gld-1, a Tumor Suppressor Gene Required for Oocyte Development in *Caenorhabditis elegans*

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ABSTRACT

We have characterized 31 mutations in the *gld-1* (defective in germ*l*ine *d*evelopment) gene of *Caenorhabditis elegans*. In *gld-1(null)* hermaphrodites, oogenesis is abolished and a germline tumor forms where oocyte development would normally occur. By contrast, *gld-1(null)* males are unaffected. The hermaphrodite germline tumor appears to derive from germ cells that enter the meiotic pathway normally but then exit pachytene and return to the mitotic cycle. Certain *gld-1* partial loss-of-function mutations also abolish oogenesis, but germ cells arrest in pachytene rather than returning to mitosis. Our results indicate that *gld-1* is a tumor suppressor gene required for oocyte development. The tumorous phenotype suggests that *gld-1(+)* may function to negatively regulate proliferation during meiotic prophase and/or act to direct progression through meiotic prophase. We also show that *gld-1(+)* has an additional nonessential role in germline sex determination: promotion of hermaphrodite spermatogenesis. This function of *gld-1* is inferred from a haplo-insufficient phenotype and from the properties of gain-of-function *gld-1* mutations that cause alterations in the sexual identity of germ cells.

GERMLINE development in multicellular organisms represents a specialized example of cell determination and differentiation. In most metazoans, primordial germ cells are set aside early in development and subsequently expanded by proliferation. At later stages, germ cells enter the meiotic pathway and begin gametogenesis. At a minimum, germline development thus requires mechanisms to (1) control germ cell proliferation and entry into the meiotic pathway, (2) specify sexual identities and (3) direct the differentiation of germ cells as either sperm or oocytes.

Studies of germline development in *Caenorhabditis elegans* have begun to provide detailed models for the processes that control germ cell sexual identity and entry into the meiotic pathway. Sexual fates in the nematode soma and germline are governed by similar regulatory hierarchies that are set in response to the *X* chromosome to autosome ratio (*X*:A ratio) (MADL and HERMAN 1979; reviewed by VILLENEUVE and MEYER 1990). Animals with a single *X* chromosome (*X*:A ratio = 0.5) develop as males, whereas animals with two *X* chromosomes (*X*:A ratio = 1) develop as self-fertile hermaphrodites.

In both sexes, the somatic gonad plays a critical role in regulating the decision between mitotic proliferation and meiotic development in the germline. Of importance to this paper is the hermaphrodite gonad that consists of two U-shaped gonad arms (Figure 1). Located at one end of each gonad arm is a single somatic cell, the distal tip cell (DTC), that supplies a signal necessary to maintain continued germ cell proliferation (KIMBLE and WHITE 1981). The *glp-1* gene encodes the likely germline receptor for the DTC signal (AUSTIN and KIMBLE 1987), a protein that belongs to the *lin-12/Notch* family of transmembrane receptors (AUSTIN and KIMBLE 1989; YOCHEM and GREENWALD 1989; CRITTENDEN *et al.* 1994). By late larval stages, the restriction of proliferative signals to the distal end of the gonad imposes a polarity to germline development along the distal/proximal axis. Germ cells located most distally express *glp-1* protein and proliferate mitotically in response to signaling by the DTC. As germ cells move more proximally, they lose cell surface expression of *glp-1* protein (CRITTENDEN *et al.* 1994), enter the meiotic pathway and progress to the pachytene stage of meiotic prophase (HIRSH *et al.* 1976). Gametogenesis and further meiotic prophase progression occur as germ cells enter the proximal half of the germline.

Although the controls for germline proliferation and entry into meiosis are similar in the two sexes, the types of gametes produced differ. Males produce only sperm, whereas hermaphrodites produce both sperm and oocytes. In hermaphrodites, each gonad arm produces ~160 sperm during late larval growth and then switches to oogenesis. This switch in sexual fate is controlled by a germline sex determination pathway composed of ≥14

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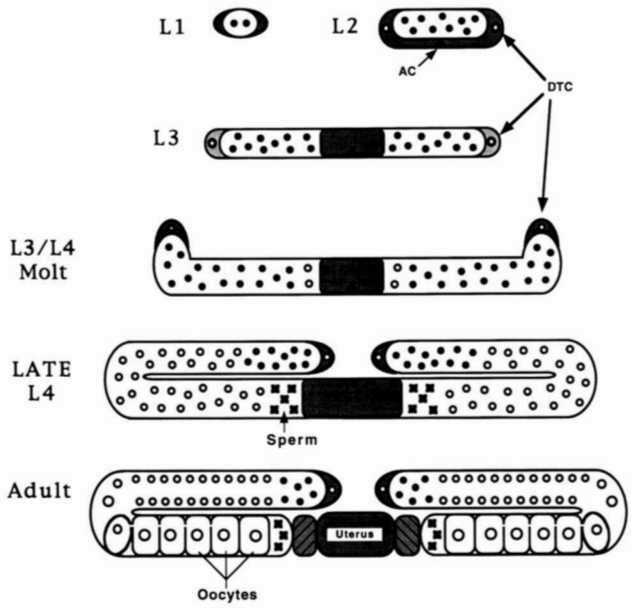

FIGURE 1.—Wild-type hermaphrodite gonad development. The organization of the gonad is shown at adulthood and selected larval stages. Germline proliferation occurs throughout most of larval development (L1–L4) and continues in the adult (mitotic nuclei indicated by closed circles). By early L3, germ cells become partitioned in two gonads arms that join to the developing somatic gonad (shaded tissue). At the distal end of each arm is a single somatic cell, the distal tip cell (DTC), which is necessary for germline proliferation. DTC migration results in the formation of two U-shaped gonad arms by L4. By late L4 and throughout adulthood, proliferation is limited to the distal end of the gonad. Moving proximally, germ cells enter meiotic prophase and then form gametes in the proximal half of each arm (meiotic prophase nuclei indicated by open circles). The germline makes sperm (indicated by ×) in late L4 larvae and switches to oogenesis in young adults. The *C. elegans* hermaphrodite is considered to have a female soma and a hermaphrodite germline that makes first sperm and then oocytes. Most of the germline is syncitial (HIRSH *et al.* 1976); each germline nucleus together with its surrounding cytoplasm and membranes is called a germ cell (AUSTIN and KIMBLE 1987). Adapted from KIMBLE and HIRSH (1979) and HIRSH *et al.* (1976).

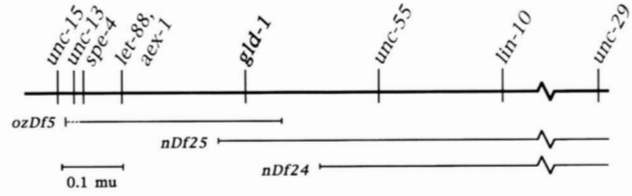

FIGURE 2.—Genetic map of the *gld-1* region. Map positions are based on data presented in Table 1 and on the *C. elegans* genetic map (J. HODGKIN, R. DURBIN and M. O'CALLAGHAN, personal communication). The defined limits of the deletions *nDf24*, *nDf25* and *ozDf5* (derived from complementation tests) are indicated by solid lines. Both *ozDf5* and *nDf25* delete *gld-1* function entirely as shown by three criteria. First, both deletions fail to complement all *gld-1* alleles tested. Second, *ozDf5/nDf25* has an embryonic lethal phenotype, showing that one or both deficiencies must delete essential genes to each side of *gld-1*. Third, assays using the polymerase chain reaction have shown that both deficiencies delete *gld-1* sequences and sequences on each side of the locus (A. JONES, personal communication). *ozDf5* and *nDf24* complement.

genes (reviewed by KUWABARA and KIMBLE 1992; CLIFFORD *et al.* 1994). The known terminal regulators of the pathway are a set of five genes, *fem-1*, *-2* and *-3* and *fog-1* and *-3*, that act together to repress the female fate (oogenesis) and specify the male fate (spermatogenesis) (DONIACH and HODGKIN 1984; HODGKIN 1986; BARTON and KIMBLE 1990; ELLIS and KIMBLE 1995). When any one of these genes is inactivated by mutation, *XX* and *XO* germ cells that would normally form sperm instead form oocytes. In *XO* males, which produce sperm throughout adulthood, the terminal *fem/fog* genes are thought to be active continuously. In the hermaphrodite germline, these genes are active only transiently to direct the brief period of hermaphrodite spermatogenesis. The switch to oogenesis is driven by a mechanism that involves negative regulation of one or more of the terminal *fem/fog* genes (*e.g.*, see AHRINGER and KIMBLE 1991) by the upstream genes *tra-2*, *tra-3* (HODGKIN 1980; DONIACH 1986; SCHEDL and KIMBLE 1988; KUWABARA *et al.* 1992) and *mog-1* (GRAHAM and KIMBLE 1993). Although *tra-2* and *-3* and *mog-1* are required for the switch to oogenesis, these genes have no direct role in specifying the oocyte fate. Moreover, no regulatory genes have yet been identified that act to direct the early stages of oogenesis.

In this and an accompanying paper (FRANCIS *et al.* 1995), we describe a novel *C. elegans* gene, *gld-1* (for defective in *g*erm*l*ine *d*evelopment), that is essential for oogenesis. Results presented here focus on the characterization of six phenotypic classes of *gld-1* alleles that, for the most part, affect only hermaphrodite germline development. We show that only one class, the *gld-1(Tum)* alleles, meets the genetic criteria expected for null and strong loss-of-function mutants. In homozygous *gld-1(Tum)* hermaphrodites, germ cells fail to undergo oogenesis and instead form a germline tumor of ectopically proliferating cells. Our results suggest that germ cells that give rise to tumors progress through the early stages of meiotic prophase but then exit meiosis and return to a mitotic cycle. Because this phenotype occurs only when the sex determination pathway is set in the female mode (FRANCIS *et al.* 1995), we suggest that the major function of *gld-1* is to direct oogenesis by either specifying the oocyte fate or executing an early step in oocyte development.

We also characterize the remaining five classes of *gld-1* alleles and show that these mutations can affect *gld-1* function in qualitatively different ways. Three mutant classes are comprised of partial reduction-of-function alleles that either block oogenesis completely or cause defects at later stages of oogenesis. Two other classes

TABLE 1

Three-factor mapping

Parental genotype[a]	Recombinant phenotype	Recombinant genotype	Number
A. Positioning of *gld-1* on LG *I*			
gld-1/dpy-5 unc-13	Unc nonDpy	*unc-13/dpy-5 unc-13*	30/30
gld-1/unc-13 lin-10	Unc nonLin	*unc-13 gld-1/unc-13 lin-10*	10/25
gld-1/spe-4 lin-10	Lin nonSpe	*gld-1 lin-10/spe-4 lin-10*	10/16
unc-55/gld-1 lin-10	Lin nonGld	*unc-55 lin-10/gld-1 lin-10*	8/15
unc-55/spe-4 lin-10	Lin nonSpe	*unc-55 lin-10/spe-4 lin-10*	4/11

Class	Allele	Lin-10 nonLet-88 recombinants carrying *gld-1*/total Lin-10 nonLet-88 recombinants[c]
B. *gld-1* alleles all map to the same region of the *let-88 lin10* interval on LG *I*[b]		
A	*q268*	23/34 (68)
	q365	17/24 (71)
	q485	13/19 (68)
	q93oz49	15/20 (75)
B	*q93oz12*	14/22 (64)
C1	*q62*	21/30 (70)
	q93	16/22 (73)
	oz17	16/23 (70)
	oz34	25/33 (76)
	oz35	12/20 (60)
C2	*oz16*	22/27 (81)
	oz29	24/35 (69)
	oz30	13/20 (65)
	oz33	22/32 (69)
C3	*oz10*	27/35 (77)
D	*q126*	11/16 (69)
E	*q266*	14/20 (70)

[a] *gld-1(q268)* was used in all cases.

[b] Lin-10 nonLet-88 recombinants segregating from a heterozygote of the genotype *gld-1(x)/unc-13 let-88 lin-10* were picked. Recombinants were cloned and scored for whether they carried a *gld-1* mutant allele by examining their self-progeny.

[c] The recombinant ratios were not significantly different from one another ($P < 0.05$, *z*-test) (FREUND 1973). Values in parentheses are percents.

of alleles are defined by gain-of-function (*gf*) mutations that affect germline sex determination: one group of mutations causes all germ cells to develop as sperm, whereas the second causes all germ cells to develop into oocytes. These *gf* alleles and the gene's haploinsufficient phenotype suggest that *gld-1* plays a role in promoting hermaphrodite spermatogenesis. Although the *gf* alleles produce dramatic effects on germline sexual identity, *gld-1* is not absolutely required for specification of the male germ cell fate. Our genetic analysis therefore shows that *gld-1* performs an essential role in oogenesis and a nonessential function in promoting the male sexual fate in the hermaphrodite germline.

MATERIALS AND METHODS

Nematode strains, nomenclature and general methods: General methods for *C. elegans* culture, manipulation and examination were as described (BRENNER 1974; SULSTON and HODGKIN 1988). Experiments were carried out at 20° unless otherwise noted. Mutagenesis with ethyl methanesulfonate (EMS) was as described (BRENNER 1974) using EMS concentrations of 25–50 mM.

The wild-type reference strain is the *C. elegans var. Bristol* isolate N2. Genetic nomenclature follows HORVITZ *et al.* (1979). To distinguish *gld-1* alleles of different phenotypes, we use *gld-1(Tum)* to identify alleles with a *tum*orous *X X* germline (Tum) phenotype, *gld-1(Fog)* to identify alleles with a *f*eminization *o*f the *g*ermline (Fog) phenotype (production of oocytes at the expense of sperm) and *gld-1(Mog)* to identify alleles with a *m*asculinization *o*f the *g*ermline (Mog) phenotype (production of sperm at the expense of oocytes). In tests assaying maternal contribution of gene product, m(+ or −) is used to designate maternal genotype and z(+ or −) is used to designate zygotic genotype. The following genes and mutations, described in HODGKIN *et al.* (1988), the *C. elegans* genetic map (J. HODGKIN, R. DURBIN and M. O'CALLAGHAN, personal communication) or the cited references, were used:

LGI: *fog-1(q180)* (BARTON and KIMBLE 1990), *dpy-5(e61)*, *unc-15(e1214)*, *unc-13(e51* or *e1091)*, *spe-4(q347)* (L'HERNAULT *et al.* 1988), *aex-1(sa9)* (THOMAS 1990), *let-*

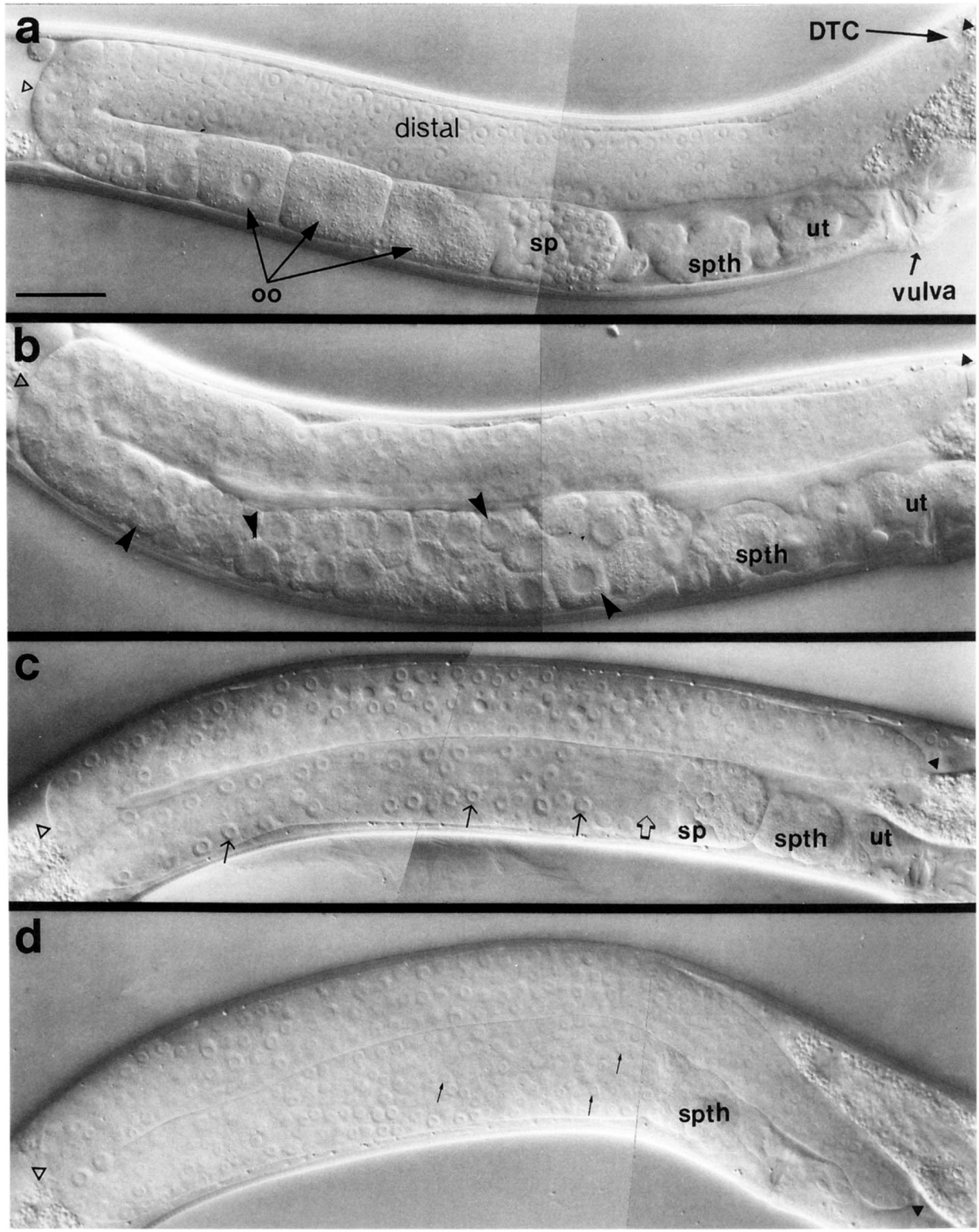

FIGURE 3.—Germline phenotypes of wild-type hermaphrodites and selected *gld-1* mutants. Each panel shows one gonad arm of a young adult hermaphrodite as viewed with Nomarski DIC microscopy. Indicated morphological landmarks (from proximal to distal) are vulva, uterus (ut), spermatheca (spth), loop region (small open triangles), distal region (distal), distal tip (small filled triangles) and distal tip cell (DTC). (a) Wild type: sperm (sp) are present proximally, followed by a single row of oocytes (oo). The distal half of the gonad consists of germ cells in meiotic prophase and proliferating germ cells (at the distal end). (b) Abnormal oocyte phenotype of class E *gld-1(q266)* hermaphrodites. Note the absence of sperm (Fog phenotype) and the accumulation of small abnormal oocytes (arrowheads) in the proximal half of the germline. (c) Undifferentiated germ cell phenotype of class B *gld-1(q93oz50)* hermaphrodites. Sperm (sp) and primary spermatocytes (open arrow) are present proximally

88(s132), *gld-1* (all alleles, this paper), *unc-55(e402)*, *lin-10(e1439)*.
LGIII: *unc-45(e286ts)*.
LGIV: *dpy-13(e184)*, *unc-5(e53)*, *unc-24(e138)*, *fem-1(hc17ts)*, *fem-3(e1996* or *e2143)* (HODGKIN 1986), *dpy-20(e1282)*.
LGV: *fog-2(q71)* (SCHEDL and KIMBLE 1988), *rol-9(sc148)* (SCHEDL and KIMBLE 1988).
Rearrangements: *hT2(I)[bli-4]*, *hT2(III)[dpy-18]* (MCKIM *et al.* 1992), *nDp4 (I;V)* (MCKIM *et al.* 1992).

gld-1 alleles that confer a hermaphrodite-sterile phenotype were generally maintained as balanced heterozygous stocks. Balancer chromosomes included *hT2* and *LGI* chromosomes marked with *unc-13*, *unc-15*, *unc-13 let-88*, *unc-55* or *lin-10*. In addition, most *gld-1(Tum)* alleles were also maintained in *X0* male/*XX* female strains of the genotype *gld-1(Tum)/unc-13 gld-1(q126)*.

Isolation of *gld-1* Mutations

All but three *gld-1* alleles described here were obtained after EMS mutagenesis. The exceptions were *gld-1(q343* and *oz127)*, which arose spontaneously in hybrid mutator backgrounds (see below), and *gld-1(q485)* (kindly provided by D. S. MILLER), which was isolated after psoralen mutagenesis. Typically, *gld-1* alleles were isolated either in screens for recessive self-sterile mutants or in screens biased toward the isolation of mutations that either feminize or masculinize the hermaphrodite germline. All mutations were outcrossed to the N2 strain at least four times and all mutations failed to complement the tumorous alleles *gld-1(q485*, *q268* or *q365)*.

Class A *gld-1(Tum)* alleles: Of 11 *gld-1(Tum)* alleles, four (*q365*, *q485*, *q495* and *oz127*) were isolated in visual screens for recessive self-sterile mutants. Three additional *gld-1(Tum)* alleles (*q268*, *q361* and *oz89*) were isolated as part of screens for mutations that fail to complement *fog-2(lf)* or *fog-1(lf)* mutations that confer a recessive Fog phenotype (SCHEDL and KIMBLE 1988; T. SCHEDL and M. K. BARTON, unpublished data). *gld-1(Tum)* mutations were presumably identified in these screens because of their dominant but incompletely penetrant Fog phenotype (this paper). Four other *gld-1(Tum)* alleles (*q93oz49*, *q93oz53*, *q93oz55* and *oz17oz47*) are double mutants; these were generated, as described below for class B *gld-1* alleles, by isolation of *cis*-revertants of the *gld-1(Mog)* alleles *q93* or *oz17*. *gld-1(oz127)* was kindly provided by J. PRIESS, whereas *gld-1(q495)* was kindly provided by S. MANGO.

Class C *gld-1(Mog)* alleles: Six *gld-1(Mog)* alleles were isolated based on their ability to dominantly suppress *fem-1(hc17ts)*, a temperature-sensitive *fem-1* allele that causes *XX* animals to develop as females when grown at 25° (NELSON *et al.* 1978). Reversion experiments using *fem-1(hc17ts)* were done as described (BARTON *et al.* 1987) and yielded six *gld-1(Mog)* alleles (*q62*, *q93*, *oz10*, *oz29*, *oz30* and *oz33*) at a rate of $\sim 1 \times 10^{-5}$ mutagenized chromosomes.

Four additional *gld-1(Mog)* alleles were obtained by selecting for suppressors of a *fog-2(q71);fem-3(e2143)* double mutant. Neither *fem-3(e2143)*, which is a weak *fem-3* allele (HODGKIN 1986), nor *fog-2(q71)* significantly affect *X0* males, although either mutation causes *XX* animals to develop as females. Similarly, *fog-2(q71);fem-3(e2143) XX* animals are female, whereas *X0* animals are cross-fertile males. The double mutant can thus be maintained as a *X0* male/*XX* female strain, a property that allows self-fertile revertants to be selected for by growth in liquid culture (DONIACH 1986). We chose to isolate suppressors of the double mutant because prior experiments in which *fog-2(q71)* alone was used yielded a high background of *tra-2(lf)* mutations (T. SCHEDL, unpublished data). For these experiments, a semisynchronous population *fog-2(q71);fem-3(e2143)* larvae was obtained from bleached eggs and mutagenized with EMS at the L4 stage. Thirty-six to 42 hr later, F1 eggs were purified by bleach treatment and transferred to 100 ml liquid cultures ($\sim 1 \times 10^5$ eggs/culture) maintained on a shaking platform (SULSTON and HODGKIN 1988). To block the rare successful mating that can occur in liquid culture, we relied on either of two strategies. In one, worms were grown to the L4 stage, at which point the cholinergic agonist levamisole (LEWIS *et al.* 1980) was added (to 0.2 mM) to each culture. In the other, the mutagenized *fog-2; fem-3* animals were also homozygous for *unc-45(e286ts)*, a conditional muscle-affecting mutation that interferes with male mating behavior at 20°. Both types of cultures were harvested after 4 days growth by making the cultures 0.4 mM in levamisole and centrifuging to pellet worms and eggs. Worms were killed by alkaline/bleach treatment, and the eggs and debris were dispersed onto 10-cm plates (1 plate per 100 ml culture). Plates were screened over several days to recover progeny produced by suppressed self-fertile hermaphrodites that arose in liquid culture. Four independent *gld-1(Mog)* alleles (*oz16*, *oz17*, *oz34* and *oz35*) were isolated by this method at a frequency of 5×10^{-6}/haploid genome.

Class B alleles and *gld-1(Tum)* double mutants: Of six class B alleles, one (*oz116*) was obtained in an F2 screen for recessive self-sterile hermaphrodites. The remaining class B mutants are all double mutants that contain a *gld-1(Mog)* mutation (*q93* or *oz17*) in *cis* with a second *gld-1* mutation. These were generated by reverting the ability of *gld-1(Mog)* alleles to dominantly suppress the *XX* female phenotype of *fog-2(q71)* in balanced hermaphrodite strains of the genotype *unc-13 gld-1(q93* or *oz17)/hT2;fog-2*. Hermaphrodites of this genotype are 100% self-fertile and segregate nonUnc-13 non-Dpy-18 hermaphrodites, Dpy-18 females (*hT2* [*dpy-18*]) and sterile *unc-13 gld-1(Mog)* animals. After EMS mutagenesis, F1 progeny were screened for nonUnc, non-Dpy females, some of which were expected to result from intragenic *gld-1* mutations. Ten candidate intragenic revertants were obtained; one was shown to carry the deficiency *ozDf5*, which deletes *gld-1* and the genes *spe-4*, *aex-1* and *let-88*, which lie to the left of *gld-1*. Physical mapping experiments based on the polymerase chain reaction have demonstrated that DNA to each side of the *gld-1* coding region is deleted by *ozDf5* and also by the deficiency *nDf25* (A. JONES, personal communication). The remaining nine isolates were homozygous viable and were identified as intragenic mutations based on their (1) tight linkage to the original *gld-1(Mog)* mutation, (2) failure to complement the tumorous allele *gld-1(q268)* and (3) displaying a previously identified *gld-1* recessive phenotype. Four alleles (*q93oz49*, *q93oz53*, *q93oz55* and *oz17oz47*) confer the same tumorous germline phenotype as do *gld-1(Tum)* single mutants, whereas five others (*q93oz12*, *q93oz45*, *q93oz50*,

in the germline but are then followed by undifferentiated cells (arrows) that are arrested in pachytene (see Figure 5e). (d) Tumorous phenotype of class A *gld-1(q268)* hermaphrodites. The proximal germline fills with mitotically active germ cells that have compact nuclear morphology (small arrows) and show no cytological evidence of oogenesis. The morphology of the tumorous phenotype is indistinguishable for all eleven *gld-1(Tum)* alleles. Scale bar, 10 μm.

TABLE 2

Germline phenotypes of *gld-1* homozygous *XX* animals[a]

Class	Alleles		Germline phenotypes
gld-1(+)	+		Sperm, then oocytes (Herm.)
A			Tumorous germ line (Tum)
A1	*q485*		
A2	*q268*	*q93oz49*	
	q361	*q93oz53*	
	q365	*q93oz55*	
	q495[b]	*oz17oz47*	
	oz89	*oz127*	
B	*q93oz12*	*q93oz52*	Sperm, then germ cells arrested in meiotic prophase (pachytene)
	q93oz45	*q93oz56*	
	q93oz50	*oz116*	
C			Sperm overproduction (Mog), no oogenesis
C1	*q62*	*oz34*	
	q93	*oz35*	
	oz17		
C2	*oz16*	*oz30*	
	oz29	*oz33*	
C3	*oz10*[c]		
D	*q126*[d]		Oocytes only (Fog) or sperm, then oocytes
	oz142[e]		
E	*q266*		Fog, abnormal oocytes
F	*q343*		Sperm, then variably abnormal oocytes

[a] See MATERIALS AND METHODS and RESULTS for details.

[b] This allele was placed in subclass A2 based on the molecular lesion being identical to that of *q268* (A. JONES, personal communication).

[c] Seventy-two percent make only sperm, 28% make excess sperm and then begin oogenesis, with ~2% of *oz10* animals eventually becoming self-fertile.

[d] Seventy-six percent make only oocytes and 24% make sperm then oocytes and are self-fertile.

[e] Seventy-eight percent make only oocytes and 22% are self-fertile.

q93oz52 and *q93oz56*) confer a phenotype similar to that of the class B mutant *oz116*.

The nine new *gld-1* mutants marked with *unc-13* were placed in a *fog-2*(+) background and balanced over *unc-55*. To remove the *unc-13* marker and linked deleterious mutations, *unc-13 gld-1(revertant)/unc-55* males were crossed to *unc-13 unc-55* hermaphrodites. From these crosses, nonUnc recombinants were picked and *gld-1(intragenic revertant)/unc-13 unc-55* animals were identified by segregation analysis. For dosage and complementation tests, it was necessary to mark these (and all other) *gld-1* alleles with an unmutagenized *unc-13* allele. This was done by picking Unc nonLet recombinants segregating from *gld-1(x)/unc-13 let-88* hermaphrodites.

Class B alleles were tested for the presence of residual *gld-1(Mog)* masculinizing activity by assaying for suppression of *fog-2(q71)* to self-fertility. This was accomplished by determining whether *fog-2 rol-9* animals segregating from *unc-13 gld-1*(class B)/+;*fog-2(q71) rol-9*/+ hermaphrodites are always female (no suppression) or sometimes hermaphrodite (suppression). Only *q93oz12* and *q93oz45* retained the ability to dominantly suppress *fog-2(q71)*. The alleles *oz116* and *q93oz50* do not suppress *fog-2*, either dominantly or recessively, even though as *XX* homozygotes they produce more sperm than wild type (see Table 3).

Class D, E and F *gld-1* alleles: The class D *gld-1(Fog)* allele *oz142* was isolated in screens for recessive self-sterile mutants based on its *XX* Fog phenotype. Other mutations representing classes D (*q126*), E (*q266*) and F (*q343*) were isolated as part of screens for mutations that fail to complement the Fog phenotype of *fog-2(q71)* (SCHEDL and KIMBLE 1988). The class F allele, *q343*, appeared in a cross using hybrid males produced from the mutator strain TR679 (COLLINS *et al.* 1987). The *q343* allele was outcrossed 12 times to marked *var. Bristol* strains to remove mutator elements. A linked *unc-13* mutation was then introduced by recombination and removed as described for class B *gld-1* alleles. The resulting *q343* allele shows no evidence of dominant or recessive Fog phenotypes.

Experiments with the *gld-1(Fog)* allele *q126* and *gld-1* alleles that confer a dominant Fog phenotypes failed to reveal maternal effects in the following three tests. (1) Penetrance of the homozygous *q126* Fog phenotype was the same in m(−/−) z(−/−) and m(−/+) z(−/−) animals. This indicates an absence of maternal rescue. (2) Penetrance of the *q126* dominant Fog phenotype was not significantly different between m(−/−) z(−/+) and m(+/+) z(−/+) animals, indicating no maternal absence effect. (3) For all alleles that display a Fog phenotype, the frequency of z(−/+) Fog gonad arms in m(+/+) z(−/+) and m(−/+) z(−/+) animals was indistinguishable (see Tables 4 and 5). Because *XX gld-1(Tum)* animals are sterile, we were unable to analyze progeny from homozygous mothers.

Mapping of *gld-1* alleles: *gld-1* was positioned on *LGI* between *spe-4* and *lin-10* as described in Table 1 and shown in Figure 2, using the allele *gld-1(q268)*. The 10 *gld-1(Mog)* alleles and one or more alleles of each other class were then

Class	Allele							
Df(gld-1)	*nDf25 ozDf 5*	Let						
A1 & A2 Tum	*q485, q268, q93oz49, q365*	Tum	Tum					
B Sp, then UD Germ cells	*q93oz50*	Sp, then UD	Sp, then UD	Sp, then UD				
C1 & C2 Mog	*q93, oz17, oz30*	Mog	Mog	Mog	Mog			
D Fog (80%)	*q126*	Fog	Fog	Fog (78%) Herm (22%)	Herm	Fog		
E Fog; Abn oocytes	*q266*	Fog; Abn oocytes	Fog; Abn oocytes	Sp, then UD & Abn oocytes	Sp, then UD & Abn oocytes	Fog	Fog; Abn oocytes	
F Sp, then Abn oocytes	*q343*	Sp, then Abn oocytes	Sp, then Abn oocytes	Sp, then UD or Abn oocytes	Sp, then UD	Fog (35%) Herm (65%)	Fog (64%) Herm (36%)	Sp, then Abn oocytes
		nDf25 ozDF5	*q485, q268, q93oz49, q365*	*q93oz50*	*q93, oz17, oz30*	*q126*	*q266*	*q343*

FIGURE 4.—Complementation analysis of the six classes of *gld-1* alleles. Complementation tests were performed for all combinations of *trans*-heterozygotes (see MATERIALS AND METHODS) to determine which class behaves identically to the deletions (*ozDf5* and *nDf25*). All pairwise tests were performed for each of the listed alleles. In general, the germline phenotype observed in *gld-1(x)/gld-1(y)* hermaphrodites was similar or identical to that of the *gld-1(x)* or *gld-1(y)* homozygote. The indicated phenotypes include Tum (tumorous; class A phenotype), Mog (continued spermatogenesis, no oogenesis; class C phenotype), Fog (no sperm, normal oogenesis; class D phenotype), Sp (sperm) and then UD (undifferentiated, pachytene arrested germ cells; class B phenotype), and Sp (sperm) then Abn (abnormal) oocytes (similar to class E abnormal oocyte phenotype but with sperm made). Note that only *gld-1(Tum)* alleles gave the same phenotypes as *gld-1* deficiencies in all combinations of *trans*-heterozygotes. Several cases of intragenic complementation are discussed in the text.

positioned between *let-88* and *lin-10* by picking nonLet, Lin-10 recombinants segregating from *gld-1(x)/unc-13 let-88 lin-10* hermaphrodites (Table 1).

Complementation tests: The complementation data summarized in Figure 4 were generated by making all possible *trans*-heterozygous combinations with representative alleles of different classes of *gld-1* mutations. Several strategies were used to ensure unambiguous identification of *gld-1(x)/gld-1(y)* *trans*-heterozygotes in specific crosses. Most frequently, the two *gld-1* alleles were marked with *unc-13*, and crosses were done using either females or purged hermaphrodites that had exhausted their self-sperm. In these cases, *unc-13 gld-1(x)/+* males were crossed with female/purged hermaphrodites of one of the following general genotypes: (1) *unc-13 gld-1(y)/unc-55* or *unc-15*, (2) *unc-13 gld-1(y);nDp4/+* or (3) *unc-13 gld-1(q126)*. Male and hermaphrodite Unc-13 cross-progeny were picked *en masse* as L4 larvae, and 40 or more adults of each sex were examined 1 and 2 days later by Nomarski differential interference contrast (DIC) microscopy (SULSTON and HODGKIN 1988). To eliminate possible effects of *unc-13*, many complementation tests were repeated using males heterozygous for an unmarked *gld-1* allele. In these crosses, *gld-1(x)/+* males were mated to *unc-13 gld-1(y)/unc-13* let-88 hermaphrodites or to females/purged hermaphrodites that were *unc-13 gld-1(y);nDp4/+* or *unc-13 gld-1(q126)*. For these crosses, nonUnc *XX* cross-progeny were picked *en masse* as L4 animals and examined by Nomarski DIC the following day. Crosses requiring a *gld-1(Mog)* allele were usually done with this allele provided by the male. Alternatively, *unc-13 gld-1(x)/+* males were crossed with *unc-13 gld-1(Mog)/hT2;dpy-20* hermaphrodites to generate Unc-13 nonDpy-20 *trans*-heterozygotes.

Three deletions in the *gld-1* region (*ozDf5*, *nDf25* and *nDf24*) were examined for complementation with each class of *gld-1* allele and with one another. For *ozDf5*, *unc-13 gld-1(x)/+* males were crossed to *unc-13 ozDf5/unc-15* or *unc-13 ozDf5;nDp4/+* hermaphrodites and Unc-13 cross-progeny were scored. Most tests with *nDf25* and *nDf24* were done by crossing the *gld-1(x)/+* males with *nDf25/unc-13 let-88* or *nDf24/spe-4* hermaphrodites and examining the next generation for viable self-sterile hermaphrodites. All tested *gld-1* alleles, including those listed in Figure 4, failed to complement *nDf25* (and also *ozDf5*) as indicated by the appearance of self-sterile *gld-1(x)/Df* hermaphrodites. These sterile animals always displayed the same germline phenotype as *gld-1(x)/gld-1(Tum)* hermaphrodites. In contrast, sterile animals were

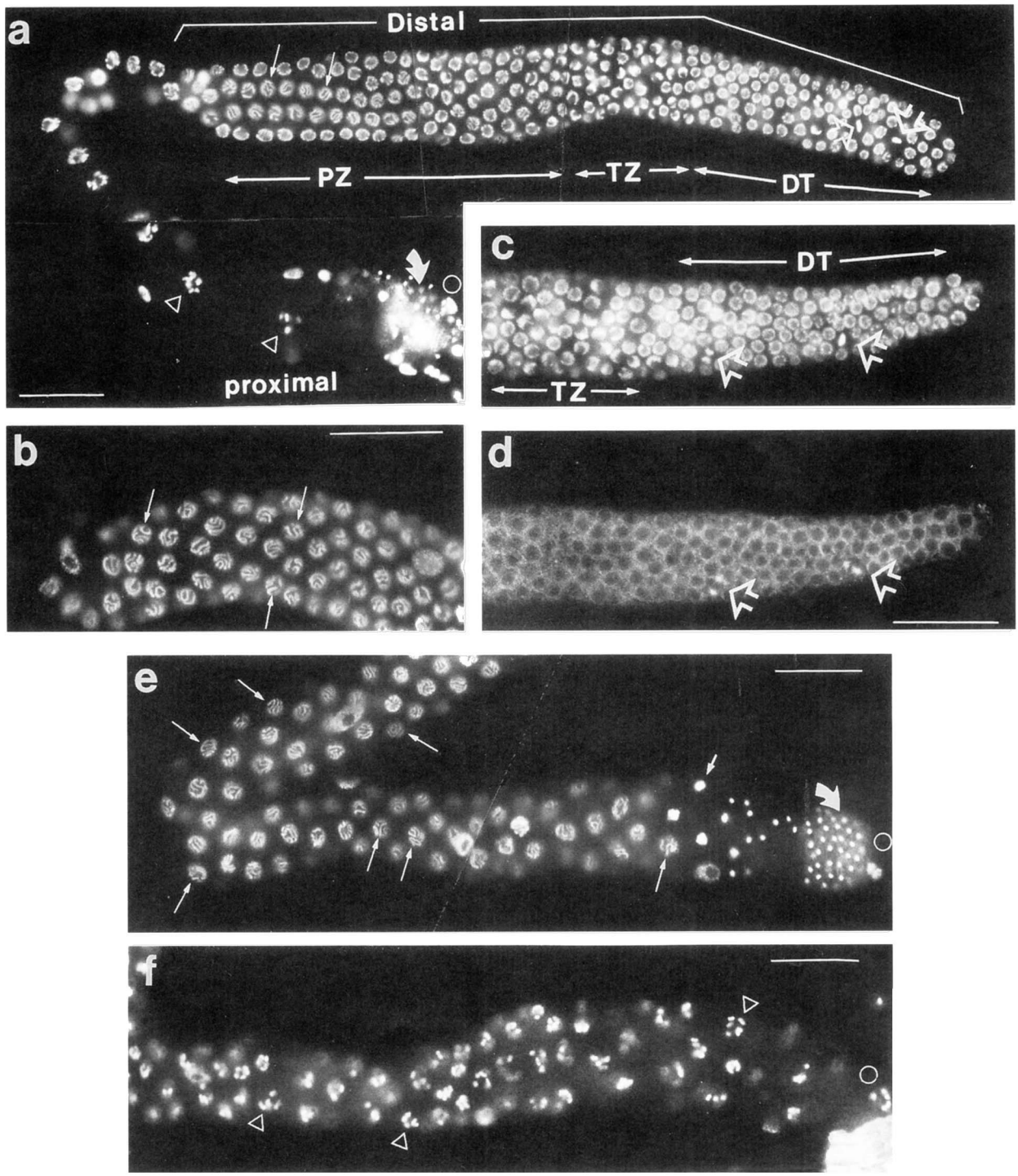

FIGURE 5.— Morphology of germline nuclei in wild-type and class B and E *XX gld-1* mutant adults. Nuclear morphologies visualized by DAPI staining (a–c, e, f) and microtubule organization visualized by anti-tubulin antibody staining (d) in gonad arms released by microdissection. Surface view shown in all panels. DAPI staining of a wild-type gonad arm (a) shows nuclei at all stages of adult germline differentiation; the distal tip mitotic region (DT, also shown in c) is followed first by the transition zone (TZ), where nuclei undergo the transition from the mitotic cell cycle through early stages of meiotic prophase, and then by the pachytene zone (PZ), where nuclei have entered the pachytene stage of meiotic prophase (arrows in a, b and e). Oocytes form in the proximal germline and arrest at diakinesis of meiosis I prophase (open arrowheads in a and f). The open circle indicates the proximal end of the gonad (a, e and f). Sperm (curved arrow in a and e) appear as small dots. Shown in c and d are typical appearances of wild-type mitotic cells in the distal tip region stained with DAPI (c) and anti-tubulin antibodies (d). Shown in b are wild-type pachytene stage nuclei that exhibit a characteristic thread-like chromatin morphology. For the class B mutant (e), *gld-1(q93oz50)*, the germline makes sperm (curved arrow, short arrow indicates a condensed primary spermatocyte nucleus) but never makes oocytes. Instead the proximal germline fills with undifferentiated germ cells that are

not obtained in crosses with *nDf24*, and for each class of allele we recovered a self-fertile *gld-1(x)/nDf24* strain. A similar cross also yielded self-fertile *unc-13 ozDf5/nDf24* hermaphrodites, indicating that *ozDf5* and *nDf24* are nonoverlapping. In contrast, crosses of *unc-13 ozDf5/unc-15* males to *unc-13 nDf25/spe-4* hermaphrodites failed to give viable Unc-13 cross-progeny; *unc-13 ozDf5/unc-13 nDf25* therefore is lethal, indicating that at least one essential gene is eliminated by both deficiencies.

Assessing Dominant Effects of *gld-1* Alleles on Germline Sex Determination

***gld-1* deficiencies and *gld-1* alleles with a dominant Fog phenotype:** Class A, D and E *gld-1* alleles all confer a dominant but incompletely penetrant Fog phenotype in which one or both gonad arms of *XX gld-1(x)/+* animals fails to make sperm. To quantitate the penetrance of the Fog phenotype, we examined *unc-13 gld-1(x)/dpy-5 unc-13 XX* animals produced by crosses of *unc-13 gld-1(x)/+* males with *dpy-5 unc-13* hermaphrodites. *XX gld-1* heterozygotes were picked as nonDpy, Unc-13 L4 larvae and examined within 18 hr using Nomarski DIC microscopy. Individual hermaphrodite gonads were scored for the presence of sperm or the absence of sperm and the premature appearance of oocytes. *unc-13 ozDf5/unc-13 dpy-5* animals were generated and scored similarly. For tests of haplo-insufficiency using *nDf25*, spermatogenesis was scored in *XX nDf25/unc-13 let-88* adults produced by mothers of the same genotype. In germlines that failed to make sperm, we found no evidence for presumptive male germ cells that die, remain undifferentiated or exit meiotic prophase and reenter the mitotic cycle. Therefore the dominant Fog phenotype is a *bona fide* feminization of the germline, like that observed for *fem-1*, *-2* and *-3* and *fog-1*, *-2* and *-3* gene mutations. The dominant Fog phenotype is somewhat cold sensitive, but this aspect has not been investigated in detail.

Because of the small percentage of *gld-1(q485)/+* worms that exhibited the Fog phenotype (see RESULTS and Tables 4 and 5), we considered the hypothesis that *XX* animals of any genotype express the Fog phenotype at a low frequency. An estimate of this frequency is 4/653 [the number of *q485/+* Fog animals divided by total (sum of all *q485/+* and *+/+* animals; Table 4)]. The hypothesis was tested by determining the exact binomial probability of observing zero Fog animals among 450 *+/+* hermaphrodites and the probability of observing as many as four Fog animals among 203 *q485/+* animals, assuming a frequency of 4/653 in each case. These probabilities are 0.003 and 0.037, respectively. Thus, the hypothesis was rejected for *q485/+*. Similar calculations show that this hypothesis can also be rejected for *ozDf5/+* and *nDf25/+*.

Dosage-tests of class A, D and E alleles were performed by comparing the penetrance of the Fog phenotype in *m/m/+*, *m/+* and *m/+/+* animals. For the first two dosages, the Fog phenotype was scored in individual gonad arms of *unc-13 gld-1(x);nDp4/+* or *unc-13 gld-1(x)/unc-15* hermaphrodites, respectively, segregating from mothers of the same genotype. *m/+/+ XX* animals were generated by crossing *unc-13;him-8;nDp4/+* males to *unc-13 gld-1(x);nDp4/+* females or purged hermaphrodites. The nonUnc *XX unc-13 gld-1(x)/unc-13;nDp4/+* cross-progeny were scored. Dosage tests with an *unc-13ozDf5* chromosome were done exactly as described for *gld-1(x)* alleles. Animals were scored, as described above, for spermatogenesis in hermaphrodite gonad arms.

Class C *gld-1(Mog)* alleles: Dominant effects associated with *gld-1(Mog)* alleles were evaluated in *unc-13 gld-1(Mog)/dpy-5 unc-13 XX* hermaphrodites produced by mating *unc-13 gld-1(Mog)/+* males with *dpy-5 unc-13* hermaphrodites. The Unc-13 nonDpy-5 [*gld-1(Mog)/+*] animals produced in these crosses were picked as L4 larvae and scored 1 day and 4 days later. Young adult hermaphrodites examined after 1 day were scored for the presence or absence of oocytes to determine whether the switch to oogenesis was delayed relative to wild type (oogenesis begins in wild-type hermaphrodites within hours of their reaching adulthood). Animals examined after 4 days were scored for whether the germline continued to produce oocytes (as in wild type) or instead displayed one or both of the following aberrant phenotypes: reinitiation of spermatogenesis and/or the presence of undifferentiated germ cells in the proximal half of the germline. The dominant Mog phenotype is somewhat heat sensitive, but this aspect of the phenotype has not been investigated in detail.

gld-1(Mog) alleles of each subclass (C1-*q93*, C2-*oz30* and C3-*oz10*) were examined for dose-dependent effects by comparing the phenotypes of *m/m*, *m/m/+* and *m/+ XX* animals. The *gld-1(Mog)/gld-1(Mog)* animals examined were the sterile nonUnc self-progeny of *gld-1(Mog)/unc-13* hermaphrodites. For *m/m/+* genotypes, nonUnc-13 animals [*unc-13 gld-1(Mog)/unc-13 gld-1(Mog);nDp4/+*] segregating from self-fertile mothers of the same genotype were examined. For *m/+*, nonUnc-13 animals [*unc-13 gld-1(Mog)/unc-15*] segregating from self-fertile mothers of the same genotype were examined. To score germline differentiation in *m/m/+* and *m/+* animals, 50 or more animals of each genotype were picked as L4 larvae and placed on separate plates. These animals were then scored intermittently over the following 4 days. On days 1, 3 and 4, each individual adult was mounted on its own agar pad, examined by Nomarski DIC microscopy, and then recovered from the mount and returned to a growth plate. Data for *m/m* animals were obtained by picking L4 larvae *en masse* and examining different populations of animals on each of the following 4 days.

Determining sperm number in homozygous and heterozygous *gld-1* animals: The extent of spermatogenesis in the selected *gld-1* mutants was determined by counting sperm nuclei in young adult *XX gld-1(x)* animals stained with DAPI. Sperm, recognized by their small nuclear size, were counted twice in eight or more gonad arms. The mean number of sperm made was calculated, excluding any gonad arms that failed to make sperm. Dominant effects of *gld-1(q485)* and *gld-1(q93)* on the number of hermaphrodite sperm produced were evaluated by counting the brood sizes of 11 or more *unc-13 gld-1(x)/dpy-5* hermaphrodites. For comparison, brood size was also determined for 12 *dpy-5 unc-13/+* hermaphrodites. Mean brood sizes were divided by two to obtain an estimate of the number of sperm produced per gonad arm (see Table 3).

In experiments with *gld-1(Tum)* alleles, the only significant marker effects that we observed involved sperm formation in homozygous *gld-1(Tum)* germlines. *gld-1(q485) XX* homozygotes fail to make sperm in an unmarked background but sometimes make sperm when also homozygous for *unc-13(e51)* or *unc-32(e189)*. Similarly, spermatogenesis in homozygous *gld-1(q268* or *q365) XX* germlines occurs more fre-

arrested in the pachytene stage (arrows). A cytoplasmic core is not observed, either proximally or distally. For the class E mutant (f), *gld-1(q266)*, the germline makes a large number of small abnormal oocytes (Figure 3b) that, like wild-type oocytes, arrest at diakinesis of meiotic prophase I (open arrowheads). Scale bars, 10 μm.

quently in an *unc-13* or *unc-32* background than in an otherwise wild-type background. The reason for this marker effect is unclear.

Gonad dissections and cytology: For staining of nuclei with DAPI, intact worms were fixed in cold (−20°) methanol for 5 min. Fixed worms were washed twice in modified M9 buffer (M9 [SULSTON and HODGKIN 1988] with no added Mg^{2+}), incubated 30 min in 100 ng/ml DAPI in modified M9 and washed two to three times in modified M9.

To prepare dissected gonad preparations, animals of the desired age were picked onto a fresh plate containing no bacteria, immersed in 2 ml of phosphate-buffered saline (PBS) containing 0.25 mM levamisole and transferred to a circular glass dish (3 cm diam and 1.5 cm deep). Worms were decapitated by slicing with two 25-gauge syringe needles in the head region, which results in gonad extrusion. The preparations were fixed in 3 ml of 3% formaldehyde, 0.1 M K_2HPO_4 (pH 7.2) for 2 hr. For anti-tubulin staining, the dissection and fixative solutions contained 1 nM taxol (Molecular Probes, Inc.). Fixed dissected worms were washed once in PBS, postfixed for 5 min in 3 ml of cold (−20°) 100% methanol and washed two times in PBS. For staining with DAPI alone, samples were incubated for 15 min in PBS/100 nM DAPI and then mounted for microscopy as follows. Using a capillary pipette, worms with attached extruded gonads were transferred onto a 2% agarose pad covering most of a glass slide. After removing excess liquid with a capillary, extruded gonads were manipulated for optimal positioning with a drawn capillary and then overlaid with a 25 × 50-mm coverslip.

Tubulin distribution in fixed gonads was visualized using the commercially available monoclonal antibody, N357 (Amersham, Inc.), directed against β-tubulin. Sperm were visualized by staining with DAPI and, in some experiments, by staining with SP56 (kindly supplied by S. STROME), a monoclonal antibody directed against epitopes expressed only in sperm and spermatocytes (WARD *et al.* 1986). Antibody incubations were generally done by transferring 100 or more dissected worms to a small glass tube (6 × 50 mm) and adding 200 μl of antibody diluted in PBS/0.1% Tween 20/1% bovine serum albumin. SP56 was used at a 1:20 dilution of culture supernatant and anti-β-tubulin at a 1:400 dilution of ascites fluid. After a 4- to 12-hr incubation, samples were washed three times in PBS/0.1% Tween 20 and incubated 4 hr in fluorescein-labeled goat-anti-mouse IgG (7.5 μg/ml; Chemicon, Inc.). After three washes in PBS/0.1% Tween 20, the preparations were placed in PBS containing 100 ng/ml DAPI and mounted for microscopy as described above. All staining was visualized using a Zeiss Axioskop microscope equipped with epifluorescence optics.

Evaluation of germline proliferation and entry into meiotic prophase in *gld-1(Tum)* mutants: Comparisons of germline proliferation in wild-type and *gld-1(Tum)* hermaphrodites were made using animals homozygous for *unc-32*. Heterozygous *unc-32/hT2* and *gld-1(Tum)/hT2(I);unc-32/hT2(III)* strains were used as a source of *unc-32* or *gld-1(Tum);unc-32* hermaphrodites, respectively. In addition, to eliminate the spermatogenesis that sometimes occurs in marked *gld-1(Tum);unc-32 XX* animals, we also examined *gld-1(Tum);unc-32* animals homozygous for *fog-1(q180)*, a mutation that abolishes the male germline fate (BARTON and KIMBLE 1990). The triple mutants were obtained from a balanced *fog-1(lf) gld-1(Tum)/hT2(I);unc-32/hT2(III)* stock constructed as described in FRANCIS *et al.* (1995). To obtain animals of known age, eggs produced by + or *gld-1(Tum)* or *fog-1(lf) gld-1(Tum)/hT2(I);unc-32/hT2(III)* hermaphrodites were picked *en masse* as late-stage embryos and transferred free of contaminating larvae to fresh plates. L1 larvae that hatched in the next hour were picked onto fresh plates and allowed to develop until the desired age. Unc-32 hermaphrodites were grown for 38, 48, 72 or 96 hr past hatching and then fixed and stained with DAPI. For each genotype and time point, germline nuclei were counted twice at successive z-axis focal plains in nine or more gonad arms to obtain a mean number of germ cells per gonad arm. To gauge proliferation in self-fertile *unc-32* hermaphrodites, we first determined the number of self-progeny produced by each animal up to the time of fixation. This number was added to the germ cell counts determined by DAPI staining, after correcting for the effects of male meiotic divisions and fertilization.

A time course study was also performed to determine when meiotic development is first observed in the *XX* germlines of *gld-1(+)*, *gld-1(q485)* and *fog-1(q180) gld-1(q485)* animals marked with *unc-32*. Tightly staged larvae were obtained as described above and fixed at successive 1-hr intervals through L4 larval growth. After staining with DAPI, animals were scored for the presence or absence of pachytene-stage nuclei in the proximal germline region of unobstructed gonad arms. For the data in Figure 9, animals were scored as positive for entry into meiotic prophase if pachytene germ cells were observed in one or both gonad arms and negative if no pachytene-stage germ cells were observed in at least one unobstructed gonad arm. Therefore some animals that contained pachytene nuclei in only one arm would be scored as negative if those nuclei were obstructed by somatic tissue. For each data point in Figure 9, ≥10 animals were scored.

RESULTS

Using several different screens and selections (see MATERIALS AND METHODS), we have isolated 31 mutations that define a new gene, *gld-1*. All 31 alleles disrupt development of the hermaphrodite germline; *XX gld-1* hermaphrodites are self-sterile and exhibit germline phenotypes associated with defects in oogenesis, meiotic prophase progression and/or sex determination. There is no obvious effect on somatic development and, for the most part, germline development in *gld-1 X0* males is unaffected. Based on the nature of their hermaphrodite-specific phenotypes, *gld-1* alleles have been placed in six classes (Table 2, classes A–F). Our assignment of the six mutant classes to a single locus rests on three lines of evidence. First, the mutations map to the same small genetic interval on chromosome *I* (Table 1 and Figure 2). Second, most mutations fail to complement one another and deficiencies of the *gld-1* region. Third, class C *gld-1* alleles, which are gain-of-function (*gf*) mutations, have been reverted to *gld-1* loss-of-function (*lf*) mutations by the introduction of *cis*-dominant intragenic lesions (see below).

We show that, of the six classes of *gld-1* alleles, only class A *gld-1(Tum)* alleles meet the genetic criteria expected for null and strong *lf* alleles. *gld-1(Tum) XX* animals have a sterile phenotype characterized by an absence of oogenesis and the formation of a germline tumor (Tum for a *tum*orous germline phenotype). Based on the absence of oogenesis in null/*lf* mutants, we infer that *gld-1(+)* is required for oocyte develop-

ment. In addition to a recessive tumorous phenotype, *gld-1(Tum)* alleles have a dominant but incompletely penetrant effect on germline sex determination: some *gld-1(Tum)/+ XX* germlines fail to make sperm because of a defect in specifying the male fate (Fog for a *f*eminization *o*f the *g*ermline phenotype). For one *gld-1(Tum)* allele (*q485*), the dominant Fog phenotype is shown to be attributable to a haplo-insufficiency, indicating that *gld-1(+)* may also function to promote hermaphrodite spermatogenesis.

The five remaining classes of *gld-1* alleles (Table 2) all confer distinct germline phenotypes. In class B mutants, germ cells that would normally form oocytes arrest at the pachytene stage of meiotic prophase; in contrast, in class E and F mutants, germ cells progress through meiosis normally but form small abnormal oocytes. Evidence presented below indicates that these are partial *lf* phenotypes. Finally, class C and D mutants exhibit opposite transformations in sexual fate: class C mutants have a germline that only makes sperm (Mog for a *m*asculinization *o*f the *g*ermline phenotype), whereas class D mutants have a germline that only makes oocytes (Fog phenotype). Both types of sexual transformation are shown to result from *gf* alterations in *gld-1* activity.

Below, we first describe the genetic and phenotypic properties of class A *gld-1(Tum)* alleles. This is followed by analyses of the other classes in the order: D (Fog phenotype), C (Mog phenotype), B (undifferentiated pachytene arrest phenotype) and E and F (abnormal oocyte phenotype).

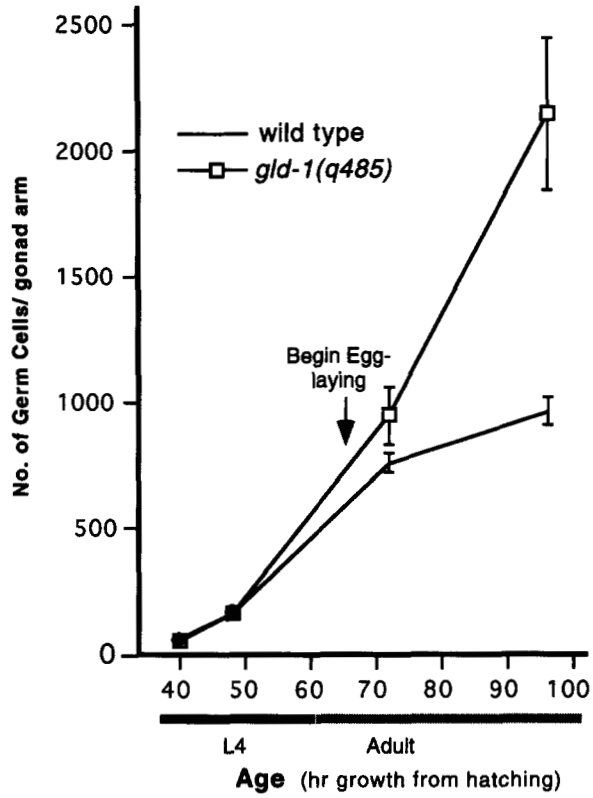

FIGURE 6.—Comparison of germline proliferation in wild-type and *gld-1(Tum)* hermaphrodites. The number of nuclei per gonad arm is plotted versus developmental time (see MATERIALS AND METHODS). Ectopic proliferation in *gld-1(q485)* mutant germlines continues throughout adulthood, producing tumors with significantly more germ cells than wild type. Error bars, ±1 SD. Similar proliferation profiles were obtained with the tumorous alleles *q268* and *q365* (data not shown).

Class A *gld-1(Tum)* Alleles Define the *gld-1* Null Phenotype

Class A includes seven single mutants and four intragenic revertants of class C alleles (see below and MATERIALS AND METHODS). Adult *XX* animals homozygous for any class A allele show no cytological evidence of oogenesis (Figure 3, compare a and d) and display a tumorous germline phenotype that is completely penetrant. To determine the extent to which wild-type gene activity is eliminated in *gld-1(Tum)* mutants, we compared the effects of *gld-1(Tum)* alleles and *gld-1* deficiencies when these mutations are placed in *trans* to different classes of *gld-1* alleles. Identical results were obtained with two homozygous lethal deficiencies (*ozDf5* and *nDf25*) and each of four *gld-1(Tum)* alleles (Figure 4). For all possible combinations, we find that *gld-1(x)/Df(gld-1)* and *gld-1(x)/gld-1(Tum) trans*-heterozygotes display essentially identical germline phenotypes. This is in contrast to the five other classes of *gld-1* alleles, each of which behaves differently from *gld-1* deficiencies in at least three combinations of *trans*-heterozygotes (Figure 4). We conclude that since *gld-1(Tum)* alleles behave identically to deficiencies in this test, these alleles retain little or no gene activity. Results presented below show that the *gld-1(Tum)* allele *q485* also behaves similarly to deficiencies in gene dosage tests. Therefore *q485* was chosen as the canonical *gld-1* null allele for use in subsequent experiments.

Characterization of the *gld-1* Tumorous Phenotype

Germ cell proliferation in wild-type adult hermaphrodites is limited to the distal region of the germline (Figure 5, c and d) and is dependent on signaling by the somatic DTC. As germ cells move proximally, they enter meiotic prophase (Figure 5b) and on reaching the proximal half of the germline begin gametogenesis (Figures 3a and 5a). In *gld-1(Tum)* hermaphrodites, proliferation appears normal until midway through the L4 larval stage. L4 *gld-1(Tum)* larvae contain approximately the same number of germ cells as wild type (Figure 6), for example, and sperm are sometimes made at the normal time in L4 larvae (also see below). However, oogenesis, which normally begins in young adults, is never observed. In-

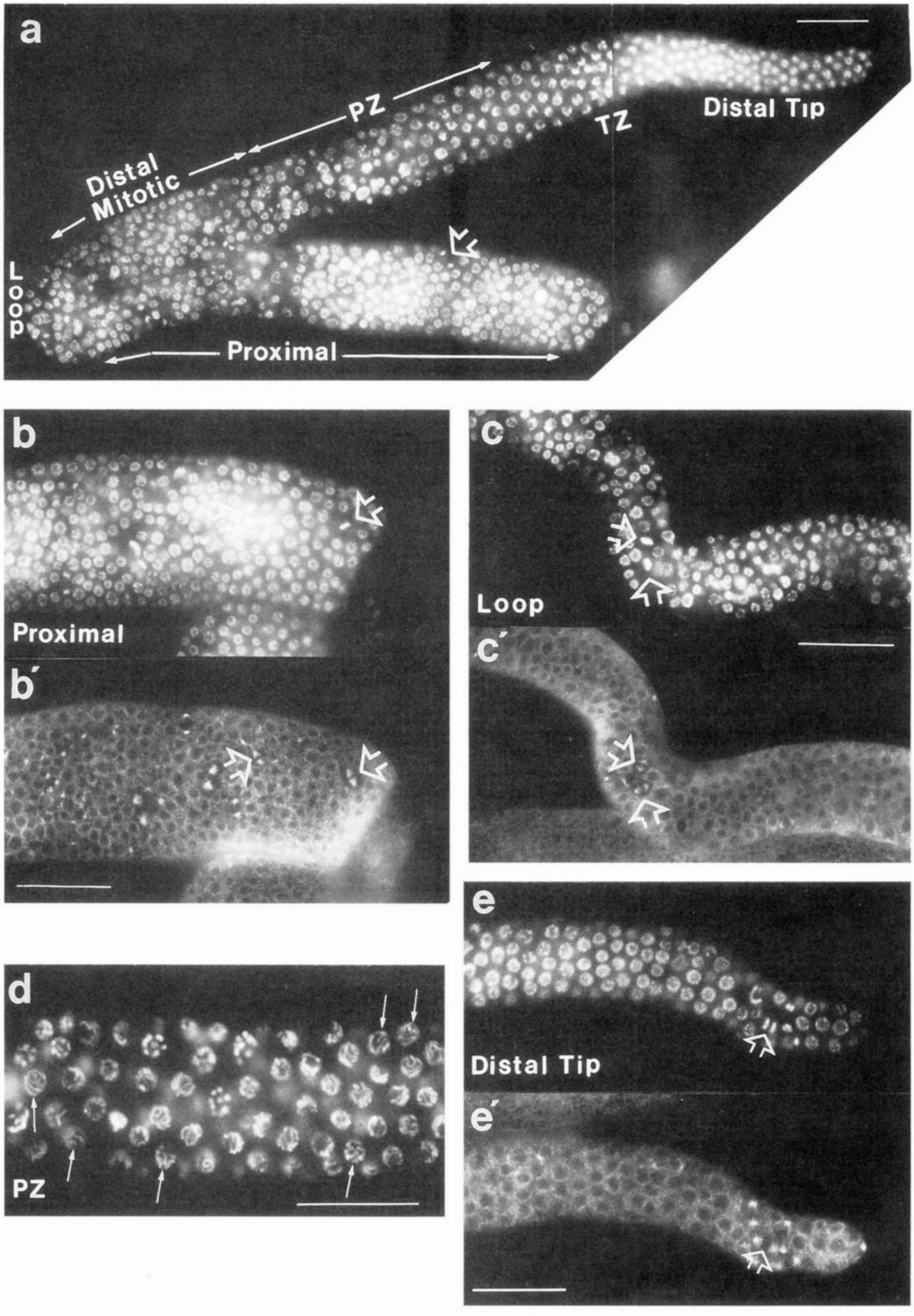

FIGURE 7.—Ectopic proliferation in the tumorous germlines of *gld-1(Tum) XX* adults. Nuclear morphologies visualized by DAPI staining (a–e) and microtubule organization visualized by anti-tubulin antibody staining (b′, c′ and e′). Surface view for all panels. Shown in a is an entire *gld-1(q268)* dissected gonad arm stained with DAPI. The other panels show specific regions of tumorous germlines dissected from different animals. Ectopic proliferation is demonstrated by the presence of DAPI-stained

stead, the proximal germline fills with small undifferentiated germ cells (Figure 3d). Examination of *gld-1(Tum)* *XX* gonads stained with DAPI and anti-tubulin antibodies demonstrates that germ cells proliferate ectopically through much of the germline (Figure 7). Proliferation continues during adulthood, producing in excess of 2000 germ cells per gonad arm, as compared with ~900 germ cells per gonad arm in wild type (Figure 6). Although there is no evidence of metastasis, proliferation leads to swelling of the gonad and eventual leakage of germ cells into the spermatheca and uterus. Old adults often leak germ cells from the vulva, which can lead to eversion of the vulva and premature death.

Close examination of germlines in young adult hermaphrodites suggests that *gld-1(Tum)* alleles have little or no effect on the entry of germ cells into the meiotic pathway. As in wild type, the extreme distal end of *gld-1(Tum)* mutant gonads contains proliferating germ cells. Moving proximally from this region, *gld-1(Tum)* germlines consistently exhibit the same changes in nuclear morphology that are seen in DAPI-stained preparations of wild-type gonads. The distal mitotic region is followed first by the transition zone in which germ cells exhibit a unique nuclear morphology (Figures 5c and 7e) (CRITTENDEN *et al.* 1994). Germ cells then progress to pachytene of meiotic prophase, a stage characterized by a distinctive thread-like nuclear morphology (Figure 7d, compare with wild type in Figure 5b). Although both the distal proliferative region and transition zone appear normal in size, the pachytene region is generally smaller than the corresponding region of wild type (Figure 7a, compare with wild type in Figure 5a). Proximal to the pachytene region, the germline consists mainly of proliferating cells (Figure 7, a–c′). Figure 8 summarizes the pattern of proliferation observed in *gld-1(Tum)* adults. These observations suggest that germ cells in *gld-1(Tum)* germlines enter meiotic prophase but are unable to progress beyond the pachytene stage. Instead, germ cells may exit meiotic prophase and return to mitotic proliferation.

As a test of this idea, we examined *gld-1(Tum)* larvae to determine whether tumorous germ cells enter meiotic prophase at the normal time during larval growth. *gld-1(Tum)* and *gld-1(+)* L4 larvae of known age were examined at 1-hr intervals through the L4 stage to determine the precise time at which proximal germ cells first display a pachytene nuclear morphology. For these experiments, we also needed to eliminate spermatogenesis that sometimes occurs in *gld-1(Tum)* L4 larvae marked with *unc-32* or *unc-13* (see MATERIALS AND METHODS), because any pachytene-stage *gld-1(Tum)* germ cells that later form sperm would not contribute to the tumorous phenotype. Accordingly, we also examined *gld-1(Tum)* animals that were homozygous for a *fog-1(lf)* mutation that causes germ cells to differentiate exclusively in the female mode (BARTON and KIMBLE 1990). A time course study showed that in wild type and *fog-1* single mutants, proximal germ cells first reach pachytene during mid-L4 (Figure 9); within a few hours these cells differentiate as either sperm (in wild type) or oocytes [in *fog-1(lf)*] (BARTON and KIMBLE 1990) (data not shown). Pachytene-stage nuclei were first observed in *gld-1(Tum)* and *fog-1(lf) gld-1(Tum)* animals at the same time (45–47 hr) as in wild type; therefore a *gld-1(Tum)* allele has no measurable effect on the timing with which germ cells first enter meiotic prophase. In most *fog-1(lf) gld-1(Tum)* germlines at mid-L4 (45–47 hours), all proximal germ cells display a pachytene morphology (Figure 10a). These germ cells never complete meiotic development, however, but instead appear to return to mitosis. This is indicated by the appearance, in mid to late L4 larvae (50–54 hr), of mitotic figures intermixed among proximal pachytene nuclei (Figure 10b). As animals mature to adulthood, pachytene-stage germ cells disappear from the proximal germline and are replaced entirely by proliferating germ cells. This ectopic proliferation eventually expands into the distal half of the germline, giving the mature adult tumorous phenotype illustrated in Figures 7 and 8. We believe it unlikely that proximal mitotic germ cells in *gld-1(Tum)* mutants reenter meiotic prophase for the following reasons. First, during late L4, the number of proximal pachytene nuclei steadily decreases. Second, in the adult, no meiotic prophase nuclei are observed in the proximal region.

Based on morphology, it is likely that tumorous germ cells proliferate using a mitotic rather than a meiotic spindle. Ectopically dividing *gld-1(Tum)* germ cells display a metaphase plate similar to that of distal mitotic cells. Further, anti-tubulin staining (data not shown) reveals that the spindle poles contain asters, structures that are not present in germ cells undergoing female meiotic divisions (ALBERTSON 1984; ALBERTSON and THOMSON 1993).

These data reinforce the idea that germ cells in *XX* *gld-1(Tum)* larvae and adults enter meiotic prophase

mitotic nuclei (b and c, open arrows) and the corresponding mitotic spindles (b′ and c′, open arrows) in the most proximal region of the germline (b and b′), the loop region (c and c′) and the part of the distal germline near the loop (distal mitotic in a). A cytoplasmic core is not observed. The distal tip region (e and e′) contains proliferating cells as in wild type (see Figure 5, c and d). Significantly, both distal transition zone (TZ) and a region of pachytene nuclei [(d) and PZ in a] are recognizable in their normal positions. Pachytene nuclei (arrows in d) in *gld-1(Tum)* germlines usually appear somewhat disorganized relative to wild type. Often intermixed with pachytene nuclei are nuclei in which chromosomes appear to be desynapsed; these nuclei contain 12 DAPI-staining dots that probably represent single chromosomes. These germ cells may have already exited pachytene. An identical adult tumorous morphology was observed for *gld-1* alleles *q485*, *q268* and *q365*. Scale bars, 10 μm.

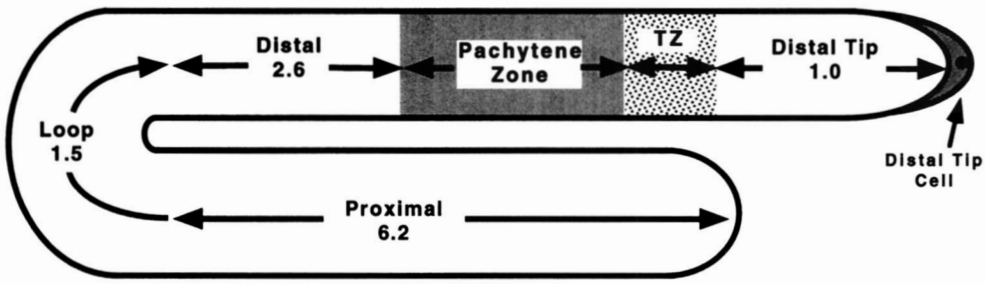

FIGURE 8.—Schematic summary of germline proliferation and polarity in *gld-1(Tum)* hermaphrodites. Diagrammed are the proximal, loop and distal regions where ectopic proliferation occurs, the distal tip region where normal germline proliferation occurs and the intervening region where germ cells enter meiotic prophase [transition zone (TZ) and pachytene zone]. Thus, there are two populations of proliferating germ cells: a population of ectopically proliferating germ cells that are the result of exit from meiotic prophase and a population of proliferating germ cells in the distal tip region (that are similar or identical to wild type) that are "premeiotic" as they have not entered the meiotic pathway. Each number represents the fold increase in proliferation relative to the distal mitotic region; the total number of mitotic nuclei (DAPI and tubulin-stained mitotic figures) were counted in a given region and divided by the total number of mitotic nuclei seen in the distal tip region. The data were derived from mitotic counts in 20 adult *gld-1(q268)* germlines. The proximal mitotic counts are likely to be underestimates due to difficulties in reliably scoring internal mitotic figures.

normally but are unable to progress beyond the pachytene stage. Instead, germ cells appear to exit meiotic prophase and return to mitosis. Additional support for this interpretation is presented in the accompanying paper (FRANCIS *et al.* 1995). Because *gld-1(Tum)* germ cells developing in animals of either sex can execute spermatogenesis (see below and MATERIALS AND METHODS) (FRANCIS *et al.* 1995), *gld-1(+)* is not required for male meiotic development. Instead, the return to mitosis phenotype may arise from a failure in *gld-1(Tum)* hermaphrodites to specify or execute the oocyte fate.

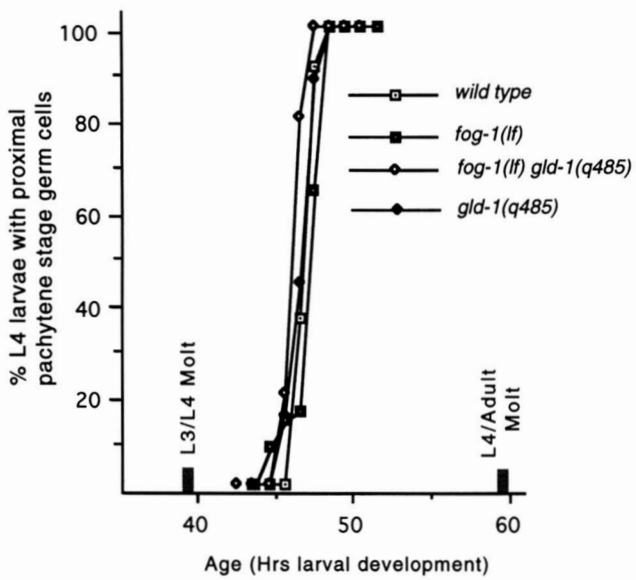

FIGURE 9.—*gld-1(Tum)* germ cells enter meiotic prophase at the normal time during larval development. Hermaphrodite larvae marked with *unc-32* were synchronized to within 1 hr (see MATERIALS AND METHODS) and examined at 1-hr intervals by DAPI staining. The ordinate represents the fraction of L4 larvae at each time point that had pachytene nuclei in the proximal germline of one or both gonad arms. Each data point is based on examination of 10 or more animals. The timing of initial entry of proximal germ cells into meiotic prophase is unaffected in a *gld-1(q485)*, a *fog-1(q180)*, or a *fog-1(q180)gld-1(q485)* background. A similar pattern of entry into meiotic prophase was observed for *gld-1(Tum)* alleles *q268* and *q365*, although fewer time points were analyzed.

Disruption of Germline Sex Determination by *gld-1(Tum)* Alleles

***gld-1(Tum)* mutations affect spermatogenesis in hermaphrodites but not in males:** *gld-1(Tum)* alleles have no apparent effects on *XO* males. For the three alleles examined in most detail (*q485*, *q268* and *q365*), homozygous males appear normal in both germline and somatic development and they sire cross-progeny efficiently. Thus *XO* male spermatogenesis is not dependent on *gld-1* activity. In contrast, *gld-1(Tum)* alleles have variable effects on spermatogenesis in *XX* hermaphrodites. As summarized in Table 3, animals homozygous for certain *gld-1(Tum)* alleles sometimes make sperm of normal morphology at the appropriate time (L4 stage). However, for these alleles, only a small fraction (<10%) of Tum gonads make sperm, and for other alleles, spermatogenesis is not observed. Further, when spermatogenesis does occur, *XX gld-1(Tum)* germlines make significantly fewer sperm than does wild type (Table 3). One explanation for these effects is that *gld-1(Tum)* alleles may feminize the *XX* germline by disrupting the processes required for germ cells to adopt the male fate. This hypothesis cannot be evaluated directly, however, because we are unable to assess sexual fates in the tumorous germlines. Therefore we have relied on the analysis of dominant effects of *gld-1(Tum)* alleles to assess the normal role of *gld-1* with regard to sex determination in the hermaphrodite germline.

Dominant effect of *gld-1(Tum)* alleles on hermaphro-

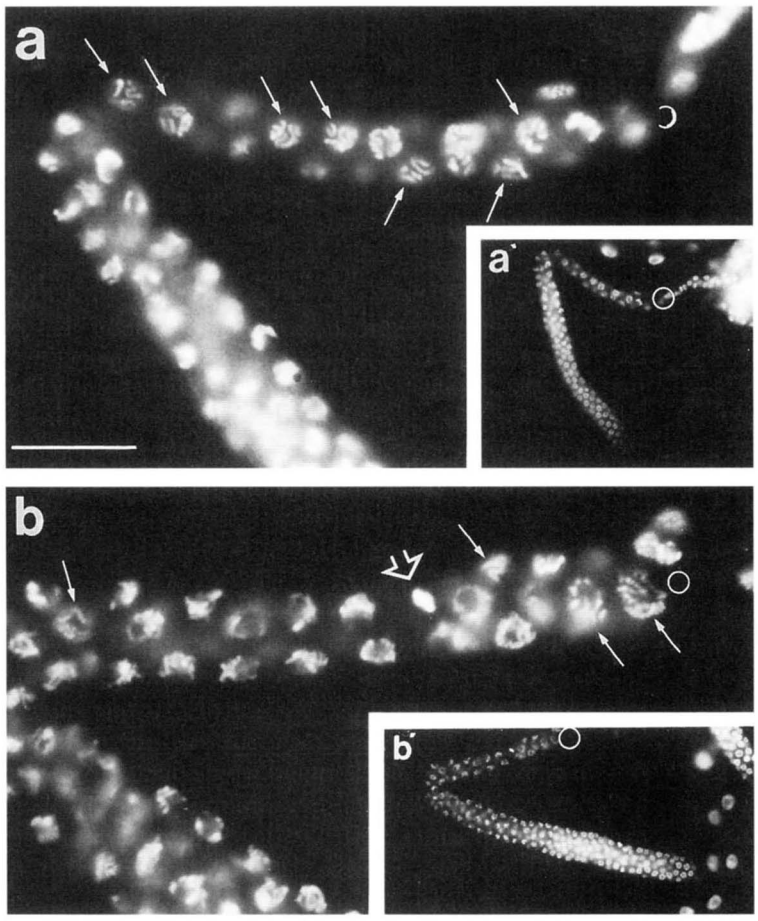

FIGURE 10.—Pachytene germ cells in *gld-1(Tum)* L4 larvae exit meiotic prophase and return to mitosis. Dissected gonads from *fog-1(q180) gld-1(q268)* L4 hermaphrodite larvae were stained with DAPI to assess nuclear morphology. High- and low-magnification (a and a′) views of a mid-L4 stage gonad. All proximal nuclei (arrows) show the threadlike chromosomal organization diagnostic of the pachytene stage of meiotic prophase. High-and low-magnification (b and b′) views of a gonad from an animal 6–8 hr older than that shown in a. Pachytene nuclei (arrows) are still evident, but some nuclei have lost the typical pachytene appearance; in addition, one or more mitotic figures (open arrows) are typically seen in late L4 larvae, indicating a return to mitosis. Similar results were obtained with the *gld-1(Tum)* alleles *q485* and *q365* (data not shown). Scale bar, 10 μm.

dite spermatogenesis: Analysis of *gld-1(Tum)* alleles, as well as two deficiencies of the *gld-1* region, indicate that *gld-1* exhibits a haplo-insufficiency (a dominant effect of eliminating one gene dose) (HODGKIN 1993) for hermaphrodite spermatogenesis. For the two deficiencies (*ozDf5* and *nDf25*), *XX Df(gld-1)/+* animals display a low-penetrance Fog phenotype in which 1–2% of hermaphrodite gonad arms make only oocytes (Table 4). A low-penetrance Fog phenotype is also observed in *gld-1(Tum)/+* hermaphrodites heterozygous for any one of eight *gld-1(Tum)* alleles (Table 4). Because Fog gonad arms begin oogenesis earlier than normal, late in L4 when only sperm would normally be made (see MATERIALS AND METHODS), the absence of sperm represents a transformation in sexual fate from male to female. We also find that brood size, which is a measure of the number of functional sperm produced (WARD and CARREL 1979), is significantly reduced in *gld-1(q485)/+* hermaphrodites, as compared with wild-type animals (Table 3). The low-penetrance Fog phenotype thus represents one extreme of a general reduction in the number of germ cells that undergo spermatogenesis in *Df(gld-1)/+* and *gld-1(Tum)/+ XX* animals. The dominant effects of *gld-1* deficiencies and Tum alleles are most simply interpreted as a haplo-insufficiency associated with reduced *gld-1(+)* activity (also see DISCUSSION). This interpretation implies that *gld-1(+)* activity promotes specification of the male fate in the *XX* germline. Because *gld-1(+)* is clearly not essential for male germline development (*X0* mutants are unaffected), we refer to this *gld-1* function as "promotion of hermaphrodite spermatogenesis."

To more fully investigate the basis for the dominant Fog phenotypes of *gld-1(Tum)* alleles, gene-dosage studies (MULLER 1932; MAINS *et al.* 1990) were performed using an attached duplication that carries wild-type alleles of the *gld-1* and *unc-13* genes (Table 5). Surprisingly, we find that only one *gld-1(Tum)* allele, *q485*, behaves similarly to a deficiency in dosage tests. For both *q485* and the deficiency *ozDf5*, the penetrance of the dominant Fog phenotype is unaffected by adding an extra dose of the mutant allele (*m/m/+ = m/+*) and completely suppressed by adding an extra dose of wild type (*m/+/+ = +/+*). Thus, like a *gld-1* deficiency, *q485* shows no dose-dependent effects on hermaphrodite spermatogenesis, as would be expected for a null allele.

In contrast to *q485*, nine other *gld-1(Tum)* alleles are characterized by dominant effects that are dose dependent. This conclusion is based on two aspects of the

TABLE 3

Extent of spermatogenesis in selected *XX gld-1* mutants

Class	Allele[a]	% Gonad arms[b,c] that produced sperm	Mean number of sperm/gonad arm[d]
	gld-1(+)	100	168[e] (±25, *n* = 12)
A1	*q485/+*	98	108[e,f] (±26, *n* = 11)
	q485	0	
A2	*oz89*	0	
	q93oz55	0	
	q361	0	
	oz17oz47	1	ND[g]
	q93oz49	3	58 (±27, *n* = 21)
	q365	6	51 (±25, *n* = 11)
	q268	18	55 (±27, *n* = 16)
B	*q93oz50*	100	311[h] (±42, *n* = 10)
	oz116	100	265[h] (±49, *n* = 10)
C	*q93/+*	100	272[e] (±46, *n* = 12)
	q93	100	1430 (±307, *n* = 8)

[a] Column entries are unmarked *gld-1(x)/gld-1(x)* mutants unless indicated otherwise.

[b] Greater than 150 gonad arms were scored for each genotype. Data for *q485/+* is from Table 4.

For class A1 and A2 homozygotes there is a marker effect on the extent of spermatogenesis. When marked with *unc-13* or *unc-32*, *gld-1(q485)* homozygotes can sometimes make sperm. Similarly, *unc-13* or *unc-32* marked *gld-1(q268* or *q365)* homozygotes have an increased number of gonad arms that undergo spermatogenesis (see MATERIALS AND METHODS).

[d] The number of sperm and primary spermatocytes (=4 sperm) per gonad arm was counted in DAPI-stained young adults. For *q93*, sperm number was determined 2 days after L4. (±1 SD, *n* = sample size).

[e] Sperm number per gonad arm is mean brood size divided by two for *dpy-5 unc-13/+*, *unc-13 gld-1(q485)/dpy-5* or *unc-13 gld-1(q93)/dpy-5* hermaphrodites. In the case of *q485/+* and wild type, the brood size is not limited by the number of functional oocytes produced, as mating of purged hermaphrodites with wild-type males yields large numbers of cross-progeny. In the case of the *gld-1* Mog allele *q93/+*, the brood size may have been limited by a failure to maintain oogenesis.

[f] Only animals where both gonad arms were self-fertile are included.

[g] Not determined.

[h] Although *q93oz50* and *oz116* make more sperm than wild type, these mutations do not behave like typical masculinizing mutations (*e.g.*, *tra-2(lf)/+*, *fem-3(gf)*) (see SCHEDL and KIMBLE 1988), as they are unable to dominantly or recessively suppress *fog-2(q71)* [see MATERIALS AND METHODS].

TABLE 4

Semidominant feminization of the *XX* germ line by *gld-1* alleles

Class	Allele[a]	% Female gonad arms[b,c]	
gld-1(+)	+	0	(450)
Deficiency	*nDf25*	0.7*	(276)
Deficiency	*ozDf5*	2*	(129)
A1	*q485*	2*	(203)
A2	*q93oz53*	1*	(159)
	q495	1*	(208)
	oz127	2*	(258)
	q268	2*	(125)
	q365	2*	(174)
	oz17oz47	2*	(119)
	q93oz49	3*	(207)
	q361	8†	(206)
	q93oz55	12†	(145)
	oz89	14†	(166)
B	*q93oz12*	0	(123)
	q93oz45	0	(164)
	q93oz50	0	(103)
	q93oz52	0	(124)
	q93oz56	0	(140)
	oz116	0	(106)
D	*q126*	2*	(161)
	oz142	2*	(210)
E	*q266*	24†	(132)
F	*q343*	0	(148)

[a] Dominance was scored in animals of the genotype *unc-13 gld-1(x)/dpy-5 unc-13*, where the *gld-1(x)* allele (and *ozDf5*) was paternally derived. For *nDf25*, heterozygotes segregating from *unc-13 nDf25/unc-15* were scored (see MATERIALS AND METHODS for details).

[b] L4 larvae were picked *en masse* and scored within 18 hr by Nomarski microscopy for the presence or absence of sperm and proximal oocytes in each gonad arm.

[c] Entries with the superscript * differ significantly ($P < 0.025$, *z*-test) (FREUND 1973) from entries with superscripts † but not from one another. Values in parentheses are number scored.

data in Table 5. First, with each of these alleles, adding an extra dose of the mutant allele increases the penetrance of the dominant Fog phenotype, that is, *m/m/+* gonad arms are more likely to be Fog than are *m/+* gonad arms. Second, for seven of the nine alleles, *m/+/+* hermaphrodites sometimes possess a Fog gonad arm. Based on these comparisons, different *gld-1(Tum)* alleles are shown ranked in Table 5 according to the penetrance of their Fog phenotypes. The five alleles with the weakest feminizing effects behave similarly to a deficiency in *m/+* animals but confer a more highly penetrant Fog phenotype in *m/m/+* animals. In contrast, the alleles with the strongest effects (*e.g.*, *oz89*, *q361* and *q93oz55*) are more strongly feminizing in *m/+* animals than is the haplo-insufficiency associated with deficiencies of the *gld-1* region.

The above results show that only the *gld-1(Tum)* allele *q485* meets the criteria expected for a mutation that completely eliminates *gld-1* function. For *q485*, the dominant Fog phenotype is dose independent (*i.e.*, *m/+ = m/m/+*), indicating that it results simply from a haplo-insufficiency of *gld-1* function. Therefore *q485* has been put in a separate subclass (A1) to distinguish it from other *gld-1(Tum)* alleles (class A2). The A2 *gld-1(Tum)* alleles also appear to strongly reduce the *gld-1(+)* function necessary to direct oogenesis, because three A2 alleles (*q268*, *q365*, *q93oz49*) were found to

TABLE 5

Dosage dependence of the semidominant germline feminization conferred by *gld-1* alleles

Class	Allele	% Female gonad arms[a]		
		m/m/+[b]	*m/+*[c]	*m/+/+*[d]
	+			0 (209)
	ozDf5	1 (201)	1 (205)	0 (219)
A1	*q485*	1 (222)	1 (207)	0 (230)
A2	*oz127*	10 (712)	0.5 (227)	0 (201)
	q365	17 (208)	1 (206)	0 (287)
	q93oz53	17 (220)	2 (242)	0.9 (230)
	oz17oz47	19 (202)	2 (204)	0.8 (241)
	q268	41 (228)	5 (201)	2 (239)
	q93oz49	60 (213)	2 (429)	0.8 (237)
	oz89	62 (215)	12 (211)	7 (213)
	q93oz55	65 (211)	12 (217)	9 (216)
	q361	90 (208)	21 (213)	10 (202)
D	*q126*	37 (212)	2 (212)	0.5 (212)
	oz142	33 (218)	2 (243)	0 (217)
E	*q266*	56 (206)	22 (209)	5 (232)

[a] Animals with the indicated *gld-1(x)* dosage were picked *en masse* as L4 larvae and scored by Normarski microscopy within 18 hr for the presence or absence of sperm. For all alleles except *q485* and *ozDf5*, entries *m/m/+* and *m/+* are significantly different from one another ($P < 0.025$; *z*-test) (FREUND 1973). Values in parentheses are number of gonad arms scored.

[b] Column entries have the genotype *unc-13 gld-1(x)/unc-13 gld-1(x);nDp4/+*.

[c] Column entries have the genotype *unc-13 gld-1(x)/unc-15* and were obtained as segregants from mothers of the same genotype. Data for *m/+* is not significantly different from that shown in Table 4, where the allele is introduced by crossing *gld-1(+)* mothers with heterozygous males (see MATERIALS AND METHODS).

[d] Except for *gld-1(+)*, column entries have the genotype *unc-13 gld-1(x)/unc-13;nDp4/+*. For *gld-1(+)*, the genotype was *unc-13/unc-13;nDp4/+*.

behave similarly to deletions in complementation tests (Figure 4). However, A2 alleles also display dose-dependent effects on hermaphrodite spermatogenesis that suggest these alleles are *gf* with respect to promotion of hermaphrodite spermatogenesis. The dose-dependent Fog phenotype of A2 alleles is suppressed by wild type (Table 5), consistent with an antimorphic or dominant-negative poisoning effect. One possible explanation for how A2 mutations can have both *lf* and *gf* properties is that they may encode nonfunctional *gld-1* products with antimorphic effects; that is, the mutant products may interfere with products of the *gld-1(+)* allele and thereby reduce the level of *gld-1(+)* activity available to promote spermatogenesis.

Class D: Feminization of the Germline (Fog)

The class D alleles, *q126* and *oz142*, exhibit two properties that are unique among *gld-1* mutations. First, these alleles eliminate hermaphrodite spermatogenesis but do not affect oogenesis: most *XX gld-1(q126* or *oz142)* homozygotes are functional females (Fog phenotype, 75% penetrant, Table 2) that produce cross-progeny on mating. Second, both mutations also affect germline sex determination in males: *X0 gld-1(Fog)* animals have a normal male soma but possess a germline that makes sperm and then oocytes. Mutant males exhibit normal mating behavior and can sire small broods. Thus, in both sexes, class D alleles disrupt the decision of germ cells to adopt the male fate but have no effect on other germline processes (oogenesis, progression through meiotic prophase) that are disrupted by other classes of *gld-1* alleles. Based on their similarity in phenotype to the *fog* genes (SCHEDL and KIMBLE 1988; BARTON and KIMBLE 1990; ELLIS and KIMBLE 1995), we designate the class D mutations as *gld-1(Fog)* alleles.

Several properties of the *gld-1(Fog)* alleles are informative as to how these alleles affect *gld-1* activity. First, because *XX gld-1(Fog)* homozygotes and *gld-1(Fog)/Df(gld-1)* animals make functional oocytes, class D alleles must retain significant *gld-1(+)* activity. Second, the ability of *gld-1(Fog)* alleles to feminize the *X0* male germline indicates that these alleles must be *gf* lesions, because a *gld-1* null allele has no effect on males. Gene dosage tests confirm the *gf* nature of *gld-1(q126)*: the penetrance of its dominant Fog phenotype is greater in *m/m/+* animals than in *m/+* animals (Table 5). Third, the phenotypes of *gld-1(Fog)* alleles are enhanced in *trans* to a *gld-1* deficiency. Whereas *XX gld-1(Fog)* homozygotes sometimes make sperm, *gld-1(Fog)/Df(gld-1)* animals never make sperm (Figure 4). This genetic behavior is inconsistent with the class D alleles producing a Fog phenotype by increasing *gld-*

1(+) activity and further argues that *gld-1(Fog)* alleles are partial *lf* mutations with respect to promoting hermaphrodite spermatogenesis. Taken together, these observations suggest that the *gld-1(Fog)* product possesses (1) substantial *gld-1*(+) function needed for meiotic prophase progression and oogenesis, (2) residual *gld-1*(+) activity that acts to promote hermaphrodite spermatogenesis and (3) *gf* activity that poisons a component of the sex determination pathway required for spermatogenesis in both sexes.

Class C: Masculinization of the Germline (Mog)

Class C alleles confer a semidominant Mog phenotype in which the *XX* germline produces a vast excess of sperm. These 10 alleles were isolated as dominant suppressors of the Fog self-sterile phenotype conferred by either *fem-1(hc17)* or *fog-2(q71)* mutations (see MATERIALS AND METHODS). When homozygous in an otherwise wild-type background, nine of the *gld-1(Mog)* mutants display a hermaphrodite-specific sterile phenotype: the *XX* germline makes a vast excess of sperm [>1000 sperm per gonad arm for the allele *q93* (Table 3)] and never switches to oogenesis. Animals homozygous for the remaining allele, *oz10*, also make excess sperm, but they sometimes make oocytes and become self-fertile as older adults. Thus all 10 class C alleles masculinize the *XX* germline so that excess spermatogenesis occurs at the expense of oogenesis. The class C alleles do not affect the soma of *XX* or *X0* animals or the *X0* germline. Based on their similarity in phenotype to *fem-3(gf)* (BARTON *et al.* 1987) and *mog* gene mutations (GRAHAM and KIMBLE 1993; GRAHAM *et al.* 1993), we designate the class C mutations as *gld-1(Mog)* alleles.

Two types of dominant phenotypes are observed in *gld-1(Mog)*/+ animals. First, heterozygotes make more sperm than do wild type before switching to oogenesis (Table 3). This partial masculinization presumably accounts for the dominant suppression of *fem-1* and *fog-2* females by *gld-1(Mog)* alleles. Second, in some *gld-1(Mog)*/+ adults, oogenesis ceases prematurely and is replaced by either renewed spermatogenesis or the production of undifferentiated germ cells in the proximal germline (Table 6). The undifferentiated germ cells reach the pachytene phase of meiotic prophase but fail to undergo either spermatogenesis or oogenesis. This failure to maintain oogenesis is at least partially attributable to the dominant masculinizing effects of *gld-1(Mog)* alleles because it can be suppressed by feminizing mutations at other loci (*e.g.*, *fog-2*, *tra-2(gf)*) (FRANCIS *et al.* 1995). Thus *gld-1(Mog)* mutations not only disrupt the initial switch to oogenesis but also the mechanism that stably maintains repression of spermatogenesis.

The *gld-1(Mog)* mutations have been placed into three subclasses based on distinct phenotypes. The five C1 alleles confer a homozygous Mog phenotype at all temperatures and display dominant effects that are ≥10% penetrant (Table 6). The four C2 alleles confer a Mog phenotype at 20 and 25° but at 15° result in a phenotype similar to that of class B alleles: sperm then undifferentiated germ cells arrested in meiotic prophase (see below and data not shown). Additionally, C2 alleles show a lower level of semidominant masculinization (<5%) than do C1 alleles (Table 6). The single C3 allele *oz10* differs from C1 and C2 alleles in two respects. First, *oz10* homozygotes sometimes make functional oocytes (Table 2). Second, the gonad arms of *oz10*/+ heterozygotes often show a significant delay in the switch to oogenesis (Table 6).

Based on the semidominant Mog phenotype, which is opposite that of the semidominant effects of *gld-1* deficiencies, *gld-1(Mog)* alleles must be *gf* mutations. Consistent with this idea, *gld-1(Mog)* alleles were isolated at a frequency (1×10^{-5} per haploid genome, see MATERIALS AND METHODS) significantly lower than that of loss-of-function mutations in other genes (10^{-3} to 10^{-4}) (BRENNER 1974; GREENWALD and HORVITZ 1980). To demonstrate that these rare mutations are in fact *gld-1* alleles, we sought to isolate intragenic revertants that reduce or abolish the *gf* Mog phenotype; for this purpose, we reverted the ability of *gld-1(Mog)*/+ to suppress the self-sterile Fog phenotype of *fog-2 XX* animals (see MATERIALS AND METHODS). Ten suppressors of the *gld-1(Mog)* alleles *q93* and *oz17* were isolated (Table 2) that appear to be intragenic mutations based on several criteria. First, the mutations are tightly linked to the original *gld-1(Mog)* allele and were isolated at a high frequency (2×10^{-3} per haploid genome), consistent with the generation of loss-of-function events. Second, one new mutation, *ozDf5*, is a small deficiency of the *gld-1* region, demonstrating that reversion can result in the elimination of *gld-1*(+) function and that our method was not biased against lethal events. Third, the other nine revertants are homozygous viable and display either of two preexisting *gld-1(lf)* phenotypes: four confer class A tumorous phenotypes and five confer class B phenotypes (Table 2 and see below). Finally, in complementation tests, the viable revertants behave similarly to class A or class B alleles that are single mutants. For example, *q93oz49* and *q485* both give the same spectrum of phenotypes when in *trans* to other classes of *gld-1* alleles (Figure 4). Collectively, these properties indicate that the revertants are *cis* double mutants in which the new mutation reduces or eliminates the *gf gld-1(Mog)* activity. Although intragenic revertants have only been isolated for the C1 alleles *q93* and *oz17*, we classify all 10 alleles as *gld-1(Mog)* mutations based on their similar map positions and phenotypes.

To investigate the nature of *gld-1(Mog)* mutations, we used gene dosage studies. These tests demonstrate that the Mog phenotypes of *gld-1(Mog)* mutations of each subclass are suppressed by the presence of a *gld-1*

TABLE 6

Semidominant masculinization of the *XX* germline by *gld-1(Mog)* alleles

Class	Allele[a]	% Gonad arms not switched to oogenesis[b]: 1-day adults	% Gonad arms that reinitiated spermatogenesis[c]: 4-day adults	% Gonad arms containing proximal undifferentiated cells[c]: 4-day adults
	+	0 (189)	0 (208)	0 (208)
C1	*q62/+*	0 (134)	8 (100)	14 (100)
	q93/+	1 (102)	6 (106)	13 (106)
	oz17/+	0 (110)	8 (112)	7 (112)
	oz34/+	0 (114)	4 (121)	6 (121)
	oz35/+	6 (124)	6 (117)	11 (117)
C2	*oz16/+*	0 (108)	0.8 (108)	4 (108)
	oz29/+	0 (106)	2 (102)	2 (102)
	oz30/+	0 (102)	0.9 (109)	4 (109)
	oz33/+	0 (112)	0.8 (118)	3 (118)
C3	*oz10/+*	19 (100)	0.8 (121)	0.8 (121)

Values in parentheses are number of gonad arms scored.

[a] Dominance was scored in animals of the genotype *unc-13 gld-1(Mog)/dpy-5 unc-13*, where the *gld-1(Mog)* allele was paternally-derived (see MATERIALS AND METHODS for details).

[b] L4 Larvae were picked *en masse* and scored 24 hr later by Nomarski microscopy.

[c] L4 Larvae were picked *en masse* and scored 1 and 4 days later by Nomarski microscopy. All gonad arms included in these two columns were observed to first make sperm and then switch over to oogenesis. Subsequently, oogenesis failed to continue, and either spermatogenesis was reinitiated (third column) or undifferentiated germ cells (fourth column) were observed.

(+) allele (Table 7, *m/m* > *m/m/+*). In view of the proposal that *gld-1(+)* activity helps promote spermatogenesis in *XX* hermaphrodites, it is possible that *gld-1 (Mog)* alleles encode poisonous products that inactivate some component of the sex determination machinery needed to make and maintain the switch from spermatogenesis to oogenesis (see DISCUSSION). Alternatively, *gld-1(Mog)* alleles might disrupt the switch to oogenesis by increasing *gld-1(+)* activity. If this is the case, *gld-1(Mog)* alleles cannot be simple hypermorphic mutations (as defined by MULLER 1932) because their mutant phenotypes are not enhanced by the addition of a wild-type gene dose.

A property common to the C1 and C2 *gld-1(Mog)* mutants is that *XX* homozygotes make only sperm and never show signs of oogenesis. To ask whether these alleles are defective for oogenesis, we examined whether they could complement a recessive class E *gld-1* allele. As described below, animals homozygous for the class E allele, *q266*, make abnormal oocytes and fail to make sperm. The C1 */q266* and C2 */q266 XX* animals display similar phenotypes: both make sperm, undifferentiated germ cells and abnormal oocytes like those seen in *q266* homozygotes (Figure 4). Thus C1 and C2 *gld-1(Mog)* alleles are unable to complement the class E abnormal oocyte phenotype, suggesting they lack *gld-1* functions required for oogenesis. In contrast, the C3 allele, *oz10*, is not defective for oogenesis (Table 2) and it is able to fully complement *gld-1(q266)* (C3/*q266* is fertile, data not shown).

Class B: Sperm and then Germ Cells Arrested in Meiotic Prophase

Class B includes one single mutant (*oz116*) and five intragenic revertants of *gld-1(Mog)* alleles (Table 2). *XX* homozygotes for each class B allele make sperm but fail to make oocytes. In place of oocytes, the proximal germline fills with undifferentiated germ cells that show no evidence of oocyte differentiation (Figure 3c). Examination by DAPI staining indicates that the undifferentiated germ cells reach the pachytene stage but fail to progress further in meiotic prophase (Figure 5e). Based on this phenotype, we designate the class B phenotype as arrested in meiotic prophase (Table 2). Because male germ cell differentiation occurs normally in class B mutants, we infer that the undifferentiated pachytene arrested germ cells are either intersexual, sexually uncommitted or arrested female germ cells. As class B alleles show some phenotypic heterogeneity (see below), we have used two alleles, *q93oz50* and *oz116*, which show intermediate phenotypes, as representative alleles.

Four class B alleles are likely to represent partial loss-of-function mutations. For these mutations, the most proximally located undifferentiated germ cells in adults sometimes exit meiotic prophase and proliferate ectopically. The penetrance of this partial tumorous phenotype is variable, ranging from ~5% (for *q93oz50* and *oz116*) to 20–30% (for *q93oz53* and *q93oz56*) affected gonad arms. These four alleles thus share three proper-

TABLE 7

Dosage dependence of germline phenotypes conferred by *gld-1(Mog)* alleles

Class	Pattern of gametogenesis	% gonad arms with indicated gametogenesis pattern[a]			
		q93/q93[b]	*q93/q93/+*[c]	*q93/+*[d]	+/+
C1	Sperm, then oocytes	0	21	80	100
	Sperm, then oocytes, then sperm[e]	0	48	11	0
	Sperm only (Mog)	100	19	2	0
	Sperm, then undifferentiated[f]	0	12	7	0
		oz30/oz30	*oz30/oz30/+*	*oz30/+*	
C2	Sperm, then oocytes	0	88	100	
	Sperm, then undifferentiated[f]	0	9	0	
	Sperm only (Mog)	100	3	0	
		oz10/oz10	*oz10/oz10/+*	*oz10/+*	
C3	Sperm, then oocytes	33	66	98	
	Sperm, then oocytes, then sperm[e]	1	4	2	
	Sperm only (Mog)	66	30	0	

[a] Animals were picked individually as L4 larvae and scored by Nomarski microscopy on each of the next 4 days. The progression of germ cell phenotypes observed over this period fell into one of the four indicated classes. *n* is greater than 90 for all genotypes.

[b] Column entries have the genotype *gld-1(x)/gld-1(x)*.

[c] Column entries have the genotype *unc-13 gld-1(x)/unc-13 gld-1(x);nDp4/+*.

[d] Column entries have the genotype *unc-13 gld-1(x)/unc-15*.

[e] Germlines that ceased oogenesis and reinitiated spermatogenesis. Some germlines that reinitiated spermatogenesis also contained undifferentiated germ cells in the proximal gonad arm.

[f] Gonad arms in which sperm and oocytes were observed on the first day and then followed by the appearance of undifferentiated germ cells proximally on the third or fourth day.

ties with the strong loss-of-function class A tumorous mutants: an absence of oogenesis, failure of germ cells to progress past pachytene of meiotic prophase and ectopic proliferation in the proximal germline.

The undifferentiated pachytene arrest phenotype of class B mutants could reflect an intersexual phenotype in which germ cells attempt to undergo both male and female differentiation. This possibility is especially relevant to class B alleles isolated as intragenic revertants of *gld-1(Mog)* mutants because these alleles might retain residual *gf* masculinizing activity. Such activity could, for example, block oogenesis while being insufficient to drive continued spermatogenesis. To examine whether the arrested germ cells in class B mutants are intersexual, two tests were performed. First, we examined whether the meiotic arrest phenotype of *q93oz50* and *oz116* could be suppressed by a mutation in *fem-3*, a gene required for the male germ cell fate [*XX fem-3(lf)* animals are female] (HODGKIN 1986). In the *q93oz50;fem-3(lf)* and *oz116;fem-3(lf)* double mutants, male germline development (spermatogenesis) was eliminated and all germ cells arrested as undifferentiated cells in pachytene (data not shown). Removal of *fem-3* function thus has no effect on the class B phenotype, other than eliminating spermatogenesis. Second, we examined *q93oz50* and *oz116* homozygotes for staining with a monoclonal antibody (SP56) directed against a set of proteins expressed specifically in sperm and primary spermatocytes (WARD *et al.* 1986). This antibody decorated sperm and spermatocytes in both mutants but failed to stain the pachytene-arrested germ cells (data not shown). We conclude that the germ cells that arrest at pachytene are unlikely to be intersexual but are either sexually uncommitted or blocked in an early step of oogenesis.

Classes E and F: Abnormal Oocytes

Classes E (*q266*) and F (*q343*) are each represented by a single mutant allele that disrupts oogenesis (Table 2). These two mutants exhibit similar yet distinct hermaphrodite-specific phenotypes. In both, proximal germ cells begin oogenesis but form only small abnormal oocytes (shown for *q266* in Figure 3b). Although abnormal, the oocytes formed in these mutants appear to arrest at diakinesis of meiotic prophase (Figure 5f), as occurs in wild-type oocytes. Thus, class E and F alleles appear to disrupt late steps in oogenesis without blocking nuclear progression through meiotic prophase. The recessive nature of these mutations, as well as their less severe effects on oogenesis as compared with *gld-1(Tum)* mutants, suggests that class E and F alleles may be partial *lf* mutations.

Although similar in phenotype, the class E and F alleles display several properties that indicate they belong in different classes. First, these alleles complement one another: *XX q266/q343* animals are self-fertile hermaphrodites or cross-fertile females (Figure 4). This suggests that *q266* and *q343* disrupt different aspects of *gld-1* function. Second, the abnormal oocyte phenotypes of the two mutants are morphologically distinct. Whereas *q343* hermaphrodites produce variably sized oocytes that sometimes appear to be fertilized, *q266* hermaphrodites produce smaller oocyte-like cells that are never fertilized. Third, although *q343* has no apparent effect on germline sex determination, *q266* confers a hermaphrodite-specific Fog phenotype. *XX q266* homozygotes never make sperm and heterozygotes often fail to make sperm. The dominant Fog phenotype conferred by *q266* is highly penetrant, dosage-dependent and competed by a *gld-1(+)* allele (Table 5). Therefore, like the class D *gld-1(Fog)* alleles described above, *q266* is associated with a *gf* alteration in *gld-1* activity that eliminates hermaphrodite spermatogenesis. In contrast to class D alleles, however, *q266* has no effect on *X0* males.

Complementation Behavior of *gld-1* Alleles

Complementation tests were performed using one or more representative allele of each class (Figure 4). In addition to the results discussed above, the complementation data reveal three general points.

1. For all classes of *gld-1* alleles except class D, homozygous phenotypes are unaltered in *trans* to a deletion of the *gld-1* region. In other words, the phenotypes of *gld-1(x)/gld-1(x)* and *gld-1(x)/Df* animals are qualitatively similar and usually indistinguishable. For other genes (though clearly not for *gld-1*) equivalent phenotypes as homozygotes and in *trans* to deletions has often been a useful criterion for defining null mutations.
2. We are unable to arrange the six different classes into a simple allelic series that might reflect quantitative differences in the amount of *gld-1* activity remaining in the mutants. This is in part due to the property that each class confers a qualitatively distinct phenotype. A further complication is that alleles of some classes (*e.g.*, classes C and E) have both *lf* and *gf* characteristics.
3. Intragenic complementation is observed in a number of cases. As discussed above, class E *(q266)* and F *(q343)* mutants have similar abnormal oocyte phenotypes, yet 100% of the *trans*-heterozygotes make normal oocytes (Figure 4). Intragenic complementation is also observed between class C Mog mutations and the class D Fog mutations *(q93/q126* and *oz17/q126* are 100% self-fertile), which have opposite effects on sex determination. The complementation between class C and D alleles is reminiscent of the complementation observed between masculinizing and feminizing alleles of the *fem-3* locus (BARTON *et al.* 1987). An interesting possibility is that *gld-1(Fog)* and *gld-1(Mog)* mutations may affect germline sex by two distinct mechanisms. Complementation would then represent a balancing of masculinizing and feminizing activities analogous to the effects that are observed when masculinizing and feminizing mutations in separate loci are combined (BARTON *et al.* 1987; SCHEDL and KIMBLE 1988). Alternatively, either example of intragenic complementation is consistent with *gld-1* gene products acting as a multimer in which mixing of subunits compensates for the effects of mutant gene products.

DISCUSSION

Germline development requires the precise control and coordination of at least three processes: entry and progression through meiosis, sex determination and gametogenesis. In this paper, we describe a novel gene, *gld-1*, that regulates several aspects of hermaphrodite germline development. *gld-1* can mutate to yield distinct germline-specific phenotypes associated with defects in oogenesis, progression through meiotic prophase and/or sex determination. We have characterized 31 *gld-1* mutations and placed them into six phenotypic classes. Table 8 summarizes the properties of the six classes of *gld-1* mutations with regard to overall phenotype, specific germline processes that are disrupted and the likely genetic basis for each phenotype. The table illustrates four general features of *gld-1* genetics: (1) *gld-1* alleles of different classes vary with respect to the germline processes that are disrupted, (2) the different classes cannot be arranged in an allelic series that reflects quantitative differences in residual *gld-1* gene activity, (3) disruption of progression through meiotic prophase and/or oogenesis in the different mutant classes results from recessive loss-of-function (*lf*) or elimination-of-function (null) lesions in *gld-1* and (4) many *gld-1* alleles that display a complete or partial *lf* disruption of meiotic prophase progression and/or oogenesis also show gain-of-function (*gf*) effects on hermaphrodite spermatogenesis.

From our analysis of mutant phenotypes, we conclude that *gld-1(+)* functions in at least two aspects of germline development. First, *gld-1* is essential for oogenesis, functioning either to specify the oocyte fate or to direct the early stages of oocyte differentiation. Second, *gld-1* acts as a nonessential component of the sex determination machinery that specifies the male germ cell fate in the hermaphrodite germline. We refer to this minor *gld-1* function as "promotion of hermaphrodite spermatogenesis."

TABLE 8

Summary of the *gld-1* mutant phenotypic classes: proposed genetic basis for defects in germline processes

Class, mutant phenotypes[b]	Germline processes affected by *gld-1* mutations[a]		
	Oogenesis[c]	Progression through meiotic prophase[d,e]	Promotion of hermaphrodite spermatogenesis[f]
Wild type:			
Sperm, then oocytes	+	+	+
A			
Tumorous germline (Tum)			
A1	lf-Null	lf-Null	lf-Null
A2	lf-Null	lf-Null	gf [Fog]
B			
Sperm, then germ cells arrested in pachytene[g]	lf	lf	+
C			
Masculinization of germline (Mog)			
C1 and C2	lf	lf	gf [Mog]
C3	+	+	gf [Mog]
D			
Feminization of germline (Fog)	+	+	gf [Fog]
E			
Fog, abnormal oocytes	lf	+	gf [Fog]
F			
Sperm, then abnormal oocytes	lf	+	+
Df(gld-1)/+ (Fog)	+	+	lf

[a] +, wild-type execution of indicated germline process; lf-Null, defect in germline process is the result of a complete loss-of-function lesion in *gld-1;* lf, defect in germline process is the result of a partial loss-of-function lesion in *gld-1;* gf, defect in germline process is the result of a gain-of-function lesion in *gld-1*. gf lesions have either a feminization of the germline (Fog) phenotype or a masculinization of the germline (Mog) phenotype. See DISCUSSION and RESULTS for details and explanations.

[b] Specific allele(s) belonging to different classes (or subclasses) are listed in Table 2. Germline processes affected are for homozygous *XX* mutant animals unless indicated.

[c] Defects in oogenesis can be either the complete absence of oogenesis (classes A and B) or a block late in oogenesis (classes E and F). C1 and C2 *gld-1(Mog)* alleles are defective in both aspects of oogenesis, as revealed by complementation tests (see DISCUSSION and RESULTS).

[d] Applies only to germ cells that would normally develop as oocytes (the upstream sex determination genes are set in the female mode: *tra-2* and *-3* and *mog-1* are active and the *fem* genes and *fog-1* and *-3* are inactive) (FRANCIS *et al.* 1995). *gld-1* alleles do not affect meiotic prophase progression in germ cells that are undergoing spermatogenesis.

[e] Defects in progression through meiotic prophase can be either an exit from pachytene and a resumption of mitosis (class A) or a block in pachytene (class B). Subclasses C1 and C2 have a defect in pachytene progression, as revealed by complementation tests (see DISCUSSION and RESULTS).

[f] Refers to promotion of the male germline fate in the hermaphrodite. For all *gld-1* mutants, when male germline development occurs, differentiation of sperm (spermatogenesis) is normal.

[g] Alleles *q93oz12* and *q93oz45* retain some *gf q93* Mog activity.

Below we first discuss the role of *gld-1* in oogenesis and then consider how different classes of *gld-1* alleles affect germline sex determination and conclude by contrasting two hypotheses for how *gld-1* might direct oocyte development.

A tumorous germline is the *gld-1* null phenotype: Class A *gld-1(Tum)* alleles confer a hermaphrodite-specific phenotype in which germ cells proliferate ectopically to create a germline tumor. This phenotype is designated as tumorous based on (1) the absence of overt germ cell differentiation (Figure 3), (2) the production of a vast excess of germ cells compared with wild type (Figure 6) and (3) the presence of ectopic germ cell proliferation (Figures 7, 8 and 10). From complementation data, which show that only *gld-1(Tum)* alleles behave identically to deletions of the gene in combinations of *gld-1 trans*-heterozygotes (Figure 4), we conclude that *gld-1(Tum)* alleles are strong *lf* or null mutations with respect to the processes of oogenesis and progression through meiotic prophase.

We have designated the class A1 mutation *q485* as the canonical null allele, because it behaves identically to a deletion of the locus in gene dosage studies (also see below). Recent molecular analysis supports this assignment: *gld-1(q485)* contains a frame-shifting deletion in the amino-terminal portion of the coding region and is thus unlikely to make a *gld-1* product (A. JONES and T. SCHEDL, unpublished observations). Because the absence of gene activity results in a tumorous phenotype, *gld-1* can be considered a "tumor suppressor" locus (WEINBERG 1991).

***gld-1* is essential for oogenesis:** *gld-1(Tum)* mutations abolish oogenesis in *XX* hermaphrodites but have no apparent effects on germline development in *X0* males. Thus *gld-1* is required for oogenesis but has no essential function in any aspect of male germline development. Two lines of evidence indicate that the germ cells that give rise to the tumorous phenotype are developing along the female pathway. First, although *gld-1(Tum) XX* animals usually produce few or no sperm, spermatogenesis appears to be executed normally when it occurs. Second, analysis of mutant combinations between *gld-1(null)* and the sex determination genes (see the accompanying paper, FRANCIS *et al.* 1995) demonstrates that tumors form only when the sex determination pathway is set in the female mode [*tra-2* and *-3* and *mog-1* are active, repressing the terminal *fem/fog* genes (*fem-1*, *-2* and *-3*, and *fog-1* and *-3*)]. For female germline differentiation, *gld-1*(+) could either act to specify the oocyte fate or act at an early step in the differentiation of a cell that is already specified as an oocyte (see below for further discussion).

***gld-1* is necessary for progression through meiotic prophase:** Analysis of *gld-1(Tum)* hermaphrodites indicates that *gld-1*(+) is not required for entry of germ cells into the meiotic pathway. *XX gld-1(Tum)* germ cells first enter meiotic prophase at the normal time during late larval development (Figures 9 and 10), and distal germ cells continue to enter meiotic prophase through adulthood (Figure 7), as occurs in wild type. *gld-1(Tum)* germ cells progress to the pachytene stage of meiotic prophase but then exit meiosis and return to a proliferative cell cycle (Figure 10). This return to mitosis phenotype is further examined in the accompanying paper where we show that it also occurs when germ cells are forced to inappropriately enter the meiotic pathway (FRANCIS *et al.* 1995). These results all support the view that tumor formation arises from a return to mitosis by germ cells that inappropriately exit meiotic prophase.

The *gld-1(Tum)* return to mitosis phenotype implies a role for *gld-1* in promoting meiotic development in the hermaphrodite germline. Because *XX gld-1(Tum)* germ cells progress well into meiotic prophase (completing diplotene, zygotene and at least part of pachytene before exiting meiosis), the primary defect does not involve the initial decision between mitosis and entry into meiosis. Instead, *gld-1*(+) is required to maintain meiotic prophase progression by germ cells that are developing in the female mode (also see below, and FRANCIS *et al.* 1995). Exit from meiotic prophase is clearly not a normal feature of *C. elegans* germline development, because mitotic germ cells are never observed among cells undergoing meiotic development (HIRSH *et al.* 1976; KLASS *et al.* 1976; KIMBLE and WHITE 1981; STROME 1986; AUSTIN and KIMBLE 1987; SEYDOUX *et al.* 1990; CAPOWSKI *et al.* 1991; BEANAN and STROME 1992; CRITTENDEN *et al.* 1994; our observations). This suggests germ cells normally commit to the meiotic pathway by the pachytene stage, if not at the time of their entry into meiotic prophase. In principle, mutant *gld-1* germ cells that exit meiosis could return to a proliferative cycle similar to that of distal mitotic stem cells that have not yet entered meiosis. However, two observations argue that the ectopic proliferation that occurs in tumors is distinct from the distal premeiotic proliferation. First, unlike distal proliferation, ectopic proliferation does not require the *glp-1*-based signaling pathway (FRANCIS *et al.* 1995). Second, we see no evidence that the ectopically proliferating germ cells ever reenter the meiotic pathway. These observations argue that rather than returning to a distal mitotic stem cell-like state, tumorous germ cells develop by an aberrant mechanism that circumvents normal germline control processes.

The above considerations serve to distinguish the *gld-1(Tum)* phenotype from situations in other systems where exit from meiotic prophase can be an aspect of normal development. During Drosophila oogenesis, for example, the developing 16-cell cyst contains four germ cells that have entered meiotic prophase (CARPENTER 1994). Although one of these cells goes on to form the oocyte, the other three exit meiotic prophase at the pachytene stage, undergo modified mitotic cycles and differentiate as polyploid nurse cells. Thus exit from meiotic prophase in Drosophila oogenesis represents one step in the process that generates the appropriate cell types. The meiotic pathway in diploid yeast is induced by deprivation of glucose and nitrogen (MALONE 1990). Throughout much of meiotic differentiation, yeast cells are not irreversibly committed to the meiotic pathway (HONIGBERG *et al.* 1992; HONIGBERG and ESPOSITO 1994) and can return to mitotic growth when transferred to growth medium (ESPOSITO and KLAPHOLZ 1981). For yeast, this behavior may allow rapid adaptation to changing nutrient conditions.

Disruption of meiotic prophase progression and/or oogenesis by class B, E and F alleles: Three classes of *gld-1* mutations confer recessive phenotypes characterized by either the production of abnormal oocytes (classes E and F) or undifferentiated germ cells arrested in pachytene (class B). These phenotypes are recessive and likely to result from a partial loss of *gld-1*

function. Germ cell arrest in class B mutants may occur at the same point in pachytene at which *gld-1(Tum)* germ cells exit meiosis. Alternatively, it is possible that germ cells in the class B mutants progress past this point and arrest at a later stage of pachytene. Either possibility suggests that class B alleles must retain some gene activity. At this time we are unable to distinguish whether the pachytene-arrested germ cells are sexually uncommitted or blocked at an early stage of oogenesis (see RESULTS).

The class E and F alleles retain *gld-1* functions required for meiotic prophase progression but are defective for oogenesis. Both types of mutants make small abnormal oocytes that, like wild-type oocytes, arrest at diakinesis of meiotic prophase. These mutants thus show that *gld-1* can mutate to disrupt oogenesis without affecting the *gld-1* functions required for meiotic prophase progression. The class E and F mutants raise the possibility that *gld-1* may be required for aspects of oogenesis that occur late in oocyte differentiation.

***gld-1* functions to promote hermaphrodite spermatogenesis:** Although many *gld-1* mutations cause alterations in germline sexual fates (Table 8), interpretation of the normal role of *gld-1* in sex determination is difficult for two reasons. First, for many alleles, sexual fate transformations result from *gf* alterations in *gld-1* activity. Second, because inactivation of *gld-1* function abolishes oogenesis, we are unable to use the homozygous null phenotype to assess whether *gld-1(Tum)* germ cells adopt the female fate. Therefore, to infer the role of *gld-1* in hermaphrodite spermatogenesis, we have relied on analysis of dominant effects (haplo-insufficiencies) associated with the null allele *q485* and with deletions of the gene (Tables 4 and 5). Heterozygous *gld-1(q485)/+* and *Df(gld-1)/+ XX* germlines sometimes make only oocytes; moreover, when sperm are made, *q485/+* germlines make significantly fewer sperm than do wild type (Table 3). Thus, when the *gld-1(+)* allele is present in a single dose, there is a variable failure in the promotion of hermaphrodite spermatogenesis. Because deletions are most commonly associated with a reduced amount of gene activity or product (*e.g.*, STEWART and MERRIAM 1974; JARRY 1979; BIRCHLER 1983; VAN VACTOR *et al.* 1988; SCHEJTER and SHILO 1989; ANDERSSON *et al.* 1994; FISHER and SCAMBLER 1994), the haplo-insufficient feminizing effects of *q485* and *gld-1* deletions are likely to reflect a partial reduction of *gld-1* function. From this we infer that *gld-1* acts to promote the male germline fate during hermaphrodite development. Although sensitive to gene dose, this *gld-1* function is not essential for hermaphrodite spermatogenesis (see MATERIALS AND METHODS and FRANCIS *et al.* 1995).

A surprising finding to emerge from the gene dosage studies is that the A1 allele *q485* is the only *gld-1(Tum)* mutation that behaves like the deletions with regard to promotion of hermaphrodite spermatogenesis. The remaining 10 *gld-1(Tum)* alleles are strong *lf* mutations with respect to oogenesis but have been placed in a separate subclass (A2) because they display a dominant Fog phenotype that is not solely attributable to *gld-1* haplo-insufficiency. The A2 alleles thus have a *gf* defect with respect to the *gld-1* function that promotes hermaphrodite spermatogenesis. For each A2 allele, adding an extra dose of the mutant allele increases the dominant *XX* germline feminization (*m/m/+ > m/+*; Table 5), whereas adding an extra dose of a *gld-1(+)* allele decreases feminization (*m/+ > m/+/+*). As indicated by these properties, the germline feminization caused by A2 alleles results from *gf* effects that are competed by *gld-1(+)* activity. How can A2 alleles cause a *gf* disruption in specification of hermaphrodite spermatogenesis when these alleles appear to be strong *lf* mutations based on their tumorous phenotype? One simple explanation is that A2 alleles may encode nonfunctional *gld-1* products with dominant negative or antimorphic properties; that is, the activity of *gld-1(+)* in promoting hermaphrodite spermatogenesis may be poisoned in the presence of nonfunctional A2 products. Dominant negative effects on *gld-1(+)* function by A2 mutant products might then account for why A2 alleles are more strongly feminizing in heterozygotes than is the null mutation *q485*. Alternatively, it is possible that A2 mutant products may poison another gene product in the sex determination machinery that is important for hermaphrodite spermatogenesis. Either idea could also account for the *gf* Fog phenotype associated with the class E allele *q266* (Table 5).

Mechanisms by which *gld-1(Fog)* and *gld-1(Mog)* might disrupt germline sex determination: Class D *gld-1(Fog)* alleles exhibit two properties that distinguish them from other classes of *gld-1* alleles. First, these alleles retain *gld-1* functions required for oogenesis and meiotic prophase progression, as *XX gld-1(Fog)* homozygotes are females that make functional oocytes. Second, *gld-1(Fog)* alleles partially feminize not only the *XX* germline but also the *X0* male germline. Because *X0* males homozygous for a *gld-1* null allele have a normal male germline, *gld-1(Fog)* mutations must be *gf* lesions. This idea is reinforced by gene dosage tests: the dominant effects of the *gld-1(Fog)* alleles on sex determination in *XX* animals are dose dependent and competed by a *gld-1(+)* allele. It is unlikely that the *gld-1(Fog) gf* defect leads to an increase in *gld-1(+)* activity, because *gld-1* deletions enhance rather than suppress the Fog phenotype (see RESULTS). In view of these properties, it appears likely that *gld-1(Fog)* alleles produce a product that, while retaining *gld-1(+)* activity required for oogenesis, poisons germline sex determination. Because *gld-1* has no essential function in *X0* males, *gld-1(Fog)* mutant products are unlikely to simply interfere with *gld-1(+)* function. Instead, poisoning

probably involves inactivation of another gene product that acts in specification of the male identity in both *XX* and *X0* germlines.

Class C *gld-1(Mog)* alleles are *gf* lesions that semidominantly masculinize the *XX* germline. Homozygous *XX gld-1(Mog)* animals make only sperm (C1 and C2 alleles) or make excess sperm, with a low percentage of germlines being able to switch to oogenesis (C3 allele). As shown by gene dosage tests (Table 7), the dominant masculinizing effects of *gld-1(Mog)* alleles are dose-dependent (*m/m/+* > *m/+*) and competed by a *gld-1(+)* allele [*m/Df(gld-1)* > *m/+*]. Because *gld-1(+)* activity partially suppresses rather than enhances the mutant phenotype, *gld-1(Mog)* alleles do not behave as simple hypermorphic mutations that lead to increased *gld-1(+)* activity.

gld-1(Mog) mutants disrupt the switch from spermatogenesis to oogenesis and can also cause the reinitiation of spermatogenesis in animals that have already made the switch to oocyte production (Tables 6 and 7). Spermatogenesis in wild type, as well as in *gld-1(Mog)* animals [see accompanying paper (FRANCIS *et al.* 1995)], requires the activities of all the terminal *fem/fog* genes (HODGKIN 1986; BARTON and KIMBLE 1990; ELLIS and KIMBLE 1995). When the germline switches from the production of sperm to the production of oocytes, one or more of the terminal *fem/fog* genes is negatively regulated. *gld-1(Mog)* mutations must therefore directly or indirectly interfere both with the initial negative regulation of the terminal *fem/fog* genes necessary for the switch to oogenesis and with the maintenance of this downregulation. Dosage experiments argue that *gld-1(+)* competes with the *gld-1(Mog)* product to allow some negative regulation of the terminal *fem/fog* genes and, consequently, oogenesis.

What Is the Role of *gld-1* in Oocyte Development?

The complete absence of oogenesis in *gld-1* null mutants suggests two alternative hypotheses: that *gld-1(+)* acts to specify the oocyte fate or that *gld-1(+)* acts at an early step(s) in the differentiation of a cell that is already specified as an oocyte. Data presented in this paper are fully consistent with either model. Further, both models are also supported equally by two conclusions reached in the accompanying paper (FRANCIS *et al.* 1995). First, the *gld-1* function required for oogenesis acts downstream of known sex determination genes. Second, tumor formation by *gld-1(null)* germ cells requires that the upstream germline sex determination pathway be set in the female mode that would normally result in oocyte production. Below we briefly discuss the two models in relation to *gld-1* mutant phenotypes. In the future, it should be possible to use molecular markers for early events in oogenesis to determine if *gld-1(Tum)* germ cells have adopted the female fate before tumor formation.

Model a: *gld-1* acts to specify the oocyte fate: According to this hypothesis, *gld-1(Tum)* germ cells would be sexually uncommitted when the upstream sex determination pathway is set in the female mode. This supposition is consistent with data showing that the germ cells that give rise to the tumor are unlikely to be male or intersexual and thus may be sexually uncommitted or female (FRANCIS *et al.* 1995).

If *gld-1(+)* acts to specify the oocyte fate, then the choice of sexual fate and the decision to enter the meiotic pathway are unlinked in *gld-1(Tum)* mutants: the sexually uncommitted germ cells enter meiosis at the normal time in development. This idea is consistent with our current understanding of the temporal relationship between sex determination and entry into meiosis (BARTON and KIMBLE 1990), which does not rule out the possibility that wild-type germ cells may adopt their sexual fate as late as the pachytene stage of meiosis. It is unclear why a sexually uncommitted germ cell would exit pachytene and return to the mitotic cell cycle, but one can speculate that sexual commitment is necessary for progression through meiotic prophase.

If *gld-1* acts to specify the oocyte fate, is it possible that the *gf* Fog and Mog phenotypes result solely from mutant effects on oocyte specification? If this is true, *gld-1* would have no role in promoting the male fate; instead, spermatogenesis would only occur when *gld-1* activity is turned off. The *gf* Fog phenotypes of certain *gld-1* alleles would then result from mutant *gld-1* products being insensitive to the regulatory mechanism that normally represses *gld-1* activity. In contrast, the *gf* Mog phenotypes would be explained as dominant negative poisoning of *gld-1(+)* activity, so that germ cells preferentially adopt the male fate. There are at least three problems associated with the idea that a disruption of oocyte specification can by itself account for defects in promoting hermaphrodite spermatogenesis. First, to reconcile this model with the Fog phenotype of *gld-1(null)/+* and *Df(gld-1)/+* animals, one must make the counterintuitive assumption that a decrease in *gld-1(+)* gene dosage can lead to an *increase* in *gld-1(+)* activity. Second, this model does not explain why *XX gld-1(null)* homozygotes fail to make the normal number of sperm before becoming tumorous. Third, some A2 *gld-1(Tum)* alleles have a dominant *gf* Fog phenotype even though these alleles lack the *gld-1* functions required for oogenesis and meiotic prophase progression. Thus it is unclear how A2 mutant products could cause excess specification of oocytes when these alleles are defective for this function. Because of these problems, it is simpler to propose that *gld-1* has an independent function in promoting hermaphrodite spermatogenesis, in addition to its role in specifying the oocyte fate.

Model b: *gld-1* is required for oocyte differentiation

and meiotic prophase progression: According to this hypothesis, the major role of *gld-1(+)* is to regulate the expression or activity of several gene products that direct and coordinate oogenesis and progression through female meiotic prophase. The tumorous phenotype can be explained by either of two general mechanisms. In one, *gld-1(+)* may be required for a specific meiotic prophase event whose execution is crucial for maintaining commitment of female germ cells to the meiotic pathway. In the other, *gld-1(+)* may act more generally as a negative regulator of cell cycle factors that promote mitosis. For example, *gld-1(+)* could repress the activity of cell cycle factors that are made during oogenesis for use during the female meiotic divisions and/or early embryogenesis. In *gld-1(Tum)* mutants, mitotic cell cycle factors would become inappropriately activated at the time of their synthesis in pachytene, causing a short-circuit of the meiotic pathway and a return to the mitotic cycle. If *gld-1(+)* does function in silencing maternal gene products, this would account for why *gld-1(+)* is essential for oogenesis, but is not required for the execution of the male meiotic pathway.

Ovarian germ cell tumors in other systems: Certain Drosophila genes can mutate to yield an ovarian germline tumor phenotype (SPRADLING 1993). The relationship between the mechanisms of germline tumor formation in the worm and fly is unclear because the cellular origins of Drosophila ovarian tumors are poorly understood. It is not known, for example, whether any of the Drosophila germline tumors results from germ cells exiting meiotic prophase and returning to mitosis.

Investigations of the cellular origins of ovarian teratomas in mouse (EPPIG *et al.* 1977; HASHIMOTO *et al.* 1994) and humans (CARRITT *et al.* 1982; PARRINGTON *et al.* 1984; SURTI *et al.* 1990) have been very informative. A subset of ovarian teratomas in humans may arise from germ cells exiting meiotic prophase and returning to the mitotic cycle, as occurs in *gld-1(Tum)* mutants. PARRINGTON *et al.* (1984) and SURTI *et al.* (1990) compared the genotypes of teratomas and unaffected somatic tissues. In 20% and 35% of the cases examined, respectively, these authors observed that the founding germ cell had undergone meiotic recombination but failed to execute the reductional division (the teratoma was genotypically heterozygous for centromere-linked markers but homozygous for centromere-distal markers). One possibility is that the founding germ cell skipped meiosis I and executed meiosis II, analogous to the *spo13* mutant phenotype in yeast (BUCKINGHAM *et al.* 1990). Alternatively, the founding germ cell may have exited directly from meiotic prophase, analogous to the origin of the *gld-1* tumorous phenotype. Given the multistep mode of tumorigenesis in mammals, additional mutational events are presumably necessary to form a teratoma. In contrast, elimination of *gld-1(+)* activity is sufficient for tumorigenesis in *C. elegans*. Despite these differences, similar developmental defects may play a critical role in the formation of certain germline tumors.

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