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sqv mutants of *Caenorhabditis elegans* are defective in vulval epithelial invagination

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ABSTRACT **By screening for mutations that perturb the invagination of the vulva of the *Caenorhabditis elegans* hermaphrodite, we have isolated 25 mutations that define eight genes. We have named these genes *sqv-1* to *sqv-8* (squashed vulva). All 25 mutations cause the same vulval defect, an apparent partial collapse of the vulval invagination and an elongation of the central vulval cells. Most *sqv* mutations also cause an oocyte or somatic gonad defect that results in hermaphrodite sterility, and some *sqv* mutations cause maternal-effect lethality. We propose that the *sqv* genes affect a pathway common to vulval invagination, oocyte development, and embryogenesis.**

The movement and folding of epithelial cell layers are basic processes in morphogenesis (1). For example, amphibian gastrulation is initiated by the invagination of endodermal cells (2), neurulation involves the inward folding of ectodermal cells to form a tube that will become the spinal cord and brain (3), and the development of the vertebrate eye requires the optic vesicle to bend inward to form a cup that ultimately will become the retina (4).

There are several general models of how an epithelium might initiate inward folding or invagination (5–7). In models for sea urchin gastrulation (8), *Drosophila* gastrulation (9–12), and vertebrate neurulation (13), cells in the invaginating epithelium individually undergo cytoskeletal changes that result in constriction of their apical surface relative to their basal surface and consequent bending inward of the epithelium. Other models (14–16) propose that changes in cell-cell adhesion drive invagination. In the simplest such model (14), an increase in adhesiveness between cells in the invaginating epithelium favors an increase in the extent of contact between them and, consequently, an increase in their height. If the basal surfaces of the cells remain adherent to a substrate, causing the basal surface area to remain the same, this increase in cell height is accommodated by a decrease in apical surface area and consequent inward folding of the epithelium.

A third type of model, suggested for sea urchin gastrulation, proposes that changes in the extracellular matrix drive invagination (17). Cells that are to invaginate deposit a new hygroscopic layer of extracellular matrix between their apices and an older less hygroscopic matrix. The greater hydration of the new matrix layer causes it to swell and increase in surface area relative to the old matrix, driving the bilayer to bend inward and causing the underlying epithelial sheet to bend as well. Although each of these models of invagination is based on a single cellular mechanism, it is certainly possible that multiple mechanisms can be coordinated during invagination and that different examples of invagination involve different mechanisms to different extents.

Most analyses of epithelial invagination have been limited to manipulating epithelia *in vitro*, either mechanically or by the addition of chemical reagents, and to defining the expression patterns of molecules proposed to be involved. Over the past few years, the analysis of *Drosophila* mutants defective in gastrulation has identified an *in vivo* role for G-protein signaling (10, 11) and Rho-dependent cytoskeletal changes (12) in this process. To identify additional molecules involved *in vivo* in epithelial invagination, we have begun a genetic analysis of this process in the nematode *Caenorhabditis elegans*.

The *C. elegans* body is enclosed by a single layer of epithelial cells, which underlie a collagenous cuticle (18). During the third (L3) and fourth (L4) larval stages, the descendants of the vulval precursors P5.p, P6.p, and P7.p, a specialized set of outer epithelial cells, invaginate and create a tube that connects the outer epithelium to the layer of epithelial cells that enclose the uterus (19). This vulval tube allows the adult hermaphrodite both to lay eggs and to receive sperm from males. The intercellular signaling pathways that direct P6.p to undergo a so-called primary pattern of cell division and P5.p and P7.p to undergo secondary patterns of division have been studied extensively (20, 21). During the final round of vulval cell divisions, the primary descendants and some secondary descendants detach from the cuticle, allowing the vulval sheet to bend inward and the cells within it to rearrange their cell-cell contacts. Because vulval invagination can occur in the absence of most other nearby cells, including the vulval muscles (T.H., unpublished observations) and the somatic gonad (22), it is likely that the mechanical force required is intrinsic to the epithelium or its extracellular matrix, consistent with models for other invaginations (see above), although the primary descendants must be in contact with a cell in the gonad, the anchor cell, for the invagination to have the correct shape and to attach to the uterus (22–24). In this paper we describe the results of a screen for mutations that affect vulval invagination. The molecular characterization of three of the genes defined by these mutations is described in ref. 25.

MATERIALS AND METHODS

Genetics. Strains were cultured as described (26) and, unless indicated otherwise, were grown at 20°C. Wild type refers to the N2 strain. Most mutations and chromosomal rearrangements mentioned are described in refs. 27 or 28. Exceptions are *let-253(n2412)* (M. Labouesse, personal communication), *lin-12(n302 n865)* (29), and *nDf40* (30).

Mutagenesis with ethyl methanesulfonate, genetic mapping, and complementation tests (all *sqv* mutations appeared to be completely recessive; data not shown) were performed by standard methods (26, 31). Alleles of *sqv-3* failed to complement the deficiency *nDf40*, alleles of *sqv-7* failed to complement the deficiency *mnDf30*, and alleles of *sqv-8* failed to complement the deficiency *mnDf29*.

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Abbreviations: sqv, squashed vulva; DIC, differential interference contrast.

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Phenotypic Characterization. Electron microscopy of individual animals was performed as described (32), except that sections were cut laterally (from left to right or from right to left) rather than from nose to tip.

Wild-type and mutant larvae were stained with MH27 antibodies (33) by the method described in ref. 34, and the immunofluorescence was examined with a Bio-Rad MRC-500 confocal microscope.

sqv-1(n2849), *sqv-2(n3027)*, *sqv-3(n2841)*, *sqv-4(n2840)*, *sqv-5(n3039)*, *sqv-6(n2845)*, *sqv-7(n2844)*, and *sqv-8(n2822)* mutants were examined by Nomarski differential interference contrast (DIC) microscopy at the L4 stage for the presence of HSN neurons and at the adult stage for vulval muscle contractions; for each of these mutants, HSNs and vulval contractions were observed, although the observed contractions did not result in the release of eggs.

Artificial insemination was performed by the method described in ref. 35.

Unlike *sqv-1* to *sqv-7* hermaphrodites, *sqv-8* hermaphrodites can produce viable progeny when mated with or artificially inseminated with sperm from wild-type males (data not shown; ref. 36). To test whether *sqv-8(n2822)* animals exhibited a paternal effect like that reported for *sqv-8(mn63)* animals (36), we mated N2 males with *sqv-8(n2822)* hermaphrodites, mated the resulting male cross-progeny with *sqv-8(n2822)* hermaphrodites, and examined whether any progeny from the latter were Sqv. Because none were Sqv, we retested the *sqv-8(mn63)* allele in the same way. We were unable to reproduce the finding reported in ref. 36. We did notice that *sqv-8* hermaphrodites mated with wild-type males produced a small number (<10%) of progeny that were dumpy, often twisted or rolling, and often bulging at the tail or midbody (data not shown); some did not reach adulthood. We therefore suggest that *sqv-8* causes a maternal-effect lethality that can be rescued (sometimes only partially) by providing a wild-type *sqv-8* gene in the zygote.

RESULTS

Isolation of Mutations that Perturb Vulval Invagination. To identify genes involved in vulval invagination, we undertook a genetic screen. We mutagenized the wild-type strain N2, transferred F_1 progeny to individual plates, and, using a dissecting microscope, examined the F_2 broods for animals with vulval defects. With this scheme, mutations that additionally might cause recessive sterility or maternal-effect lethality could be recovered in heterozygous siblings. F_2 hermaphrodites were examined at the mid-L4 stage, after the vulval cells normally have invaginated and during a period when the space between the invaginating vulval cells and the cuticle is largest (see Fig. 2*E*). We sought animals in which this space was absent or abnormal, indicating that the vulval cells had not invaginated or had invaginated incorrectly. Although animals with abnormal vulval cell lineages can have multiple, misshapen, or absent invaginations (37), we were interested in identifying genes specifically required for the invagination process itself and therefore demanded that candidate mutants possess the wild-type number of vulval nuclei. We later confirmed that representative mutations did not affect the pattern of cell divisions that generates the vulva (see below).

In this way we examined just over 12,000 independently mutagenized haploid genomes for mutations with recessive effects and just under 13,000 mutagenized haploid genomes for mutations with dominant effects and isolated 25 mutations that recessively perturb vulval invagination.

The 25 Mutations Isolated Correspond to Eight Genes, *sqv-1* to *sqv-8*. The 25 mutations define eight complementation groups, of which seven appear to be new (Fig. 1). We have named the latter genes *sqv-1* to *sqv-7* (squashed vulva) because of the appearance of their mutant vulval phenotype. Mutations in the eighth complementation group failed to complement a previously identified mutation, *spe-2(mn63)* (36), which we found also causes a defect in vulval invagination. Although *spe-2(mn63)* hermaphrodites previously were reported to have a sperm defect (36), we were unable to identify such a defect either with the *mn63* allele or a new allele, *n2822* (see *Materials and Methods*), and additionally found that sperm from an *n2822* homozygous hermaphrodite can produce viable cross-progeny (see below). With the permission of R. Herman (personal communication) we therefore have renamed this gene *sqv-8*.

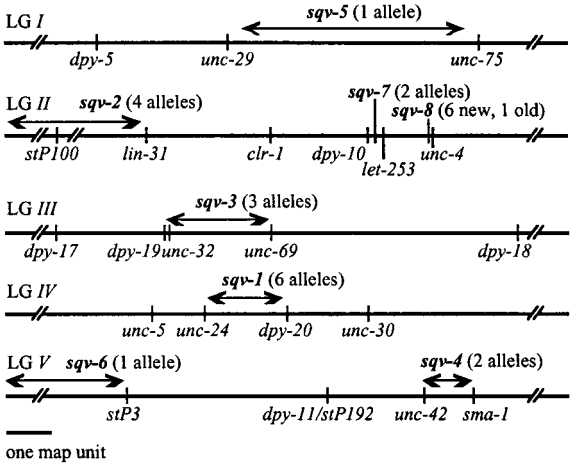

FIG. 1. Locations of *sqv-1* to *sqv-8* on the genetic map. The positions of *sqv-1* to *sqv-7* are based on the three-factor data in Table 1 and on linkage analysis. The position of *sqv-8* was determined by Sigurdson *et al.* (36), who identified its first mutant allele (the "1 old" allele). A list of alleles is provided in Table 2.

Because the *sqv* mutations were not rare, the phenotypes they cause are recessive to wild type, and the vulval phenotypes caused by those mutations tested (all alleles of *sqv-3*, *sqv-7*, and *sqv-8*) are similar whether the mutations are homozygous or hemizygous, it is likely that these mutations cause a loss of gene function.

Mutations in *sqv-1* to *sqv-8* Cause a Partial Collapse of the Vulval Invagination and Affect Both Primary and Secondary Vulval Cell Descendants. All 25 newly isolated mutations and *mn63* appear to cause identical vulval phenotypes, as judged by Nomarski DIC microscopy. Before the final round of vulval cell divisions, both wild-type and *sqv* mutant vulval cells lie along the ventral cuticle in a plane with the surrounding outer epithelium, hyp7 (Fig. 2 *A* and *B*). In the wild type, during the final round of vulval cell divisions, a defined subset of the vulval cells detaches from the cuticle and begins to invaginate, leaving the plane of the surrounding epithelium and creating a space between the apices of these vulval cells and the cuticle (Fig. 2*C*). In the *sqv* mutants, however, although the appropriate cells appear to detach from the cuticle, the resulting invagination space is considerably reduced in size (Fig. 2*D*). This abnormality persists, and later the height of the mutant vulval invagination appears to be slightly decreased relative to that of the wild type, suggesting that the mutant invagination may be partially collapsed (Fig. 2 *E* and *F*). The vulval phenotype caused by homozygous or hemizygous alleles of *sqv-3*, *sqv-7*, and *sqv-8* (the only mutations tested as hemizygous) appeared to be identical and hence may correspond to the null phenotype of these genes. Three mutations, *sqv-2(n2821)*, *sqv-2(n2826)*, and *sqv-7(n2839)*, cause a slightly weaker vulval defect than that depicted in Fig. 2 (i.e., a smaller reduction in the size of the invagination space) and therefore may cause only a partial loss of gene function.

To confirm that the Sqv vulval phenotype was not the result of abnormal vulval cell lineages, we directly observed the pattern of vulval cell divisions in at least one hermaphrodite of

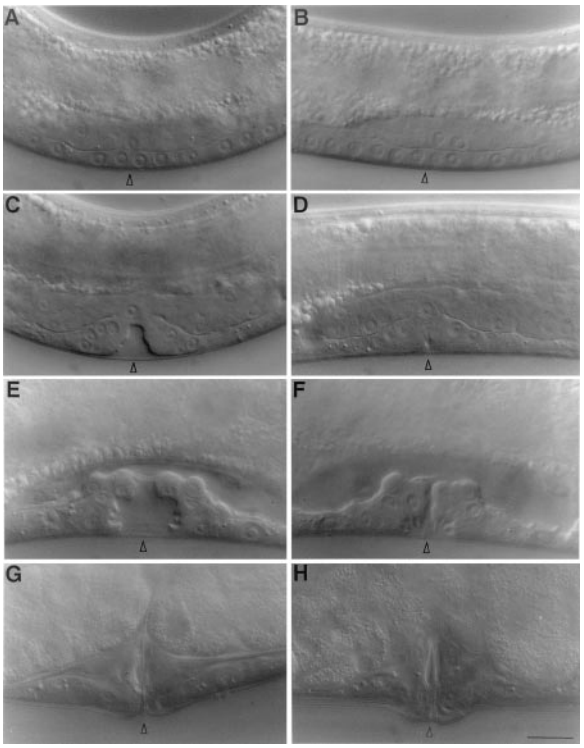

FIG. 2. The *sqv* mutations result in a partially collapsed vulval invagination. Nomarski micrographs of wild-type (*A*, *C*, *E*, and *G*) and *sqv-3(n2841)* (*B*, *D*, *F*, and *H*) vulvae before, during, and after vulval invagination. (*A* and *B*) Late L3 stage. In both wild-type and *sqv* mutant vulvae the vulval precursors P5.p, P6.p, and P7.p all have given rise to four cells, which lie in a plane with the surrounding epithelium, hyp7. The arrowhead points to the center of the vulva, between cells P6.pap and P6.ppa. In the wild type, the gonadal anchor cell eventually takes a position directly above and between these cells, as seen in *A*. The anchor cell in the *sqv-3(n2841)* animal in *B* has not yet taken this position and lies slightly posterior. The animal in *B* is also homozygous for *unc-69(e587*am*)*. (*C* and *D*) Early L4 stage. Vulval cell division is complete. In both the wild type and the *sqv* mutant the appropriate cells have detached from the cuticle and left the plane of the surrounding epithelium, but the space between the vulval cells and cuticle is considerably smaller in the *sqv* mutant than in the wild type. Again, the arrowhead points the center of the vulva. (*E* and *F*) Mid-L4 stage. The *sqv* mutant invagination appears to extend less dorsally than does the wild type, suggesting that the former is partially collapsed and that the space between the vulval cells and cuticle is reduced at least in part as a consequence. (*G* and *H*) Adult. The wild-type and *sqv* mutant vulvae do not obviously differ in appearance, although in later adulthood, when *sqv* mutants become bloated with eggs, their vulvae often protrude abnormally. The bar at the lower right represents 10 μm, and the animals are oriented such that anterior is to the left, and ventral is down.

each of the following genotypes: *sqv-1(n2819)*, *sqv-2(n2826)*, *sqv-3(n2841) unc-69(e587)*, *sqv-4(n2840)*, *sqv-5(n3039) unc-75(e950)*, *sqv-6(n2845)*, *sqv-7(n2844) unc-4(e120)*, and *sqv-8(mn63) unc-4(e120)*. In every case this pattern was wild type (data not shown). In addition, we have observed that these and the remaining 18 *sqv* mutations do not appear to affect the number and gross arrangement of the vulval nuclei at the mid- to late-L4 stage (data not shown).

To determine which invaginating vulval cells are affected by the Sqv mutant phenotype, we took advantage of the cell lineage transformations caused by loss-of-function and gain-of-function mutations in the gene *lin-12*. In *lin-12(n302 n865)* animals no vulval precursor cells undergo a secondary pattern of divisions and instead P5.p, P6.p, and, often, P7.p undergo primary patterns of divisions, whereas in *lin-12(n137)* animals no vulval precursor cells undergo a primary pattern of divisions, and instead P3.p to P8.p undergo secondary patterns of divisions (29). We found that when animals of either *lin-12* genotype were also homozygous for a *sqv* mutation [*sqv-3(n2841)*] their vulval invagination spaces were severely reduced in size, indicating that invaginating vulval cells derived from both the primary and secondary lineages are affected by the Sqv mutant phenotype. It remains possible, however, that only particular descendants from each type of lineage are affected.

The Vulval Invagination Space Is More Electron Dense and the Central Invaginating Vulval Cells Are More Elongated in a *sqv* Mutant Than in the Wild Type. To examine directly the invaginating vulval cells at the early L4 stage, when the difference between wild-type and *sqv* mutant vulvae is first detectable by Nomarski DIC microscopy, we prepared serial sections of N2 and *sqv-3(n2842)* mutant hermaphrodites for electron microscopy. *sqv-3(n2842)* appears to cause a vulval defect identical to that of all other *sqv* mutants except *sqv-2(n2821)*, *sqv-2(n2826)*, and *sqv-7(n2839)* (see above) and was chosen arbitrarily. At this stage, the central wild-type vulval cells have detached from the cuticle and surround an invagination space of minimal electron density (Fig. 3*A*). The central vulval cells of the *sqv-3(n2842)* mutant also were detached from the cuticle but surrounded an invagination space that is not only considerably reduced in size but also more electron dense than that of the wild type (Fig. 3*B*), a difference that was observed in several N2 and *sqv-3(n2842)* animals. The increased electron density may reflect a qualitative difference in the composition of the mutant extracellular space or simply may be the result of concentrating wild-type material into a smaller volume.

To compare the arrangement and shapes of the wild-type and *sqv-3* vulval cells, we traced their plasma and nuclear membranes as well as the cuticle in electron micrographs of our serial sections; four such tracings are shown in Fig. 3 *C–F*. Cell identifications were made on the basis of the known arrangement of vulval nuclei

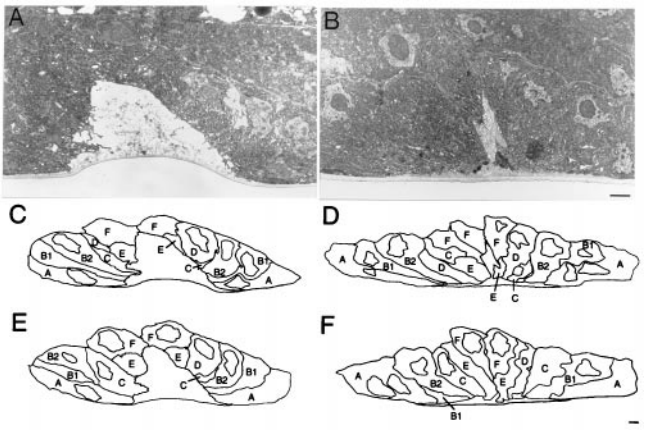

FIG. 3. The vulval invagination space is more electron dense, and the central vulval cells are more elongated in a *sqv* mutant than in the wild type. Electron micrographs of a wild-type (*A*) and a *sqv-3(n2842)* mutant (*B*) early L4 vulva in the plane at which the invagination space is largest. In each case the invagination space is the region of decreased electron density, and the cuticle is the ventral-most narrow gray band. Sections were cut so that each was parallel to the plane of the Nomarski micrographs in Fig. 2. The appropriate *sqv-3(n2842)* vulval cells have detached from the cuticle, but the resulting invagination space is reduced in size and more electron dense than that of the wild type. Tracings of vulval plasma and nuclear membranes and the cuticle from electron micrographs of a wild-type animal (*C* and *E*) and a *sqv-3* mutant animal (*D* and *F*). Cell identifications were made on the basis of the known arrangement of vulval nuclei as judged from Nomarski DIC microscopy, and each cell is labeled with the letter name of the corresponding late L4 toroid (42). Tracings *C* and *D* are of the micrographs partly shown in *A* and *B*, and tracings *E* and *F* are of sections located roughly the same lateral distance from those traced in *A* and *B*, respectively. The central *sqv-3* vulval cells are abnormally elongated, extending into and reducing the size of the invagination space. The bars at the lower right of *B* and *F* represent 1 μm.

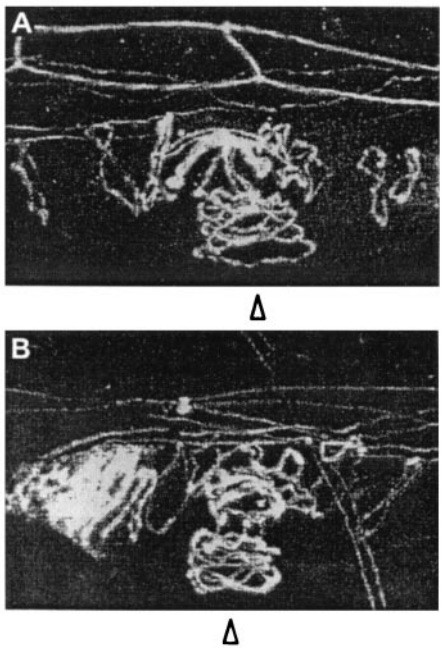

FIG. 4. *sqv* mutant vulval cells can form toroids. Confocal images of wild-type (*A*) and *sqv-3(n2841)* mutant (*B*) mid-L4 stage vulvae stained with MH27 antibodies and fluorescein isothiocyanate-conjugated secondary antibodies. (*A*) The MH27 antibody stains the rings of contact between wild-type vulval toroids. (*B*) The MH27 antibody also stains the *sqv-3(n2841)* vulva in a pattern of stacked rings, indicating that, as in the wild type, the invaginating *sqv-3* vulval cells can form toroids, although we did not ascertain whether their number and connection to the uterus were precisely correct. Arrowheads indicate the axes about which the vulval rings are arranged. The *sqv-3* animal shown is somewhat twisted back on itself, causing the bright gonadal staining to the left of the vulva to appear closer and therefore be visible in *B* and also accounting for the two almost vertical bands of staining not present in *A*.

as judged by Nomarski DIC microscopy, and it was possible in this way to assign cell identities in the *sqv-3* vulva consistent with its having a grossly normal arrangement of cells. However, a comparison of roughly equivalent wild-type and *sqv-3* sections shows that the central mutant vulval cells (in particular, cells F, E, and D) are abnormally elongated, extending into and reducing the size of the invagination space. This general difference also was observed in other N2 and *sqv-3(n2842)* animals examined, although in these cases we did not follow the three-dimensional shapes of the cells through electron microscopy sections.

The *sqv* Mutant Vulval Cells Form a Partially Functional Adult Vulval Tube. As wild-type vulval cells invaginate they reorganize their cell-cell contacts around the invagination space and fuse in a specific pattern that results in a stack of seven toroidal cells (34, 38, 39). The dorsal-most toroid is attached to the uterine epithelium, the ventral-most toroid remains attached to the outer epithelium, and the central hole of the stack becomes the vulval tube of the adult. We stained mid-to late-L4 stage wild-type and mutant animals with a mAb, MH27, that recognizes the desmosomal connections between epithelial cells in *C. elegans* (33): in the wild type, this antibody decorates each ring of contact between adjacent vulval toroids, as well as the vulval attachments to the uterus and outer epithelium and the connections between epithelial cells in the uterus (Fig. 4*A*). The MH27 antibody also recognizes a pattern of rings in *sqv-3(n2841)* animals (Fig. 4*B*), indicating that despite their abnormal invagination, the *sqv* vulval cells form toroids.

By the adult stage, the wild-type and *sqv* mutant vulvae appeared similar by Nomarski DIC microscopy (Fig. 2 *G* and *H*). However, although adult Sqv hermaphrodites occasionally laid some eggs, and *sqv-8* hermaphrodites could receive sperm from mating males (see *Materials and Methods*), mutant alleles of most *sqv* genes caused a defect in laying eggs (Table 1), resulting in older adult animals that were visibly bloated with unlaid eggs and often had protruding vulvae. Because the other cells required for egg laying, the HSN neurons and vulval muscles, appeared to be present and functional in these mutants (see *Materials and Methods*), their egg-laying defect is likely to be the result of the *sqv* vulval defect. Although *sqv-5* hermaphrodites did not accumulate unlaid eggs in their uteri, they also laid few, if any, eggs, suggesting that they simply produced few oocytes (see below).

The *sqv* Mutations Cause Hermaphrodite Sterility, and At Least Some *sqv* Genes Are Required for Embryogenesis. Nearly all the mutations we isolated, including at least one allele each of *sqv-1* to *sqv-8*, caused a severe reduction in hermaphrodite fertility (Table 2). The three mutations that had a weak or no effect on brood size are those that also caused weaker vulval defects (see above). We determined the stages at which progeny of mutant hermaphrodites arrest and found that most *sqv-1* to *sqv-8* mutants produced at least some eggs that failed to undergo cytokinesis. We did not determine whether these one-cell eggs underwent fertilization. They may have been unfertilized, because, like unfertilized oocytes from *spe-1(mn47) unc-4(e120)* hermaphrodites, they were dissolved by bleach (41), but they appeared to retain their shapes better than *spe-1* oocytes and so may have a partial eggshell. In artificial insemination experiments we found that sperm from *sqv-1(n2819)*, *sqv-2(n3038)*, *sqv-3(n2842)*, *sqv-4(n2840)*, *sqv-5(n3039)*, *sqv-6(n2845)*, *sqv-7(n2844)*, and *sqv-8(n2822)* hermaphrodites were able to produce adult cross-progeny when injected into *eT1* hermaphrodites, indicating that the sterility of *sqv-1* to *sqv-8* hermaphrodites was caused by a defect in the mutant oocytes or somatic gonad. In addition, when the uteri of *sqv-1* to *sqv-8* mutants were dissected to assay egg-laying ability (see Table 1), a number of small oocyte-like cells were released along with oocytes/eggs of normal size, indicating that these mutants may have a defect in oocyte formation.

Some *sqv* mutants additionally produced eggs that arrested during embryogenesis, suggesting that the corresponding genes are required for embryonic development (Table 2). *sqv-8* mutants in particular produced a large proportion of eggs that progressed beyond the one-cell stage before arresting. Although *sqv-8(n2822)* behaves genetically like a strong loss of *sqv-8* function (Table 3), it remains possible that stronger losses of *sqv-8* gene function might result in a fertility defect that resembles that of *sqv-1* to *sqv-7* mutants.

Table 1. *sqv* hermaphrodites retain eggs

Genotype	Average number of eggs in uterus	Range
N2	18.7	10–25
sqv-1(n2849)	35.1	25–45
sqv-2(n3037)	29.1	12–63
sqv-3(n2841)	31.3	10–59
sqv-4(n2840)	25.0	14–47
sqv-5(n3039)	3.2	1–5
sqv-6(n2845)	32.1	17–71
sqv-7(n2844)	34.7	21–87
sqv-8(n2822)	44.0	26–63

Fifteen mid-L4 stage hermaphrodites of each genotype were aged for 41 hr and individually dissected to release eggs from the uterus. Uteri of all mutant genotypes often contained oocyte-like cells less than half the size of normal oocytes; these cells were not counted. *sqv-5* animals produced few oocytes. At least one allele of *sqv-1, n2819*, also may reduce the number of oocytes produced, because homozygotes did not retain a significant number of eggs in this assay but also laid few, if any, eggs. The remaining *sqv-1* alleles were not tested in this assay.

Table 2. Most *sqv* mutations reduce fertility

Genotype	Average blood size	Arrested progeny			Hatched larvae	
		One-cell	Bean/comma	Two-/three-fold	Misshapen	Normal
N2 (wild type)	243	0	0	0	0	46
sqv-1						
n2819	0	34	0	0	0	0
n2824	0	42	0	0	0	0
n2828	0	38	0	0	0	0
n2848	0	25	1	0	0	0
n2849	0	30	2	0	0	0
n2820	8	18	3	0	1	21
sqv-2						
n3037	0.7	38	8	6	1	3
n3038	0.8	30	14	1	0	1
n2826	68	nd	nd	nd	nd	nd
n2821	246	nd	nd	nd	nd	nd
sqv-3						
n2823	0	40	0	0	0	0
n2841	0	33	0	0	0	0
n2842	0	45	0	2	0	0
sqv-4						
n2827	0	31	0	0	0	0
n2840	0	41	2	0	0	0
sqv-5						
n3039	0	31	0	0	0	0
sqv-6						
n2845	0	35	0	0	0	0
sqv-7						
n2844	1	36	16	1	0	0
n2839	101	nd	nd	nd	nd	nd
sqv-8						
n2822	0	4	34	0	0	0
n2850	0	6	38	1	0	0
n2847	0	5	32	5	0	0
n2851	0	5	30	2	0	0
n2843	0.05	5	35	2	0	0
mn63	0	1	12	18	2	0
n2825	0.6	6	12	12	6	9

Average brood size was calculated by counting the total number of hatched progeny of five (in the case of N2, *sqv-2*(*n2826*), *sqv-2*(*n2821*), and *sqv-7*(*n2839*)) or 100 (all other genotypes) hermaphrodites and dividing this by five or 100, respectively. In a second experiment, embryos were dissected from homozygous adults, aged for approximately 12–18 hr, and the number at each stage of development estimated. These latter numbers were not determined (nd) for the three alleles that had substantial brood sizes. The estimation of developmental stage was complicated by abnormalities in the shape of arrested embryos, but the groupings used (one-cell, bean/comma, two-/three-fold, hatched larvae; ref. 40) should have been sufficiently distinct for accuracy.

DISCUSSION

We have isolated 25 mutations that result in a partial collapse of the vulval invagination and elongation of the central invaginating cells. These mutations appear to result in a loss of gene function and define eight genes, which we have named *sqv-1* to *sqv-8*. These mutations do not prevent the formation of vulval toroids and a partially functional adult vulva, indicating that there must be other as yet unidentified genes involved in this process.

Table 3. *sqv-8*(*n2822*) is a strong loss-of-function allele

Parental genotype	Arrested progeny		
	One-cell	Bean/comma	Two-/three-fold
sqv-8(n2822)	6	53	0
sqv-8(n2822)/mnDf29	4	49	0
sqv-8(mn63) unc-4(el20)	0	12	46
sqv-8(mn63) unc-4(el20)/sqv-8(n2822)	4	44	8
sqv-8(mn63) unc-4(el20)/mnDf29	5	37	5

Embryos were dissected from homozygotes 18 hr after the mid-L4 stage, aged for 12 hr, and the stage of developmental arrest estimated. No progeny hatched.

Any of several simple models could explain the *sqv* mutant phenotype. In all of these models the *sqv* genes are required to execute invagination efficiently, while other genes cause the changes in cell-cell contacts and the cell fusions required for the formation of vulval toroids.

One possibility is that the loss of the *sqv* genes decreases the rigidity of the vulval epithelium, reducing its ability to support itself over a large invagination space and causing it instead to form a collapsed invagination over a smaller space. Such a decrease in rigidity could result from a defect within the vulval cells themselves, for instance in the cytoskeleton or in the strength of the adhesion among these cells, or from a defect in the rigidity of the extracellular matrix adjacent to the apices of the vulval cells and lining the invagination space. The plausibility of such a model is supported by the observation that when the mid-L4 vulval epithelium is artificially subjected to increased outward pressure, it can collapse and resemble an L4 Sqv vulva: such a collapse has been observed when the cuticle adjacent to the mid-L4 invagination space is punctured (P. Sternberg, personal communication) and presumably occurs because the vulval epithelium is not sufficiently rigid to support the internal hydrostatic pressure of the worm in the absence of the cuticle. This observation also would be consistent with a

model in which a leaky cuticle might cause the Sqv vulval defect; however, the cuticle of *sqv-3(n2842)* hermaphrodites appeared normal based on electron microscopy.

A second possibility is that the expansion of the invagination space, presumably by the accumulation of water, is an active process that requires *sqv* gene function.

A third possibility is that the Sqv vulval phenotype results from an inappropriate or increased adhesion between the apices of the vulval cells and abnormal material deposited in the mutant invagination space [we observed that the region between the *sqv-3(n2842)* vulval apices and cuticle was abnormally electron dense)]. If such material were not expandable and acted as a glue between the cuticle and the vulval cell apices, then as the basal side of the vulval epithelium bends away from the cuticle the central vulval cells would become stretched, resulting in the Sqv phenotype.

A fourth possibility is that the *sqv* phenotype results from an abnormally increased adhesiveness among the mutant vulval cells themselves. Such an increase would favor an increase in the extent of vulval cell-cell contact and therefore an elongation of the vulval cells, as is seen in the *sqv* mutants. Because, as proposed in ref. 14, such an increase in adhesiveness could drive invagination, it is also possible that this increase normally might occur during wild-type vulval invagination and simply be abnormally high in the *sqv* mutants.

In addition to causing a defect in vulval invagination, loss of *sqv* gene function results in a severe reduction in hermaphrodite fertility. *sqv-1* to *sqv-8* mutants appeared to produce some abnormally small oocytes and at least some eggs that fail to undergo cytokinesis and may not be fertilized, suggesting that the *sqv* genes may be required for aspects of oocyte development, function, and/or fertilization. In addition, *sqv-1*, *sqv-2*, *sqv-3*, *sqv-4*, *sqv-7*, and *sqv-8* mutants produced eggs that arrested during embryogenesis, suggesting that these genes are required for embryonic development (it also might be possible to isolate *sqv-5* and *sqv-6* alleles that cause this phenotype). Such arrested animals often were abnormally shaped: in particular, *sqv-8* embryos with well-developed pharynges often were less elongated than and lumpy in comparison with wild-type embryos at a similar stage of pharyngeal development. Hatched embryos of several *sqv* genotypes also were observed to be lumpy and poorly elongated (the "misshapen" embryos in Table 2). Finally, some *sqv-8*/+ progeny of *sqv-8* hermaphrodites were dumpy, often twisted or rolling, and often bulging at the tail or midbody (see *Materials and Methods*). Because a normal, elongated body shape is derived in part from cell shape changes in the outer epithelium during embryogenesis, we suggest that at least some of the *sqv* genes may affect other aspects of epithelial morphogenesis in addition to vulval invagination.

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