}$ system. Removal of maternal and zygotic *slam* ($M^{-}Z^{-}$ *slam*$^{waldo}$) revealed a new and unexpected phenotype: $M^{-}Z^{-}$ *slam*$^{waldo}$ mutant embryos fail to cellularize during nuclear cycle 14 (Fig. 3). Interestingly, germ cells form normally during cycle 10. The germ cells have no soma to move through, so germ cells are found distributed throughout an otherwise unstructured embryo.

In wild type, nuclei migrate to the periphery of the embryo during the ninth nuclear cycle. During each of the following four nuclear divisions, these nuclei and their associated centrioles induce structural changes in the actin cytoskeleton (actin cap) and the cortical membrane. After the 14th nuclear division, the membrane grows and invaginates between each peripheral nucleus to create an epithelium of somatic cells. This membrane invagination proceeds first slowly for 35-40 minutes (slow phase) and then rapidly for 15-20 minutes (fast phase) until each nucleus is surrounded by a cell membrane (Müller, 2000; Schejter et al., 1992).

Nuclei reach the periphery normally in $M^{-}Z^{-}$ *slam*$^{waldo}$ mutants, the primordial germ cells bud normally, and the somatic nuclei continue to divide until cycle 14. The phases of cellularization during cycle 14 were followed by live observation and by observing the progression of the cellularization front (furrow canal) using an anti-Myosin antibody in wild-type and $M^{-}Z^{-}$ *slam*$^{waldo}$ mutants (Fig. 3; data not shown). The first deviation from wild type is seen during the slow phase of cellularization. As the embryo proceeds

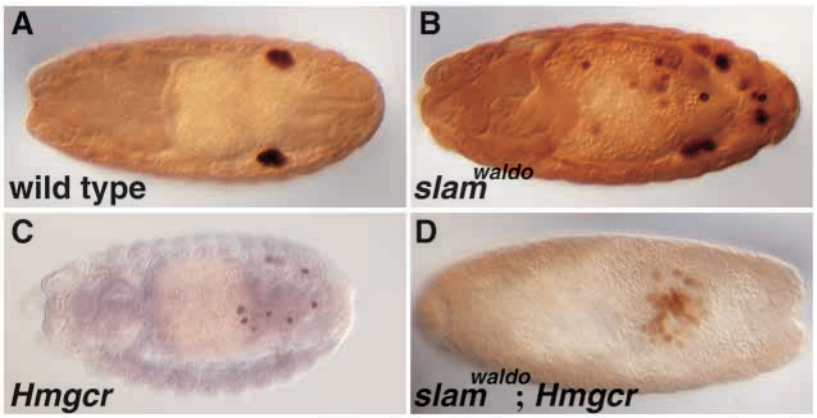


**Fig. 2.** *slam*$^{waldo}$; *Hmgcr* double mutants have a different phenotype from either single mutant. Dorsal views of stage 14 embryos, stained with α-Vasa to mark germ cells, anterior is towards the left. (A) Wild type, (B) *slam*$^{waldo}$, (C) *Hmgcr*, (D) *slam*$^{waldo}$; *Hmgcr*. In *slam*$^{waldo}$; *Hmgcr*, germ cells do not move off the gut, and the embryos are poorly differentiated.

through cycle 14, the nuclei elongate and the membrane invaginates. However, compared with wild type, membrane invagination in *slam* mutants is delayed. At the time when membranes normally enclose each nucleus basally, the incompletely invaginated membranes of $M^{-}Z^{-}$ *slam*$^{waldo}$ mutants entrap the nuclei as they pinch off basally (Fig. 3H). In wild type, after cellularization is complete, the complex

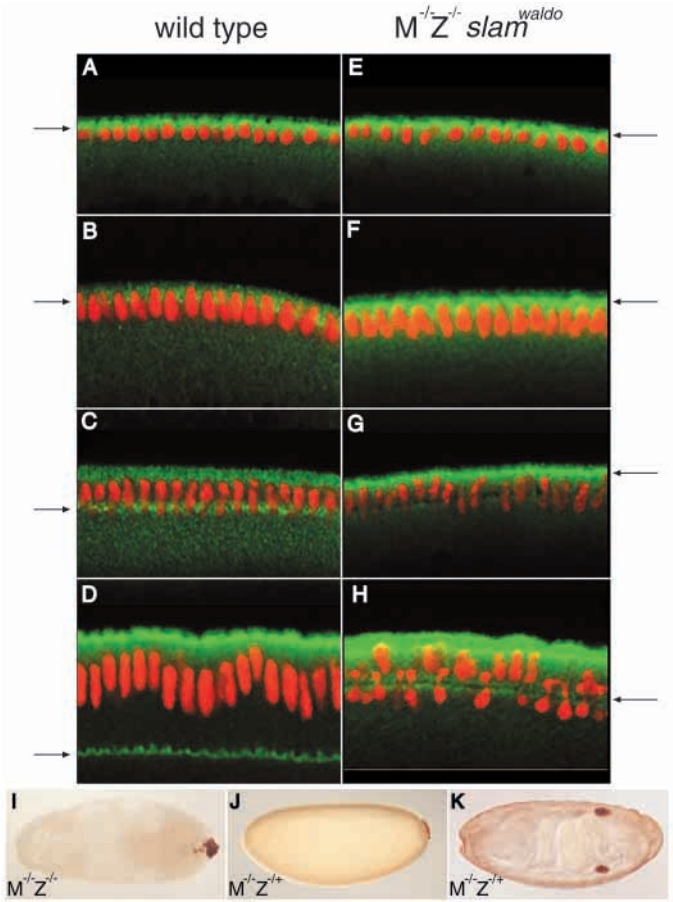


**Fig. 3.** *slam*$^{waldo}$ $M^{-}Z^{-}$ mutants have a cellularization defect. (A-D) Wild type, (E-H) *slam*$^{waldo}$ $M^{-}Z^{-}$ mutants, embryos are stained with α-myosin in green to mark the advancing basal-most part of the cell membrane, and in red with DAPI to mark the nuclei. Arrows mark cellularization front. I-K are stained with α-Vasa to mark germ cells. (A,E) Pre-cellularization embryos, the nuclei are lined up under the plasma membrane, which has not begun to invaginate. *slam*$^{waldo}$ $M^{-}Z^{-}$ is indistinguishable from wild type at this stage. (B,F) Mid-cellularization, nuclei are elongating in both wild type and *slam*$^{waldo}$ $M^{-}Z^{-}$. The membrane has begun to invaginate in wild type, but not in *slam*$^{waldo}$ $M^{-}Z^{-}$. (C,G) Mid-late cellularization, in wild type, the nuclei have continued to elongate as the membrane has advanced almost to the end of the nuclei. The membrane in *slam*$^{waldo}$ $M^{-}Z^{-}$ still has not begun to invaginate. (D,H) End of cellularization, in wild type, the membrane has now reached its final basal position, past the nuclei, and has pinched off individual cells. In *slam*$^{waldo}$ $M^{-}Z^{-}$, the membrane has begun to invaginate, but it does not advance beyond the nuclei once it begins to pinch off. The nuclei are trapped in the pinching membrane. The appearance of two lines of basal Myosin staining is due to the section being slightly tangential, revealing apposing membranes. (I) *slam*$^{waldo}$ $M^{-/-}Z^{-/-}$ mutant. (J) $M^{-/-}Z^{-/+}$ *slam* cellularizes properly. (K) Germ cell migration is normal in $M^{-/-}Z^{-/+}$ *slam*, stage 14.

movements of gastrulation lead to the invagination of the midgut primordia and the mesoderm and to the extension of the germ band. $M^{-}Z^{-}$ *slam*$^{waldo}$ mutants do attempt to gastrulate, but the vigorous movements of gastrulation disrupt the incompletely formed somatic cells; the embryos look like they fall apart and fail to develop further.

This cellularization phenotype is only seen in $M^{-/-}Z^{-/-}$ *slam* mutants (Fig. 3I). A single zygotic copy of *slam* ($M^{-/-}Z^{+/-}$ *slam*) restores both cellularization and germ cell migration (Fig. 3J,K), and a single maternal copy ($M^{+/-}Z^{-/-}$ *slam*) rescues the cellularization defect, but not the germ cell migration defect (Fig. 1; data not shown). In an independent study, Lecuit and Wieschaus identified *slam* as the zygotic cellularization gene in the 26BF genomic interval (Lecuit et al., 2002). Their and our data suggest that the cellularization defect is the null phenotype because it is the phenotype of embryos that lack maternal and zygotic *slam*$^{waldo}$ alleles (this study), embryos deficient for the genomic region containing *slam* and embryos injected with *slam* RNAi (Lecuit et al., 2002). Our *slam*$^{waldo}$ mutants are presumably hypomorphic alleles, such that maternally provided *slam* activity is sufficient for cellularization and thereby reveal a second function for Slam in germ cell migration.

## *slam* is a novel gene

To identify the gene responsible for the *slam*$^{waldo}$ mutant phenotypes, we used male mitotic recombination (Chen et al., 1998) to map *slam* to a 100 kb genomic region between two P elements (k13720 at 26 C2-3 and l(2)k07502 at 26 D1-2). The Berkeley *Drosophila* Genome Project had identified the position of these P elements in the genome and predicted 16 genes in this interval (Fig. 4A). Two of these genes, *tiggrin* and CG13982, were removed in *Df(2L) tig*$^{X}$, a deficiency strain that complemented the *slam*$^{waldo}$ mutants; this eliminated them as candidates. An important clue for the molecular identification of the *slam* gene came from the observation that the $M^{-}Z^{-}$ *slam*$^{waldo}$ cellularization phenotype was rescued zygotically. As expression of the zygotic genome is barely initiated during the syncytial blastoderm stage, we reasoned that the *slam* gene had to be one of the earliest zygotically transcribed genes and that its expression pattern may resemble that of other zygotic genes required for cellularization such as *nullo*, *serendipity α* and *bottleneck* (Schejter et al., 1992). With this information in mind, we determined the RNA expression pattern of the remaining 14 candidate genes by in situ hybridization and found three candidates that were expressed during blastoderm formation (Pez, CPR and CG9506, Fig. 4, data not shown). The predicted protein coding regions of these three genes were sequenced from DNA isolated from homozygous mutant embryos for each of the *slam*$^{waldo}$ alleles. We found single base pair changes for both alleles in CG9506: Q722stop (*slam*$^{waldo1}$) and G879K (*slam*$^{waldo2}$) (Fig. 4B). Based on the correlation between the cellularization phenotype and the expression pattern, and the fact that the mutant alleles carry a nonsense and a missense mutation in the gene, respectively, we conclude that CG9506 is *slam*.

CG9506/*slam* encodes a protein of 1173 amino acids (Fig. 4B). Despite application of a large number of sequence analysis programs [BLAST (Altschul et al., 1997), PROSITE (Hofmann et al., 1999), PFAM (Bateman et al., 2002) and SMART (Schultz et al., 2000)], we were unable to find any

homology between Slam and any proteins or protein motifs in the existing databases, except for a short 30 amino acid coiled-coil region at residues 493-523, predicted by PFAM and SMART. Thus, at this point, the two *slam* alleles provide the only evidence regarding regions of the protein important for Slam function. The *slam*$^{waldo1}$ allele is predicted to produce a truncated protein, while the *slam*$^{waldo2}$ allele has a single amino acid glycine to lysine change in the region truncated by *slam*$^{waldo1}$ (Fig. 4B).

Detailed analysis of the RNA expression pattern during embryogenesis revealed low levels of uniformly distributed *slam* RNA, which is most likely of maternal origin (Fig. 4C). During nuclear cycle 10 we first detect an increase in *slam* RNA, presumably because of new zygotic transcription. During nuclear cycle 14 as cellularization begins, *slam* RNA is highly expressed and this expression is maintained throughout cellularization (Fig. 4D). *slam* RNA is localized basally within the cytoplasm surrounding each nucleus (data not shown). Slam expression is due to transcription from the embryonic genome as it is only observed in somatic nuclei but not in the germ cells, which are transcriptionally repressed during early embryogenesis (Seydoux and Dunn, 1997; Van Doren et al., 1998a; Zalokar, 1976) (Fig. 4D and insert). At the end of cellularization, *slam* RNA is limited to three stripes (Fig. 4E); the RNA levels then rapidly decline and *slam* RNA can no longer be detected after gastrulation (stage 8 onwards) (Fig. 4F).

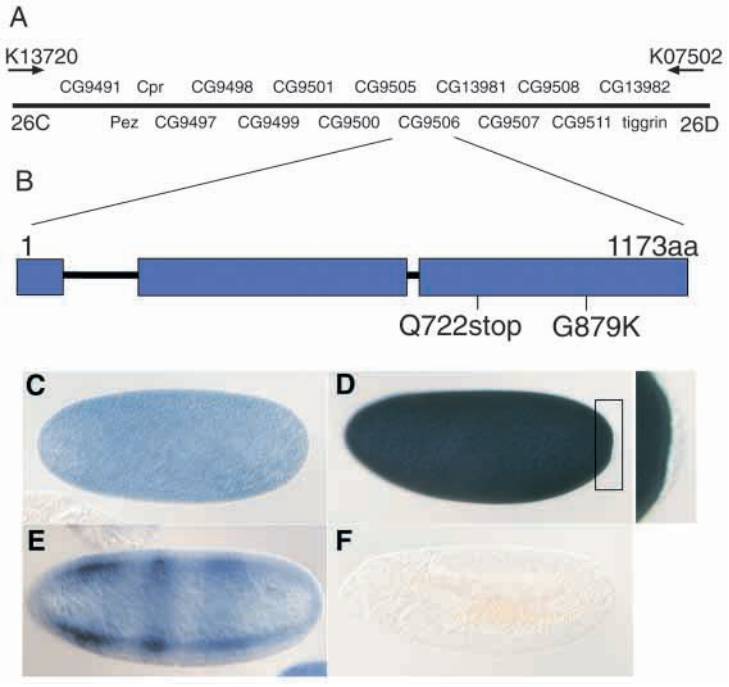


**Fig. 4.** *slam* is encoded by CG9506 and is expressed in blastoderm embryos. (A) Male mitotic recombination mapping placed *slam* between k13720 and k07502, a region with 16 predicted genes. (B) Genomic structure of CG9506. Boxes represent exons. Sequencing of the *slam*$^{waldo}$ alleles revealed a nonsense mutation at Q722 in *slam*$^{waldo1}$ and a glycine to lysine change at G879 in *slam*$^{waldo2}$. (C) *slam* RNA expression in a stage 2 embryo. There is no zygotic transcription at this stage, thus the weak expression in early embryos is maternal RNA contribution. (D) Stage 4. There are high amounts of *slam* in somatic nuclei, but not in germ cells, where zygotic transcription is repressed (inset). (E) Stage 6 embryo. By the end of cellularization, *slam* decays to three stripes in the blastoderm. (F) Stage 11 embryo. By the end of gastrulation, *slam* is no longer expressed.

## Slam is expressed in the basal-lateral membrane at cellular blastoderm

To analyze the time course and distribution of Slam protein, we raised an antibody against a peptide of the Slam protein. We used a peptide competition experiment to test the specificity of the Slam antibody. Pre-incubation of the Slam antibody with the Slam peptide blocks Slam staining in embryos (Fig. 5F). Pre-incubation with a different peptide from an unrelated protein (Nanos) (Fig. 5G) did not block Slam staining. This result shows that anti-Slam specifically recognizes the Slam protein.

Slam protein first can be detected at the plasma membrane of embryos at nuclear cycle 11, before cellularization begins, but after nuclei have migrated to the cortex (Fig. 5A). At this

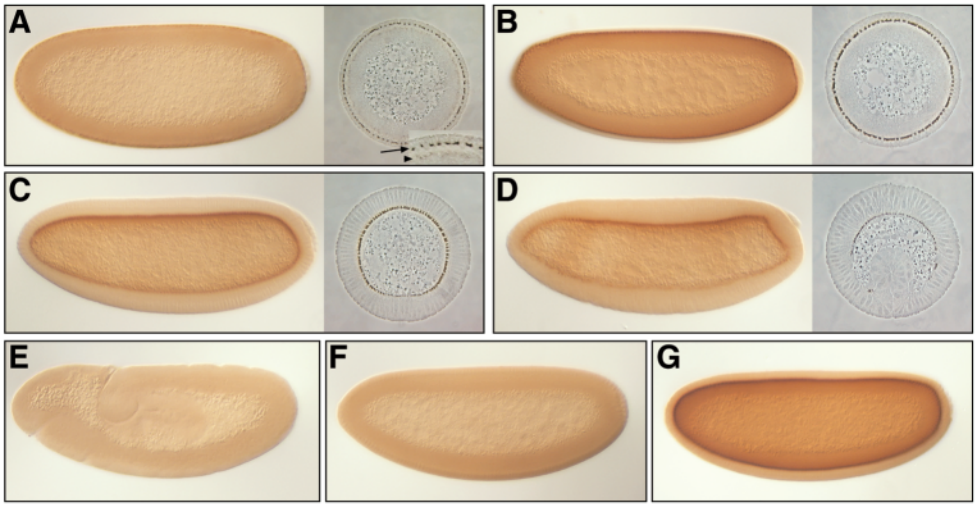


**Fig. 5.** Slam protein is expressed in the blastoderm embryo. Lateral views of embryos stained with α-Slam, with accompanying transverse 2 μm sections. Dorsal upwards, anterior towards the left in whole mounts. Dorsal upwards in sections. (A) Pre-cellularization (arrow marks Slam staining at membrane, arrowhead marks Slam staining basal to the nuclei). (B) Mid-cellularization. (C) End cellularization. Note decreased expression on ventral side of section. (D) Beginning of gastrulation. Invaginating mesoderm has lost Slam staining while Slam is still present in the dorsal epithelium of the section. (E) Stage 10, end of gastrulation. (F,G) Peptide competition experiment. Slam antibody was pre-incubated with the peptide used to generate α-Slam (F) or a control Nanos peptide (G) before incubation with wild-type embryos.

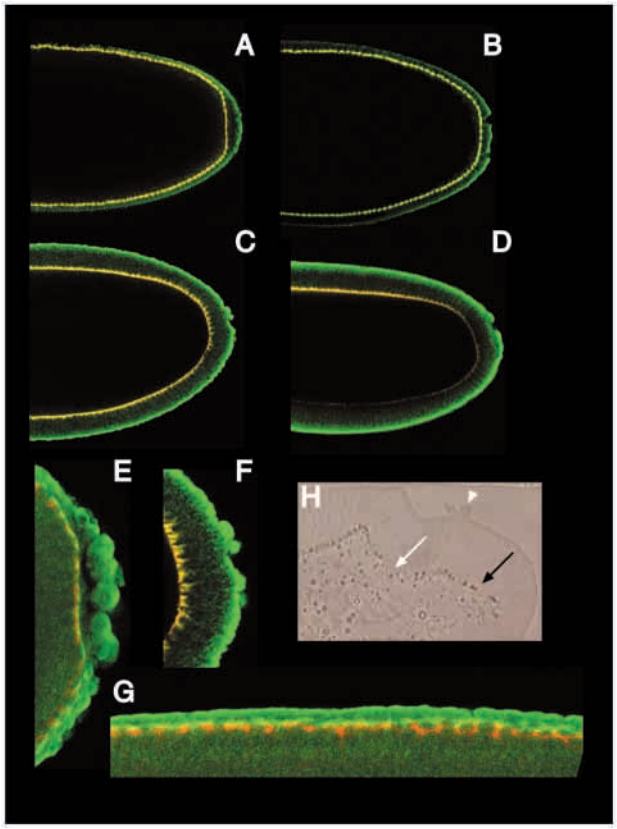


**Fig. 6.** Slam localizes to the furrow canals during cellularization. Embryos are stained in green with α-Neurotactin, which marks surfaces of membrane during cellularization. α-Slam is in red; overlap is yellow. (A-D) Slam localizes to the basal-lateral domain of the invaginating plasma membrane from early cellularization (A) to the end of cellularization (D). Note the decrease of Slam expression in the ventral part of the embryo at the end of cellularization (D). (E,F) Posterior pole during early (E) and late (F) cellularization. Slam is not expressed in the germ cells. (G) Enlarged double-labeling of Slam and Neurotactin reveals Slam (red) preceding Neurotactin (green) in growing membrane during early cellularization. (H) Section (2 μm) through gastrulating embryo. White arrow indicates lack of Slam in the farthest invaginated cells. Black arrow indicates presence of Slam in the basal membrane of cells that will become adjacent to migrating germ cells at stage 10-11. White arrowhead indicates germ cells.

stage, we observe Slam also in the cytoplasm basal to the peripheral nuclei; the position of this staining suggests that we are detecting Slam in the Golgi (Fig. 5A, arrowhead) (Sisson et al., 2000). During cellularization, Slam is localized to the most advancing membrane as it invaginates between each nucleus (Fig. 5A-C). Confocal microscopy of embryos doubly labeled for Slam protein and Neurotactin, which outlines the cell membranes, reveals that Slam is localized to the furrow canal, which marks the most basal part of the growing membrane (Fig. 6A-D,G). The germ cells do not contain any Slam protein (Fig. 6E,F).

Once the embryo begins to gastrulate, we no longer see Slam protein at the membrane of invaginating cells; however, it is maintained in the cell membranes of the rest of the epithelium (Fig. 5D, Fig. 6D,H white arrow versus black arrow). We analyzed Slam staining in thin sections, where localization to the basal-lateral membrane is clearly evident. Slam protein seems to be associated with the membrane. In thin sections of gastrulating embryos, we observe Slam protein first lost in the membranes of invaginating cells (Fig. 5D, Fig. 6H). Slam protein can no longer be detected in embryos by the end of gastrulation (Fig. 5E). The RNA and protein expression profile of *slam* correlated well with the *slam* cellularization phenotype. No RNA or protein was detected, however, during the second phase of Slam function, germ cell migration, suggesting that early Slam expression is not only necessary for cellularization but also later for germ cell migration.

## DISCUSSION

We have identified a novel gene, *slam*, which is required for the formation of the cellular blastoderm and for germ cell migration in a pathway independent of *Hmgcr*. Consistent with the cellularization phenotype, *slam* RNA is expressed at high levels in the early embryo just before cellularization and Slam protein localizes to the basal membrane of newly forming cells. Slam expression is transient and by the time of germ cell migration we can no longer detect *slam* RNA or protein. Our data suggest that signals required for the migration of germ cells during later stages of development are deposited into the cell membrane during blastoderm formation.

### *slam*, a new zygotic gene required for cellularization

We identified *slam* in a zygotic screen for germ cell migration defects and showed that lack of maternal and zygotic *slam* function causes a failure in blastoderm cellularization. *slam* was identified independently as one of a small number of genes with a zygotic cellularization phenotype (Lecuit et al., 2002; Merrill et al., 1988; Wieschaus and Sweeton, 1988). In a genome-wide screen using deletions of large genomic regions, seven regions of the genome, including the *slam* region, were identified as crucial for cellularization of early embryos (Merrill et al., 1988; Wieschaus and Sweeton, 1988). Other genes accounting for the cellularization phenotype in three of the genomic regions were identified as *nullo*, *serendipity α* and *bottleneck* (Schejter et al., 1992). These three genes share the following characteristics: first, they are expressed transiently at high levels only during blastoderm stage; second, they encode novel, small proteins, which share no homology to other genes in the *Drosophila* genome or in other genomes; third, the phenotype was only revealed in embryos deficient for that genomic region; there were no known point mutant alleles. Despite their dramatic effect on embryogenesis, these genes had escaped detection in the large-scale mutagenesis screens for zygotic embryonic lethal mutations carried out by Nüsslein-Volhard and Wieschaus (Jürgens et al., 1984; Nüsslein-Volhard et al., 1984; Wieschaus et al., 1984), presumably because of the difficulty in identifying point mutations in these genes.

Several findings identify *slam* as a fourth member of this group of cellularization genes. First, *slam* maps to region 26D, which when deleted causes a cellularization defect; second, *slam* is expressed transiently and at high levels at the cellular blastoderm stage; and third, *slam* encodes a novel protein. In addition, in a parallel study, Lecuit and Wieschaus showed that injection of *slam* RNAi produces a cellularization phenotype similar to that of a genomic deletion of the region (Lecuit et

al., 2002). However, in contrast to *nullo*, *serendipity α* and *bottleneck*, and the genomic deletion of the 26D region, our genetic analysis showed that the cellularization phenotype was produced only after removal of both maternal and zygotic *slam*. Embryos treated with *slam* RNAi or embryos missing large genomic regions containing *slam* fail to cellularize, and look like the maternal$^{-}$zygotic$^{-}$ *slam*$^{waldo}$ mutants. This suggests that our *slam*$^{waldo}$ alleles are hypomorphs, such that the maternal contribution is sufficient to rescue the cellularization defect of homozygous mutant *slam*$^{waldo}$ embryos but not that of embryos deleted for the genomic region that contains *slam*. Simultaneous removal of the maternal and zygotic *slam*$^{waldo}$ ($M^{-}Z^{-}$) contribution reduces gene activity below a threshold required for cellularization. While this scenario offers a plausible explanation for the *slam* phenotypes, it remains puzzling that *slam* is the only cellularization gene with point mutant alleles, and that these point mutations were identified on the basis of a germ cell migration phenotype and not by the cellularization phenotype.

### A role for blastoderm cellularization in germ cell migration

During cellularization the membrane surface of the embryo increases 25-fold. To account for this impressive membrane growth, it has been proposed that new synthesis of membrane and controlled vesicle fusion along the apical and lateral membrane contribute to most of the observed membrane growth. Gene products known to be associated with Golgi vesicles, such as Lava Lamp, or known components of the vesicle fusion machinery, such as Syntaxin, have been implicated in cellularization (Burgess et al., 1997; Sisson et al., 2000). We detect Slam protein in vesicles close to the membrane as well as in the membrane. Furthermore, detailed analysis of the *slam* cellularization defect observed after RNAi injection suggests that *slam* mutants are defective in directed transport of membrane vesicles (Lecuit et al., 2002).

In addition to its role in cellularization, *slam* is also required for germ cell migration. Our data strongly suggest that for germ cell migration, like for cellularization, Slam function is needed in the somatic cells and not in the germ cells. We detect *slam* RNA and protein only during blastoderm and the onset of gastrulation (stage 4-5, 2-3.5 hours after fertilization) but not later (stage 10-11, 5-7 hours after fertilization) during germ cell migration. We did not detect maternally loaded Slam protein in germ cells and zygotic expression of *slam* RNA was absent from germ cells. Furthermore, we found that the germ cell migration phenotype is only observed in homozygous mutant embryos and that a paternal *slam*$^{+}$ allele rescues both the cellularization and the germ cell migration defect. As germ cells are transcriptionally repressed until they initiate migration, the genetics of the *slam* phenotype and the *slam* RNA and protein expression pattern argue against a role of *slam* in germ cells.

*slam* RNA and protein expression profiles lead us to conclude that expression of Slam in the soma during blastoderm formation must be necessary later in development for germ cell migration. One possible explanation for the role of *slam* in germ cell migration could be that proper cellularization is required for germ cell migration and that zygotic *slam*$^{waldo}$ mutants have a subtle cellularization defect that later causes a germ cell migration defect. We did not detect any obvious cellularization defects in homozygous *slam*$^{waldo}$ mutant embryos (data not shown). Thus, as an alternative, we propose that *slam*-targeted membrane vesicles may not only accomplish rapid membrane growth but may also be used to insert a germ cell guidance signal into the growing membrane. This hypothetical guidance signal would be deposited before or during gastrulation, when Slam is present, but act after gastrulation when germ cells are migrating off the midgut (Fig. 7). Our mutant analysis suggests that Slam function in the midgut primordium is crucial for germ cell migration. We observed a delay in germ cells migrating from the midgut to the mesoderm and a delay in the epithelial to mesenchymal transition of the midgut primordium in mutant embryos. A possible model is that during blastoderm formation Slam is needed for the deposition of a germ cell guidance factor into the basal-lateral membrane. This factor could be present in all cells, or just in the posterior midgut. During gastrulation, the germ cells migrate through the posterior midgut primordium from the apical to the basal side (Fig. 7C). Once they reach the

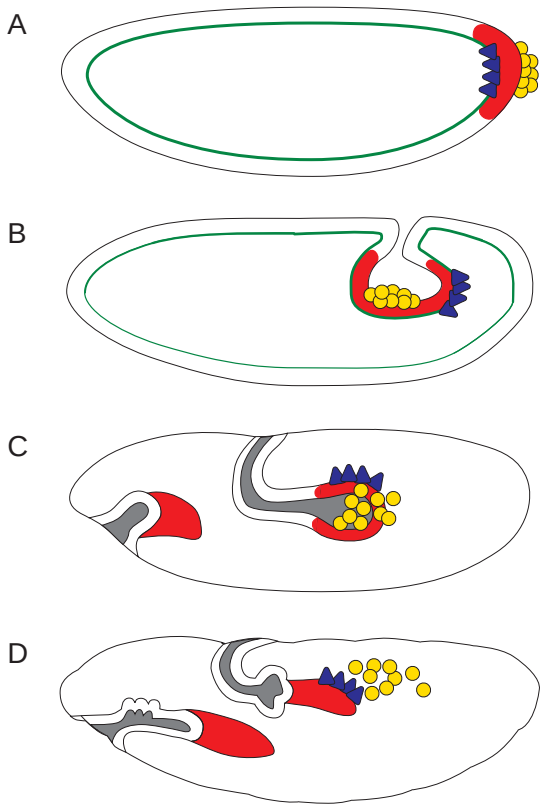


**Fig. 7.** Slam is hypothesized to deposit a germ cell guidance cue early that will act later in embryogenesis. Slam expression, green; posterior midgut primordium, red; germ cells, yellow; hypothesized guidance cue, blue triangles. (A) Blastoderm embryo, Slam is expressed on the basal-lateral membrane of all cells. A guidance cue (blue triangles) is expressed in the posterior midgut (if not in other cells as well), which is deposited on the basal-lateral membrane by Slam. (B) Gastrulating embryo, Slam expression begins to decrease. (C) Stage 9-10, germ cells are exiting the midgut pocket, moving from the apical to the basal side of the midgut cells. Slam protein is no longer present, but the guidance cue remains. (D) Stage 11, germ cells are guided off the midgut into the mesoderm by the guidance cue that requires Slam.

basal side of the epithelium, germ cells require this guidance factor to move quickly off the midgut and into the mesoderm (Fig. 7D). The nature of this guidance factor is unknown, it could be a factor very specific to germ cells or it could be a component of the basement membrane, which mediates cell migration in many systems. The guidance factor may be a germ cell repellant or a protein that promotes germ cell movement. It is likely to be independent of *Hmgcr* based on the *slam*$^{waldo}$;*Hmgcr* double mutant phenotype. It is also likely independent of *wun* and *wun 2*. The *wun*/*wun 2*-dependent germ cell reorientation on the midgut appears normal in *slam*$^{waldo}$ mutant embryos, suggesting that *slam*$^{waldo}$ mutations affect a later step in germ cell migration than *wun*/*wun 2*.

### A connection between blastoderm cellularization and morphogenesis

Blastoderm-specific genes, like *slam*, *nullo*, *bottleneck* and *serendipity-α* only affect formation of cells at the blastoderm stage. These genes are not required for germ cell formation, which happens at the same developmental stage, and are not expressed during cytokinesis at other stages of development. This suggests that blastoderm cellularization is a highly specialized form of cytokinesis. Blastoderm formation has been used as a model system to study the generation of polarized epithelia. In support of this idea, blastoderm cells form apical junctions and contain proteins in a polarized distribution typical for epithelial cells. However, it has been difficult to establish a functional link between the polarity established during the blastoderm stage and the polarity observed in epithelial cells at later stages of differentiation, because embryos that fail to form cells in the first place cannot be analyzed later. *slam*$^{waldo}$ mutations cause defects in germ cell migration and midgut morphology, but not in blastoderm cellularization. Nevertheless, the blastoderm-specific expression of Slam suggests that Slam function at this early stage is required for later development. Our findings, therefore, provide evidence that Slam may not only generate the cells from which the embryo develops but may also establish spatial cues within those cells needed for later morphogenesis.

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## REFERENCES

**Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D. J.** (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* **25**, 3389-3402.

**Bateman, A., Birney, E., Cerruti, L., Durbin, R., Etwiller, L., Eddy, S. R., Griffiths-Jones, S., Howe, K. L., Marshall, M. and Sonnhammer, E. L.** (2002). The Pfam protein families database. *Nucleic Acids Res.* **30**, 276-280.

**Boyle, M. and DiNardo, S.** (1995). Specification, migration, and assembly of the somatic cells of the *Drosophila* gonad. *Development* **121**, 1815-1825.

**Broihier, H. T.** (1998). A genetic analysis of germ cell migration. PhD thesis, Department of Biology, Cambridge, Massachusetts: Massachusetts Institute of Technology.

**Bunch, T. A., Graner, M. W., Fessler, L. I., Fessler, J. H., Schneider, K. D., Kerschen, A., Choy, L. P., Burgess, B. W. and Brower, D. L.** (1998). The PS2 integrin ligand *tiggrin* is required for proper muscle function in Drosophila. *Development* **125**, 1679-1689.

**Burgess, R. W., Deitcher, D. L. and Schwarz, T. L.** (1997). The synaptic protein Syntaxin1 is required for cellularization of Drosophila embryos. *J. Cell Biol.* **138**, 861-875.

**Chen, B., Chu, T., Harms, E., Gergen, J. P. and Strickland, S.** (1998). Mapping of Drosophila mutations using site-specific male recombination. *Genetics* **149**, 157-163.

**Eldon, E. D. and Pirotta, V.** (1991). Interactions of the Drosophila gap gene *giant* with maternal and zygotic pattern forming genes. *Development* **111**, 367-378.

**Ephrussi, A., Dickinson, L. K. and Lehmann, R.** (1991). *Oskar* organizes the germ plasm and directs localization of the posterior determinant *nanos*. *Cell* **66**, 37-50.

**Gavis, E. R. and Lehmann, R.** (1992). Localization of *nanos* RNA controls embryonic polarity. *Cell* **71**, 301-313.

**Hofmann, K., Bucher, P., Falquet, L. and Bairoch, A.** (1999). The PROSITE database, its status in 1999. *Nucleic Acids Res.* **27**, 215-219.

**Jürgens, G., Wieschaus, E., Nüsslein-Volhard, C. and Kluding, H.** (1984). Mutations affecting the pattern of the larval cuticle in Drosophila melanogaster. II. Zygotic loci on the third chromosome. *Roux Arch. Dev. Biol.* **193**, 283-295.

**Lecuit, T., Samanta, R. and Wieschaus, E.** (2002). *slam* encodes a developmental regulator of polarized membrane growth during cleavage of the Drosophila embryo. *Dev. Cell* **2**, 425-436.

**Lehmann, R. and Tautz, D.** (1994). In situ hybridization to RNA. *Methods Cell Biol.* **44**, 575-598.

**Martin-Bermudo, M. D., Alvarez-Garcia, I. and Brown, N. H.** (1999). Migration of the Drosophila primordial midgut cells requires coordination of diverse PS integrin functions. *Development* **126**, 5161-5169.

**Merrill, P., Sweeton, D. and Wieschaus, E.** (1988). Requirements for autosomal gene activity during precellular stages of Drosophila melanogaster. *Development* **104**, 495-509.

**Moore, L. A., Broihier, H. T., van Doren, M. and Lehmann, R.** (1998). Gonadal mesoderm and fat body initially follow a common developmental path in Drosophila. *Development* **125**, 837-844.

**Müller, H. A.** (2000). Genetic control of epithelial cell polarity: lessons from Drosophila. *Dev. Dyn.* **218**, 52-67.

**Nüsslein-Volhard, C., Wieschaus, E. and Kluding, H.** (1984). Mutations affecting the pattern of the larval cuticle in *Drosophila melanogaster* I. Zygotic loci on the second chromosome. *Roux's Arch. Dev. Biol.* **193**, 267-282.

**Schejter, E. D., Rose, L. S., Postner, M. A. and Wieschaus, E.** (1992). Role of the zygotic genome in the restructuring of the actin cytoskeleton at the cycle-14 transition during Drosophila embryogenesis. *Cold Spring Harb. Symp. Quant. Biol.* **57**, 653-659.

**Schultz, J., Copley, R. R., Doerks, T., Ponting, C. P. and Bork, P.** (2000). SMART: a web-based tool for the study of genetically mobile domains. *Nucleic Acids Res.* **28**, 231-234.

**Seydoux, G. and Fire, A.** (1994). Soma-germline asymmetry in the distributions of embryonic RNAs in *Caenhorhabditis elegans*. *Development* **120**, 2823-2834.

**Sisson, J. C., Field, C., Ventura, R., Royou, A. and Sullivan, W.** (2000). Lava lamp, a novel peripheral golgi protein, is required for Drosophila melanogaster cellularization. *J. Cell Biol.* **151**, 905-918.

**Starz-Gaiano, M., Cho, N. K., Forbes, A. and Lehmann, R.** (2001). Spatially restricted activity of a Drosophila lipid phosphatase guides migrating germ cells. *Development* **128**, 983-991.

**Starz-Gaiano, M. and Lehmann, R.** (2001). Moving towards the next generation. *Mech. Dev.* **105**, 5-18.

**Van Doren, M., Williamson, A. and Lehmann, R.** (1998a). Regulation of zygotic gene expression in *Drosophila* primordial germ cells. *Curr. Biol.* **8**, 243-246.

**Van Doren, M. B., Broihier, H. T., Moore, L. A. and Lehmann, R.** (1998b). HMG-CoA reductase guides migrating primordial germ cells. *Nature* **396**, 466-469.

**Wieschaus, E., Nüsslein-Volhard, C. and Jürgens, G.** (1984). Mutations

affecting the pattern of the larval cuticle in Drosophila melanogaster. III. Zygotic loci on the X-chromosome and fourth chromosome. *Roux's Arch. Dev. Biol.* **193**, 296-307.

**Wieschaus, E. and Sweeton, D.** (1988). Requirements for X-linked zygotic gene activity during cellularization of early Drosophila embryos. *Development* **104**, 483-493.

**Williamson, A. and Lehmann, R.** (1996). Germ cell development in *Drosophila*. *Annu. Rev. Cell Dev. Biol.* **12**, 365-391.

**Zalokar, M.** (1976). Autoradiographic study of protein and RNA formation during early development of Drosophila eggs. *Dev. Biol.* **49**, 425-437.

**Zhang, N., Zhang, J., Purcell, K. J., Cheng, Y. and Howard, K.** (1997). The *Drosophila* protein Wunen repels migrating germ cells. *Nature* **385**, 64-67.