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Analysis of the Multiple Roles of *gld-1* in Germline Development: Interactions With the Sex Determination Cascade and the *glp-1* Signaling Pathway

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ABSTRACT

The *Caenorhabditis elegans* gene *gld-1* is essential for oocyte development; in *gld-1(null)* hermaphrodites, a tumor forms where oogenesis would normally occur. We use genetic epistasis analysis to demonstrate that tumor formation is dependent on the sexual fate of the germline. When the germline sex determination pathway is set in the female mode (terminal *fem/fog* genes inactive), *gld-1(null)* germ cells exit meiotic prophase and proliferate to form a tumor, but when the pathway is set in the male mode, they develop into sperm. We conclude that the *gld-1(null)* phenotype is cell-type specific and that *gld-1(+)* acts at the end of the cascade to direct oogenesis. We also use cell ablation and epistasis analysis to examine the dependence of tumor formation on the *glp-1* signaling pathway. Although *glp-1* activity promotes tumor growth, it is not essential for tumor formation by *gld-1(null)* germ cells. These data also reveal that *gld-1(+)* plays a nonessential (and sex nonspecific) role in regulating germ cell proliferation before their entry into meiosis. Thus *gld-1(+)* may negatively regulate proliferation at two distinct points in germ cell development: before entry into meiotic prophase in both sexes (nonessential premeiotic *gld-1* function) and during meiotic prophase when the sex determination pathway is set in the female mode (essential meiotic *gld-1* function).

OOGENESIS in multicellular animals represents a complex developmental program in which the meiotic nuclear cycle and gametogenesis are coordinated to produce a functional oocyte. In the preceding paper, we describe the *Caenorhabditis elegans* gene *gld-1* (FRANCIS *et al.* 1995). The genetic and phenotypic properties of *gld-1* argue that it is a tumor suppressor gene that regulates oocyte development. Mutations that eliminate *gld-1* function abolish oogenesis in the hermaphrodite and result in the formation of a germline tumor. Germ cells that give rise to the tumor complete the early stages of meiotic prophase but then exit pachytene and return to a mitotic cycle. These cells subsequently undergo repeated rounds of ectopic proliferation, giving rise to a tumor that fills much of the germline. Tumor formation is a sex-specific phenotype, as *gld-1(null)* males show no abnormalities in germline development. Further, *gld-1* has no essential function in the soma.

The sex specificity of the *gld-1* tumorous phenotype has led to the proposal that *gld-1(+)* may act as an important regulator of oocyte development (FRANCIS *et al.* 1995). Tumor formation would then result from a failure of oocyte determination and/or female meiotic prophase progression that allows germ cells to return to mitosis. Data available at present, however, do not exclude the possibility that tumors result from an intersexual mode of differentiation. Ovarian tumors in certain Drosophila mutants are comprised of germ cells with intersexual traits (BAE *et al.* 1994; J. HORABIN, personal communication). In this report, we investigate the basis of the sex-specific tumorous phenotype using genetic epistasis analysis. By constructing mutant combinations with *gld-1(null)* and the sex determination genes, we ask whether tumor formation is specifically correlated with the female sexual fate in the germline. The cumulative results suggest that *gld-1(+)* acts downstream of all known sex determination genes to either specify the oocyte fate or direct oocyte differentiation.

We also use genetic epistasis to investigate a second previously inferred role of *gld-1* in germline development (FRANCIS *et al.* 1995). Although *gld-1* has no essential role in male germline development, it acts to promote spermatogenesis in the hermaphrodite germline. For several reasons, we are unable to investigate this aspect of *gld-1* function using a null *gld-1* allele. Therefore epistasis tests are done with two classes of gain-of-function alleles that cause transformations in sexual fate in the hermaphrodite germline. The data obtained are consistent with the proposal that *gld-1(+)* promotes spermatogenesis by assisting a set of sex determination loci that specify the male fate.

Finally, we examine whether germ cell proliferation in *gld-1(null)* hermaphrodites is under the same controls that regulate proliferation in the wild-type germline. Previous work has shown that germ cell prolifera-

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tion depends on the gene *glp-1*, which encodes a transmembrane protein belonging to the *lin-12/Notch* family of receptor molecules (AUSTIN and KIMBLE 1987, 1989; YOCHEM and GREENWALD 1989). *glp-1* protein acts as the likely receptor for a somatically derived signal that induces germ cell proliferation (AUSTIN and KIMBLE 1987; CRITTENDEN *et al.* 1994). We examine the dependence of germ cell proliferation in *gld-1(null)* mutants on the *glp-1*-mediated signaling pathway in two types of experiments: by ablating certain sets of somatic cells to eliminate the somatic signals and by analysis of *gld-1(null);glp-1(lf)* mutant combinations to eliminate the germline receptor. These experiments confirm the meiotic prophase origin of the tumorous phenotype and indicate that *gld-1(+)* has an additional, nonessential and/or redundant function in negatively regulating proliferation of germ cells before their entry into the meiotic pathway.

MATERIALS AND METHODS

Nematode culture and strains

General methods for *C. elegans* culture and genetic manipulation were as described (BRENNER 1974; SULSTON and HODGKIN 1988). Experiments were done at 20° unless otherwise noted. *C. elegans* nomenclature follows HORVITZ *et al.* (1979). *lf* is used for loss-of-function, *0* is used for a null allele that has been shown to fail to produce a gene product, *gf* is used for gain-of-function, *gld-1(Tum)* is used to identify alleles with a *tum*orous *XX* germline (Tum) phenotype, *gld-1(Fog)* is used to identify alleles with a *f*eminization *o*f the *g*ermline (Fog) phenotype and *gld-1(Mog)* is used to identify alleles with a *m*asculinization *o*f the *g*ermline (Mog) phenotype. Maternal and zygotic genotypes are indicated using the notation m(− or +) and z(− or +), where m represents the maternal genotype and z the zygotic genotype. All nematode strains used in this work are derived from the wild-type *C. elegans var. Bristol* isolate N2. 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* and *q187)* (BARTON and KIMBLE 1990), *unc-11(e47)*, *dpy-5(e61)*, *dpy-14(e188)*, *unc-13(e51* and *e1091)*, *gld-1* (all alleles) (FRANCIS *et al.* 1995), *fog-3(q443)* (ELLIS and KIMBLE 1995).

LGII: *dpy-10(e128)*, *tra-2(e1095)*, *tra-2(q122gf)* (SCHEDL and KIMBLE 1988), *unc-4(e120)*.

LGIII: *fem-2(e2105)*, *mog-1(q370)* (GRAHAM and KIMBLE 1993), *unc-69(e587)*, *tra-1(e1834)* and *tra-1(e1575gf)* (HODGKIN 1987), *unc-32(e189)*, *glp-1* [see text and Table 4, all described in AUSTIN and KIMBLE (1987) and/or KODOYIANNI *et al.* (1992)].

LGIV: *dpy-13(e458sd)*, *fem-1(e1991, e2003ts)*, *unc-5(e53)*, *unc-24(e138)*, *fem-3(e1996)*, *fem-3(q20gf* and *q95gf)* (BARTON *et al.* 1987), *dpy-20(e1282)*, *tra-3(e1107)*, *tra-3(bn75)* (E. CAPOWSKI, B. BRENDZA and S. STROME, personal communication).

LGV: *her-1(y101hv1)*, *unc-42(e270)*, *fog-2(q71)*.

LGX: *lon-2(e678)*.

Rearrangements: *hT2(I)[bli-4];hT2(III)[dpy-18]* (MCKIM *et al.* 1992), *nDp4(I;V)* (MCKIM *et al.* 1992).

Construction of double mutants between *gld-1* alleles and mutations in the sex determination pathway

Tables 1 and 2 describe the phenotypes of double or triple mutants made between *gld-1(Tum, Fog* or *Mog)* alleles and mutations in the major sex determination loci. Except where noted below, all doubles requiring a *gld-1(Tum)* allele were made both with the null allele *q485* and with the strong *lf* allele *q268*; identical phenotypes were observed in double mutants containing these *gld-1(Tum)* alleles. In many constructions, *gld-1* alleles were marked with *unc-13*, which lies ~0.2 map units to the left of *gld-1* (FRANCIS *et al.* 1995). In cases where the progeny segregating from a balanced strain were analyzed, the genotype of the balanced strain was verified by complementation tests. For mutations in the sex determination genes, we use *lf* to indicate that the allele is putative null or strong loss-of-function. Other types of alleles are indicated. At least 50 animals of the appropriate genotype were examined for each double or triple mutant constructed.

***fog-1(lf) gld-1(Tum* or *Mog)*:** Double mutants of *fog-1(lf)* allele *q180* were made using each of three *gld-1(Tum)* alleles (*q268*, *q365* and *q485*), as well as each of three *gld-1(Mog)* alleles (*oz10*, *oz30* and *q93*). To make recombinant *fog-1 unc-13 gld-1* chromosomes, we picked female Unc-13 nonDpy-5 recombinants segregating from *fog-1(q180) dpy-5 unc-13/unc-13 gld-1(Tum* or *Mog)* hermaphrodites. The resulting *fog-1 unc-13 gld-1* chromosomes were maintained balanced over *hT2*. Double mutants between a second *fog-1* allele (*q187*) and two *gld-1(Tum)* alleles (*q268* and *q365*) and the Mog allele *q93* were constructed by picking nonUnc-11 female progeny segregating from *fog-1 unc-11/unc-13 gld-1* hermaphrodites; the recombinant chromosome was balanced over *hT2*. *fog-1 unc-13 gld-1 X0* males were generated by crossing *fog-1 unc-13 gld-1/hT2* or + males to *fog-1 unc-13 gld-1/hT2* hermaphrodites.

***gld-1(Tum* or *Mog) fog-3(lf)*:** Double mutants with *fog-3(lf)* were constructed using *fog-3(q443)* and the *gld-1* tumorous allele *q485* and the Mog allele *q93*. Recombinant *unc-13 gld-1 fog-3* chromosomes were generated by picking recombinant nonUnc nonDpy females segregating from *unc-13 gld-1/dpy-14 fog-3* hermaphrodites. Balanced *unc-13 gld-1 fog-3/hT2* strains were used as the source of homozygous *unc-13 gld-1 fog-3* animals. Males of the same genotype were generated by crosses.

***fog-1(lf) gld-1(Tum) fog-3(lf)*:** A balanced triply mutant strain of the genotype *fog-1(q180) unc-13 gld-1(q485) fog-3(q443)/hT2* was constructed as follows. Self-progeny of heterozygous *fog-1 dpy-5/unc-13 gld-1 fog-3* hermaphrodites were screened for female recombinants that were nonDpy-5 and nonUnc-13. To identify recombinant females of the desired genotype (*fog-1 unc-13 gld-1 fog-3/fog-1 dpy-5*, resulting from recombination in the *fog-1 dpy-5* interval), single females were crossed with *unc-13/hT2* males. Only animals heterozygous for a recombinant *fog-1 unc-13 gld-1 fog-3* chromosome produced *X0* Unc-13 male progeny (genotype *fog-1 unc-13 gld-1 fog-3/unc-13*) that display the semidominant *fog-1/+* male germline phenotype (production of sperm and then oocytes) (BARTON and KIMBLE 1990). For three females that segregated such males, single *XX* L4 cross-progeny were picked onto individual plates to identify candidate *fog-1 gld-1 unc-13 fog-3/hT2* strains. Genotypes of these balanced strains were confirmed by using complementation tests to show that each was heterozygous for *fog-1(lf)*, *gld-1(Tum)* and *fog-3(lf)*.

***fog-1(lf) fog-3(lf)*:** *XX* animals of the genotype *fog-1(q180) unc-13 fog-3(q443)* were obtained from a balanced *fog-1 unc-13 fog-3/hT2* hermaphrodite strain constructed as follows. NonUnc nonDpy female recombinants segregating from *fog-1 dpy-5/unc-13 fog-3* hermaphrodites were picked and crossed individually to *hT2/unc-13* males. Recombinant females of

the desired genotype (*fog-1 unc-13 fog-3/fog-1 dpy-5*) were identified based on their segregation of *X0* Unc-13 male cross-progeny (genotype *fog-1 unc-13 fog-3/unc-13*) that display the semidominant *fog-1/+* male phenotype (production of sperm and then oocytes). From such crosses, single non-Unc-13 *XX* cross-progeny were picked onto separate plates to identify candidate *fog-1 unc-13 fog-3/hT2* animals. The genotypes of three independently isolated strains were then confirmed by showing that each carried mutations that fail to complement *fog-1* and *fog-3* mutations. Like *fog-1* and *fog-3* single mutants, *XX* and *X0 fog-1 unc-13 fog-3* animals have germlines that make only oocytes and show no obvious defects in proliferation or meiotic development.

***gld-1 (Tum* or *Mog);fem-1 (lf)*:** *fem-1(e1991)*, an amber allele (DONIACH and HODGKIN 1984), was used for the construction of double mutants with *gld-1(Tum)* alleles (*q485* and *q268*) and with *gld-1(q93)*. Approximately 25% of the Unc-24 self-progeny of *gld-1(Tum)/+;fem-1 unc-24/+* hermaphrodites were Tum, indicating *gld-1(Tum)* is epistatic to *fem-1*. Construction of *unc-13 gld-1(Mog);fem-1 unc-24* animals was facilitated by the property that *fem-1 XX* animals produced by *fem-1/+* mothers are often self-fertile hermaphrodites owing to maternal rescue. Therefore, Unc-24 hermaphrodites segregating from *unc-13 gld-1(Mog)/+;fem-1 unc-24/+* mothers were picked onto separate plates. Some hermaphrodites segregated broods consisting of three fourths female Unc-24 and one fourth sterile Unc-13 animals (*unc-13* is epistatic to *unc-24*) of the genotype *unc-13 gld-1(q93);fem-1 unc-24*. Heterozygosity for *gld-1(q93)* appears to increase the fraction of *XX fem-1(lf)* [m(+/−) z(−/−)] animals that make sperm, but this effect was not quantitated.

***gld-1 (Tum* or *Mog);fem-2 (lf)*:** *gld-1(q485)* or *gld-1(q93); fem-2* double mutants were constructed at 25° using *fem-2(e2105)*, a strong *fem-2(lf)* allele that is maternally rescued (HODGKIN 1986). Hermaphrodites segregating from *unc-13 gld-1(Tum* or *Mog)/+;fem-2/+* mothers were picked onto separate plates. Maternally rescued *unc-13 gld-1/+;fem-2* hermaphrodites segregated a 3:1 ratio of nonUnc females and sterile Unc-13 progeny of the genotype *unc-13 gld-1(Tum* or *Mog);fem-2*.

***gld-1 (Tum);fem-3 (lf)*:** Doubles of *gld-1(q268* or *q485)* with *fem-3(lf)* were made using *e1996*, a *fem-3* ochre nonsense allele (HODGKIN 1986; AHRINGER *et al.* 1992). Approximately one fourth of the Unc-24 progeny of *gld-1(Tum)/+;unc-24 fem-3 XX* animals had a Tum germline.

To generate *X0 gld-1(Tum);fem-3(lf)* animals, single *gld-1(q485)/+;unc-24 fem-3(e1996)/+ X0* males were mated with *gld-1(q485)/+;unc-24 fem-3(e1996);lon-2 XX* females. These crosses yielded Unc-24 Lon-2 *X0* hermaphrodites. About one half of these had a Tum germline.

***gld-1 (Tum);tra-2 (lf);fem-3 (lf)*:** *XX* animals of the genotype *gld-1(q485);dpy-10 tra-2(e1095);unc-24 fem-3(e1996)* were identified among the self-progeny of *gld-1(Tum)/+;dpy-10 tra-2/+;unc-24 fem-3/+* hermaphrodites. Approximately one fourth of the Dpy-10 Unc-24 self-progeny were tumorous, showing that the *gld-1(Tum)* phenotype is expressed in a *tra-2(lf); fem-3(lf)* background.

***gld-1 (Mog);fem-3 (lf)*:** Double mutants of *fem-3(e1996)* were constructed with *gld-1(Mog)* alleles representing each *gld-1(Mog)* subclass (C1, *q93*; C2, *oz30*; C3, *oz10*). In constructions with the C3 allele *oz10*, it was initially found that *oz10* can suppress the *XX* Fog phenotype of *fem-3(lf)* [m(+/−) z(−/−)] animals [*XX fem-3(lf)* is not normally rescued by maternal *fem-3(+)* activity (HODGKIN 1986)]. Thus *gld-1(oz10);unc-24 fem-3(e1996) dpy-20/+* mothers segregated many self-fertile Unc-24 Dpy-20 progeny that were subsequently shown to be homozygous for both *oz10* and *fem-3(lf)*. Homozygosity for *fem-3(lf)* was indicated by their segregation of only female progeny and homozygosity for *oz10* was determined by testing females for failure to complement *gld-1(q268)*. These results indicate that *oz10* can restore spermatogenesis in *fem-3(lf)* [m(+/−) z(−/−)] but not *fem-3* [m(−/−) z(−/−)] animals. Suppression by *oz10* was ~40% penetrant. The C1 and C2 *gld-1(Mog)* alleles *q93* and *oz30* were also found to sometimes restore spermatogenesis in *fem-3* [m(+/−) z(−/−)] *XX* animals. However, although sperm are often made in C1 or C2 *XX gld-1(Mog);unc-24 fem-3(e1996) dpy-20* animals produced by heterozygous mothers, these animals are always sterile because oocytes are never made (see RESULTS). The C1 allele, *q93*, restored spermatogenesis in 56% of *fem-3(lf)* [m(+/−) z(−/−)] gonad arms and the C2 allele restored spermatogenesis in 44% of gonad arms.

To generate *gld-1(q93* or *oz30);fem-3(lf)* animals that lack maternal *fem-3* activity, we crossed *unc-13 gld-1(Mog)/+;fem-3(lf) dpy-20/+* males with *unc-13 gld-1(Mog)/+;unc-24 fem-3(lf) dpy-20* females. From these crosses >100 Unc-13 Dpy-20 progeny were picked and their germline phenotype scored. Almost all (>97%) animals failed to make sperm and had germlines that contained only undifferentiated germ cells. The remaining animals made excess sperm in both gonad arms, indicating they were *gld-1(Mog);unc-24 fem-3 dpy-20/dpy-20* recombinants.

***gld-1 (Tum);her-1 (lf)*:** Double mutants with *her-1* were constructed using *her-1(y101hv1)*, a deletion allele that removes most of the *her-1* coding region (TRENT *et al.* 1991). To generate *XX gld-1(q485);her-1(lf)* animals, heterozygous *gld-1(Tum)/+;her-1(lf) unc-42/+* hermaphrodites were made and allowed to self. Approximately one fourth of the Unc-42 self-progeny had a Tum germline. To make *X0 gld-1(q485);her-1(lf)* hermaphrodites, *X0* males of the genotype *gld-1(Tum)/+;her-1(lf) unc-42/+* were crossed to *gld-1(Tum)/+;dpy-11 her-1(lf) unc-42;lon-2* hermaphrodites. nonDpy-11 Unc-42 Lon-2 animals, which must be *X0* cross-progeny, were picked *en masse* and their germline phenotype scored. About one fourth of the Unc-42 Lon-2 animals were Tum.

***gld-1 (Tum);tra-1 (lf)*:** *tra-1(e1834)*, a deletion allele that removes most of the *tra-1* coding region (ZARKOWER and HODGKIN 1992), was used to construct a *gld-1(q485);tra-1(lf)* double mutant. *unc-13 gld-1(Tum)/+;tra-1(lf)/+ XX* heterozygotes were constructed and their Unc-13 self-progeny with a male soma [of the genotype *unc-13 gld-1(Tum);tra-1(lf)*] were examined. Like the *tra-1(e1834)* single mutant, these animals have a completely male nongonadal soma and a gonadal soma that is sometimes male and sometimes morphologically abnormal. Germline phenotypes were scored only in animals whose somatic gonad showed no obvious morphological abnormalities.

***gld-1 (Tum);tra-1 (gf)*:** The *tra-1(gf)* allele *e1575gf* transforms both *XX* and *X0 tra-1(gf)/+* animals into females (HODGKIN 1987). However, whereas *XX tra-1(gf)/+* animals are morphologically normal females, *X0 tra-1(gf)/+* females have an abnormal, truncated tail. Therefore *XX* and *X0 tra-1(gf)/+* females can be distinguished by tail morphology. To generate double mutants, *unc-13 gld-1(q485* or *q268)/+* males were crossed with single *unc-13 gld-1(q485* or *q268)/+;tra-1(gf)/+ XX* females. All Unc-13 cross-progeny were somatic females and had a Tum germline. The Tum animals included *X0* somatic females with a truncated tail [*X0 unc-13 gld-1;tra-1(gf)/+*] and *XX* somatic females with a normal tail [50% *unc-13 gld-1;tra-1(gf)/+* and 50% *unc-13 gld-1*].

***gld-1 (Tum);tra-2 (lf)*:** The *tra-2* allele *e1095*, an ochre nonsense mutation (KUWABARA *et al.* 1992), was used to make *XX gld-1(q268* or *q485);tra-2(lf)* animals. From the self-progeny of *unc-13 gld-1(Tum)/+;tra-2(lf)/+* mothers, we exam-

ined *XX* Unc-13 pseudomales, 100% of which should be *unc-13 gld-1(Tum);tra-2(lf)* in genotype.

***gld-1(Tum);tra-2(gf)*:** The *tra-2(gf)* allele *q122gf* dominantly eliminates spermatogenesis in *XX* but not *X0* animals (SCHEDL and KIMBLE 1988). To generate *XX gld-1(q485);tra-2(gf)* animals, single *gld-1(Tum)/+;tra-2(gf) unc-4/+* males were crossed with single females of the same genotype. From crosses that gave nonUnc tumorous progeny, Unc-4 cross-progeny were scored. Of the *XX unc-4 tra-2(gf)* cross-progeny with a female soma, one fourth had a Tum germline. In contrast, all *X0 unc-4 tra-2(gf)* animals with a male soma had a normal male germline.

***gld-1(Tum);tra-3(lf)*:** Double mutants of *gld-1(q485)* were constructed with two *tra-3* alleles, *e1107* and *bn75*. *e1107* is an amber allele that transforms *XX tra-3* [m(−/−) z(−/−)] animals grown at 25° into pseudomales that have a partially masculinized soma and germline (HODGKIN 1980). *bn75* is an unusual temperature-sensitive *tra-3* allele; it has no major effect on the *XX* female soma but masculinizes the germline more strongly than do *tra-3* amber alleles (E. CAPOWSKI, B. BRENDZA and S. STROME, personal communication). The phenotype of double mutants with both *tra-3* alleles were analyzed at 25°. We first generated *XX unc-13 gld-1(q485)/+;tra-3* [m(+/−) z(−/−)] animals that, owing to maternal rescue by *tra-3*(+) activity, are self-fertile hermaphrodites. These segregated one fourth Unc-13 animals that are genotypically *unc-13 gld-1(Tum);tra-3*.

***gld-1(Tum);mog-1(lf)*:** The *XX gld-1(q485);mog-1(q370)* double mutant was examined at 25°, the temperature at which the *mog-1(lf)* phenotype is strongest (GRAHAM and KIMBLE 1993). Starting with *gld-1(Tum)/+;mog-1 unc-69/+* hermaphrodites, we scored all *XX mog-1 unc-69* self-progeny, one fourth of which should be homozygous for *gld-1(Tum)*. For the data in Table 1, the percentage of total *mog-1 unc-69* animals that showed ectopic germline proliferation was multiplied by four to obtain an estimate (7%) of the fraction of *gld-1(Tum);mog-1 unc-69* animals with ectopic proliferation. This number was subtracted from 100 to estimate the percentage *gld-1(Tum);mog-1 unc-69* animals with a Mog phenotype like that of the *mog-1* single mutant (*i.e.*, excess sperm, no ectopic proliferation).

***gld-1(Tum);fem-3(gf)*:** The *fem-3(gf)* alleles *q20gf* and *q95gf* confer a temperature-sensitive Mog phenotype in which animals grown at 25° produce only sperm, whereas animals grown at 15° usually (*q20gf*) or sometimes (*q95gf*) make both sperm and oocytes (BARTON *et al.* 1987). Double mutants of *fem-3(q20gf* and *q95gf)* with *gld-1(q485* and *q365)* were obtained from strains of the genotype *unc-13 gld-1(Tum);fem-3(gf);nDp4/+* that were maintained at 15°. These strains were made by first constructing *unc-13 gld-1(Tum)/+;fem-3(gf)/dpy-20;nDp4/+* heterozygotes at 15°. Starting with these heterozygotes, we first identified *unc-13 gld-1(Tum);nDp4/+* hermaphrodites based on their segregation of ~55% Tum animals. Animals homozygous for *fem-3(gf)* were identified by following elimination of *dpy-20*. For the data in Figure 1 and Table 1, *unc-13 gld-1(Tum);fem-3(gf) XX* animals were picked as newly hatched L1 larvae and grown for 58 hr at 25°.

Dominant suppression of *fog-2(lf)* and *tra-2(gf)* mutations by a *gld-1(Mog)* allele

The *gld-1(Mog)* allele *q93* was shown to dominantly suppress the female self-sterile phenotypes of *XX fog-2(q71)* and *XX tra-2(q122gf)* animals. For *fog-2(lf)*, *XX unc-13 gld-1(q93)/+;fog-2(lf) rol-9* are hermaphrodite in contrast to *fog-2(q71)* animals, which are always female. However, *XX unc-13 gld-1(93);fog-2 rol-9* homozygotes have a Mog phenotype like that of *XX gld-1(q93)* animals, showing that *gld-1(q93)* is epistatic to *fog-2(lf)*. Therefore *gld-1(q93)* cannot masculinize the *XX* germline by acting through *fog-2*(+).

Dominant suppression of *tra-2(gf)* by *gld-1(q93)* was demonstrated by showing that *XX unc-13 gld-1(q93)/+;tra-2(gf)* animals are self-fertile hermaphrodites instead of females. These hermaphrodites segregate sterile *unc-13 gld-1(Mog);tra-2(gf)* progeny that first make sperm and then undifferentiated germ cells.

Double mutants with the *gld-1* fog allele *q126*

XX double mutants of the genotypes *gld-1(q126);tra-1(e1832)* and *gld-1(q126);tra-2(e1095)* were obtained as *XX* self-progeny from *unc-13 gld-1(q126)/+;tra-1/+* or *gld-1(q126)/+;tra-2/+* hermaphrodites. *XX* Unc-13 Tra-2 pseudomales and *XX* Unc-13 Tra-1 males were picked and their germline and somatic phenotypes examined. The double mutant *unc-13 gld-1(q126);tra-3(e1107)* was examined at 25°. Unc-13 pseudomales segregating from *unc-13 gld-1(q126)/+;tra-3* [m(−/+) z(−/−)] mothers were examined for germline and somatic abnormalities. *gld-1(q126)* has no obvious effect on the male somatic phenotypes of *XX tra-1*, *tra-2* or *tra-3* mutants. Finally, a homozygous *gld-1(q126);unc-24 fem-3(q20gf) dpy-20* hermaphrodite strain was constructed by virtue of the ability of *gld-1(q126)* to suppress the sterile Mog phenotype of *fem-3(q20gf)* animals grown at 25°. Specifically, *XX gld-1(q126)/+;unc-24 fem-3(q20gf) dpy-20/+* heterozygotes grown at 25° segregated many self-fertile Unc-24 Dpy-20 hermaphrodites. Several of these hermaphrodites were shown to contain both *gld-1(q126)* and *fem-3(gf)* by the independent segregation of both types of mutations after outcrossing with wild-type males.

Construction of strains mutant for *gld-1* and *glp-1*

***gld-1(Tum);glp-1* double mutants:** Table 4 and Figure 8 describe the different *gld-1(Tum);glp-1(0* or *lf)* genotypes examined in this work. For each, we constructed balanced heterozygous strains of the general genotype *gld-1(Tum)/hT2(I);unc-32 glp-1/hT2(III)*. Owing to *hT2(I)*[*bli-4*];*hT2(III)*[*dpy-18*]-induced pseudolinkage, these heterozygotes segregate Unc-32 self progeny that are essentially always of the genotype *gld-1(Tum);unc-32 glp-1*. The *gld-1*(+);*glp-1*(+) control animals used for comparison in all experiments were the Unc-32 self-progeny of *unc-32/hT2* hermaphrodites. During passaging, the *hT2* chromosome *I-III* pseudolinkage was found to occasionally break down: if a single nonUnc tumorous animal or a nonDpy Bli-4 animal was observed, the plate was discarded.

To construct balanced strains heterozygous for *gld-1(Tum)* and *glp-1* alleles, *gld-1/hT2* males were crossed with *unc-32 glp-1/hT2* hermaphrodites. Of the resulting male progeny, one half were heterozygous for *hT2*, whereas one quarter had the desired genotype of *gld-1/+;unc-32 glp-1/+*. Single males were crossed with *hT2* [*bli-4;dpy-18*] hermaphrodites; sires that produced no Dpy-18 (*hT2*) male cross-progeny were identified as being *gld-1/+;unc-32 glp-1/+*. From these crosses, 25 or more L4 *XX* cross-progeny were picked onto separate plates and their self-progeny inspected in the following generation. In most cases, balanced *gld-1(Tum)/hT2;unc-32 glp-1(0)/hT2* strains were easily identified because their *gld-1(Tum);unc-32 glp-1(0* or *lf)* progeny had a germline phenotype distinct from those of both *gld-1(Tum)* and *glp-1* single mutants. The genotypes of many of these strains were confirmed by out-crossing or by complementation testing. In addition, because *gld-1(Tum);unc-32 glp-1(q172)* and *gld-1(Tum);unc-32* animals have identical Tum germline phenotypes, we verified the presence of the *glp-1(q172)* allele in double mutants by PCR analysis. PCR analysis was done using

primers that bracket the 260 bp of *glp-1* sequence that is deleted by *q172* (KODOYIANNI *et al.* 1992). The *glp-1* DNA fragment amplified from *gld-1(Tum);unc-32 glp-1(q172)* homozygotes showed the expected size reduction (compared with *gld-1(Tum);unc-32* control animals), confirming that these animals were homozygous for *q172*.

***gld-1(Tum);glp-1(0 or lf)* males and masculinized *XX* animals:** Several balanced *gld-1(q485)/hT2;unc-32 glp-1(0* or *lf)/hT2* strains were maintained as *X0* male / *XX* hermaphrodite strains to provide a source of *X0* double mutants. The male germline phenotypes of different *gld-1(q485);glp-1(0* or *lf)* double mutants varied depending on the allele, ranging from a Glp phenotype [for the *glp-1(0)* alleles *q46* and *q175* and the strong lf allele *q224*] to essentially wild type [for the strong *lf* allele *q172*]. In addition, we examined *XX gld-1(q485);glp-1(q172* or *q224)* animals that were homozygous for *fem-3(q20gf)*, a mutation that masculinizes the *XX* germline (BARTON *et al.* 1987). To construct these strains, *gld-1(q485)/hT2;unc-32 glp-1(q172* or *q224)/hT2* males were crossed with *hT2;fem-3(q20gf)* hermaphrodites at 15°. Single nonDpy-18 (non-hT2) cross-progeny were picked onto separate plates and the *fem-3(gf)* chromosome was made homozygous by testing different lines grown at 25° for expression of the *fem-3(gf)* Mog phenotype (BARTON *et al.* 1987). The *glp-1* alleles *q172* and *q224* behaved differently in the *XX gld-1(Tum);unc-32 glp-1;fem-3(gf)* triple mutants; the triple mutant with *q172* had a Mog phenotype (see RESULTS), whereas that with *q224* had a Glp-1 phenotype.

***gld-1(Tum);glp-1(0)* triple mutants bearing *lf* mutations in *fog-1*, *fem-1* or *fem-3*:** Table 4 lists the triple mutants that were constructed using a *gld-1(Tum)* allele, a *glp-1(0)* allele (*q175* or *q46*) and one of the following *lf* mutations: *fog-1(q180)*, *fem-1(e2003ts)* or *fem-3(e1996)*. In all cases, triple mutants were obtained from balanced heterozygous strains. To make triple mutants carrying *fog-1(lf)*, we first constructed a balanced *fog-1 gld-1/hT2;unc-32/hT2* strain (as described above for the construction for *gld-1/hT2;unc-32 glp-1/hT2*). A cross of *fog-1 gld-1/hT2;unc-32/hT2* hermaphrodites with *unc-32 glp-1(0)/hT2* males was then used to generate hermaphrodites of the genotype *fog-1 gld-1(Tum)/+;unc-32 glp-1(0)/unc-32*. These were then crossed with *hT2/unc-32* males, and wild-type hermaphrodite cross-progeny were picked onto individual plates. Hermaphrodites that were *fog-1 gld-1/hT2;unc-32 glp-1/hT2* were initially identified based on the unique germline phenotype of *fog-1 gld-1(Tum);unc-32 glp-1(0)* segregants (see RESULTS). These strains were outcrossed to confirm they carried both *gld-1(Tum)* and *glp-1(0)*.

Triple mutants containing *fem-1(e2003ts)* were obtained from a balanced strain of the genotype *gld-1(Tum)/hT2;unc-32 glp-1(0)/hT2;fem-1/dpy-13(e458sd) unc-5*. Heterozygous *gld-1(Tum)/hT2;unc-32 glp-1(0)/hT2* males were first crossed to *hT2;dpy-13 unc-5* hermaphrodites to generate males that were *gld-1(Tum)/hT2;unc-32 glp-1(0)/hT2;dpy-13 unc-5/+*. These males were then crossed to *hT2;fem-1* females. Cross-progeny that were partially Dpy-13, and hence *dpy-13 unc-5/fem-1* in chromosome *IV* genotype, were picked onto separate plates. Self-progeny were inspected in the following generation to ensure segregation of all phenotypes expected for the balanced strain. Triply mutant *gld-1(Tum);unc-32 glp-1(0);fem-1* animals were identified as Unc-32 animals that failed to show the semidominant *dpy-13* phenotype.

Triple mutants bearing *fem-3(e1996)* were obtained as segregants from a balanced strain with the genotype *gld-1(Tum)/hT2;unc-32 glp-1(0)/hT2;fem-3(e1996)/unc-24 fem-3(q20gf) dpy-20*. These strains were constructed by a method analogous to that used for construction of a balanced triple mutant containing *fem-1*. Approximately one third of Unc-32 nonUnc-24 nonDpy-20 segregants are homozygous for *fem-3(e1996)*.

Characterization of *gld-1(Tum);glp-1* double mutants and feminized triple mutants

Quantitation of germline proliferation: For assays of germline proliferation, the above described balanced strains were used as a source of hermaphrodites with the general genotype *gld-1(q485);unc-32 glp-1(+* or *0* or *lf)*. The feminized triple mutants *fog-1(lf) gld-1(Tum);glp-1(0)* and *gld-1(Tum);unc-32 glp-1(0);fem-1* were also examined (see Figure 6). To obtain tightly staged animals, newly hatched L1 larvae were picked as described (FRANCIS *et al.* 1995) and grown to the L4 stage or adulthood. At the times indicated in Figures 6 and 8, animals were fixed and stained with diamidinophenolindole (DAPI) (FRANCIS *et al.* 1995). For each timepoint, the number of germ cells per gonad arm was counted twice in ≥10 gonad arms, and the mean number of germ cells per gonad arm was determined. In experiments with nonconditional *glp-1* alleles (Figures 6 and 8A), animals were grown at 20°. Experiments with temperature-sensitive *glp-1* alleles (*q224, bn18, q415*) were done using animals grown from hatching at 25°.

Determination of when germ cells first enter meiotic prophase during larval growth: Data presented in the accompanying paper (FRANCIS *et al.* 1995) showed that *gld-1(q485)* does not affect the timing with which germ cells first enter meiotic prophase in a *glp-1(+)* background. For the present work, it was important to determine when *gld-1(Tum)* germ cells in various *fog-1(+* or *lf);glp-1(0* or *lf)* backgrounds first reach pachytene of meiotic prophase. This was done as described (FRANCIS *et al.* 1995) by examining DAPI-stained preparations of tightly staged larvae for the presence or absence of pachytene-stage meiotic germ cells. For each genotype, the mean age (in hours after hatching) at which pachytene nuclei were first observed was determined using 10 animals. Results were as follows: *unc-32 glp-1(0)* (23 hr, L2 stage), *fog-1;unc-32 glp-1(0)* (23 hr); *gld-1;unc-32 glp-1(0)* (31 hr, L3); *fog-1 gld-1;unc-32 glp-1(0)* (32 hr, L3); *unc-32* [46 hr, L4 (wild-type control)]; *fog-1(lf);unc-32* (47 hr); *gld-1(Tum);unc-32* (46 hr); *fog-1 gld-1;unc-32* (46 hr) and *gld-1;unc-32 glp-1(q172)* (46 hr). Entry into meiotic prophase in *fog-1(+* or *lf) gld-1(Tum);unc-32 glp-1(0)* animals is delayed relative to a *glp-1(0)* single mutant because germ cells undergo two additional rounds of cell division before entering meiosis. Note that *fog-1(lf)* has no effect on premeiotic proliferation by *gld-1(Tum)* germ cells nor does it increase the rate of tumor growth in *gld-1(Tum)* animals (FRANCIS *et al.* 1995) (data not shown)

Ablation of somatic gonad cells: Ablations of specific cells of the hermaphrodite somatic gonad were performed using a nitrogen pulse laser (Laser Sciences Inc.) set up as described by AVERY and HORVITZ (1987). The laser was coupled to a Zeiss Axioplan microscope and beam intensity was adjusted by the use of neutral density filters. Somatic cells in L1 and L2 gonad primordia were identified by their position and morphology (KIMBLE and HIRSH 1979) and ablated using 30–80 pulses from the laser. Operated animals were reexamined within 2 hr to confirm killing of the target cell(s) and to assess collateral damage.

Previous results have shown that ablation of the distal tip cell (DTC) precursors (Z1.a and Z4.p) does not produce a perfect phenocopy of the *glp-1(0)* phenotype. In the ablated animals, all germ cells enter meiotic prophase at the normal time (KIMBLE and WHITE 1981). By contrast, germ cells in *unc-32 glp-1(0)*, *gld-1(Tum);unc-32 glp-1(0)* and the feminized *gld-1(Tum);unc-32 glp-1(0)* triple mutants enter meiotic pro-

phase prematurely (see previous section) (AUSTIN and KIMBLE 1987). Furthermore, although ablation of the DTC precursors has no effect on sex determination (KIMBLE and WHITE 1981), *glp-1(lf)*-induced premature entry into meiotic prophase can cause masculinization of the germline in genetic backgrounds that are partially feminized. For example, *fem-2(ts)* mutants make only oocytes, whereas *fem-2(ts) glp-1(lf)* mutants make sperm at the restrictive temperature (E. MAINE and J. KIMBLE, unpublished observations). These differences may, at least in part, explain why DTC precursor ablations in *gld-1(null)* animals and *gld-1(null); glp-1(lf)* mutant combinations result in nonidentical phenotypes (see RESULTS).

A time-course study to evaluate the effects on killing the somatic DTC in *gld-1(Tum)* hermaphrodites was done as follows. *unc-32* and *gld-1(Tum); unc-32 XX* larvae were grown to the late L3 or young L4 stage and the DTC was killed in one or both gonad arms. Operated animals were grown for a further 24–40 hr and then prepared for dissection, fixation and staining with DAPI (FRANCIS *et al.* 1995). At least 12 operated animals were examined for each 2-hr interval between 24 and 40 hr after ablation of the DTC. Distal germ cells at the pachytene stage of meiotic prophase were generally first observed in the 28- and 32-hr intervals. Distal mitotic figures were first observed at ~32 hr after DTC ablation but were more common 34–38 hr after ablation.

Morphological evaluation of germline phenotypes: Observations of living animals by Nomarski differential interference contrast (DIC) microscopy were made as described (SULSTON and HODGKIN 1988). Generally, worms were picked as L4 larvae and examined as adults between 18 and 48 hr later. For staining with DAPI and antibodies, males and hermaphrodites were dissected, fixed and stained as described (FRANCIS *et al.* 1995). The monoclonal antibody SP56, which is directed against an epitope expressed only by sperm and primary spermatocytes (WARD *et al.* 1986), was used at a 1:20 dilution of culture supernatant.

RESULTS

In the first half of this section we describe experiments designed to investigate the sex specificity of the *gld-1* tumorous phenotype and the role of *gld-1* in promoting spermatogenesis in the hermaphrodite germline. As background for these experiments, we briefly review germline sex determination in *C. elegans*.

In *C. elegans* there are two sexes: *XX* hermaphrodites, which are considered to have a female soma and a hermaphrodite germline that makes first sperm and then oocytes, and *X0* males, which have a male soma and germline. Sexual identity in the germline is specified by a regulatory pathway of ≥14 genes whose activity is set in response to the ratio of *X* chromosomes to autosomes (refer to Figure 9) (reviewed by KUWABARA and KIMBLE 1992; CLIFFORD *et al.* 1994). The known terminal regulators of the pathway are a group of five genes (*fem-1*, *-2* and *-3* and *fog-1* and *-3*) that act together to specify the male identity in both *XX* and *X0* germ cells. In the *X0* male germline, activation of these terminal *fem/fog* genes is achieved indirectly via the *her-1* gene. *her-1*, which is active only in *X0* animals, negatively regulates the genes *tra-2* and *tra-3*, whose normal function is to negatively regulate one or more of the terminal *fem/fog* genes. As a result, the terminal *fem/fog* genes are active continuously in males, and *X0* germ cells form sperm throughout adulthood. In the hermaphrodite, the production of first sperm and then oocytes is achieved through a different mechanism. In place of *her-1*, which is not active in *XX* animals (TRENT *et al.* 1991), the *fog-2* gene (SCHEDL and KIMBLE 1988) may transiently repress *tra-2* and/or *tra-3* and thereby free the terminal *fem/fog* genes to direct a brief period of spermatogenesis. Later, increased *tra-2* activity (DONIACH 1986; SCHEDL and KIMBLE 1988; GOODWIN *et al.* 1993) and/or activation of the *mog-1* gene (GRAHAM and KIMBLE 1993) leads to negative regulation of one or more terminal *fem/fog* genes, and the germline switches to oogenesis.

Mutations in the sex determination genes allow the manipulation of germline and somatic sex independent of each other and of chromosomal sex. Therefore we have used epistasis analysis with *gld-1(Tum)* alleles and sex determination gene mutations to examine the effects of chromosomal, somatic and germline sex on tumor formation and to infer the relationship between the *gld-1(+)* function that directs oogenesis and the sex determination pathway. These data are summarized in Table 1 and detailed below. All experiments were done with the null *gld-1(Tum)* allele *q485*, and many were also performed with *q268*. Identical results were obtained with both alleles.

The *gld-1* tumorous phenotype depends on germline sexual identity

Tumor formation in *XX* germlines is independent of the male fate: Tumor formation in *XX* hermaphrodites that lack *gld-1* activity might result from a failure to specify the female germ cell fate or a defect in an early step in oocyte differentiation (FRANCIS *et al.* 1995). A prediction of either hypothesis is that inactivation of any of the five genes required for spermatogenesis (*fem-1, -2* and *-3* and *fog-1* and *-3*) should have no effect on tumor formation in *XX gld-1(Tum)* germlines. Analysis of *XX* double mutants (Table 1) shows that tumors still form when any one of the terminal *fem/fog* genes is inactivated by a null or strong *lf* allele or when both *fog-1* and *fog-3* are inactivated in the same animal. Further, quantitation of germline proliferation in a *fog-1(lf) gld-1(Tum)* double mutant indicates that *fog-1(lf)* has no effect on tumor growth (FRANCIS *et al.* 1995) (see below). These results are consistent with *gld-1* being essential for oogenesis and also argue that tumor formation does not result from an intersexual mode of development that is dependent on the terminal *fem/fog* genes.

We also examined whether *gld-1(Tum)* is similarly epistatic to several other mutations that eliminate hermaphrodite spermatogenesis. These included (1) a *fog-2(lf)* mutation (SCHEDL and KIMBLE 1988), (2) a *tra-2(gf)* allele (SCHEDL and KIMBLE 1988) and (3) a *tra-*

TABLE 1

Effect of sexual fate on the *gld-1* tumorous phenotype[a]

Genotype		Somatic phenotype	Germline phenotype	
		gld-1(+) or *gld-1(−)*	*gld-1(+)*	*gld-1(−)*
Wild type	*XX*	Female	Sperm, then oocytes	Tumorous
	X0	Male	Sperm	Sperm
Feminizing mutations				
fem-1(lf), *fem-2(lf)* or *fem-3(lf)*	*XX*	Female	Oocytes	Tumorous
fem-3(lf)	*X0*	Female	Oocytes	Tumorous
her-1(lf)	*XX*	Female	Sperm, then oocytes	Tumorous
	X0	Female	Sperm, then oocytes	Tumorous
fog-1(lf)	*XX*	Female	Oocytes	Tumorous
	X0	Male	Oocytes	Tumorous[b]
fog-2(lf)	*XX*	Female	Oocytes	Tumorous
	X0	Male	Sperm	Sperm
fog-3(lf)	*XX*	Female	Oocytes	Tumorous
	X0	Male	Oocytes	Tumorous
fog-1(lf) fog-3(lf)	*XX*	Female	Oocytes	Tumorous
	X0	Male	Oocytes	Tumorous
tra-1(gf)/+	*XX*	Female	Oocytes	Tumorous
	X0	Female	Oocytes	Tumorous
tra-2(gf)	*XX*	Female	Oocytes	Tumorous
	X0	Male	Sperm	Sperm
tra-2(lf);fem-3(lf)	*XX*	Female	Oocytes	Tumorous
Masculinizing mutations				
Tra				
tra-2(lf)	*XX*	Incomplete Male	Sperm	Sperm
tra-3(lf)[c]	*XX*	Incomplete Male	Sperm (46%) Sperm, then oocytes (54%)	Sperm (43%) Sperm plus ectopic proliferation (57%)
tra-1(lf)[d]	*XX*	Male	Sperm (63%) Sperm, then oocytes (37%)	Sperm (29%) Sperm plus ectopic proliferation (71%)
Mog				
fem-3(gf)	*XX*	Female	Sperm	Sperm (92%) Sperm plus ectopic proliferation (8%)
mog-1(lf)	*XX*	Female	Sperm	Sperm (96%) Sperm plus ectopic proliferation (4%)
tra-3(Mog)[e]	*XX*	Female	Sperm (99%) Sperm, then oocytes (1%)	Sperm (93%) Sperm plus ectopic proliferation (7%)

[a] See MATERIALS AND METHODS for details. The phenotype is completely penetrant unless indicated. The genotypes of *fem-1*, *fem-2*, *fem-3* and *tra-3* were m(−/−) z(−/−) to eliminate maternal rescue effects associated with these genes.

[b] For *fog-1(q180) gld-1(q485)*, 80% of tumorous germ lines make some sperm (see text). The percent of *X0* males making sperm was not quantitated for *fog-1(lf) gld-1(Tum)* double mutants using different alleles.

[c] The amber allele *e1107* was used as the putative *tra-3* null mutation. Only animals with a normal male somatic gonad were scored. Not included were the ~20% of animals with abnormal/intersexual somatic gonads.

[d] Only animals with a normal male somatic gonad were scored. Not included were the ~50% of total *tra-1(e1834)* animals with abnormal gonads (HODGKIN 1987; SCHEDL *et al.* 1989).

[e] *tra-3(bn75)* was used. *bn75* has a Mog phenotype at 25°C (E. CAPOWSKI, B. BRENDZA and S. STROME, personal communication).

1(gf) allele that results in constitutive *tra-1* activity (HODGKIN 1987). None of these mutations have any obvious effect on tumor formation in *XX gld-1(Tum)* germlines. This result was expected for *tra-2(gf)* and *fog-2(lf)* because these mutations may lead to increased negative regulation of the terminal *fem/fog* genes in the *XX* germline (DONIACH 1986; SCHEDL and KIMBLE 1988; GOODWIN *et al.* 1993). The role(s) of *tra-1* in germline sex determination are uncertain (HODGKIN 1987; SCHEDL *et al.* 1989).

Tumor formation is independent of the *X* chromosome dosage: *gld-1(Tum)* alleles disrupt germline de-

velopment in *XX* hermaphrodites but not in *X0* males. To determine whether sex specificity is correlated with either the sexual phenotype of the animal or its *X* chromosome dosage, we examined *XX* and *X0 gld-1(Tum)* animals whose sexual phenotype had been reversed by a mutation in the sex determination pathway. For this purpose *her-1(lf)*, *fem-3(lf)* and *tra-1(gf)* mutations were each used to transform *X0* animals into either hermaphrodites [*her-1(lf)*] or females [*fem-3(lf)* and *tra-1(gf)/+*]. When also homozygous for a *gld-1(Tum)* allele, these *X0* somatic females develop germline tumors identical to those of *XX gld-1(Tum)* animals (Table 1). Thus tumor formation is not dependent on *X* chromosome dosage but is instead correlated with a female sexual phenotype. [Although not tested, we presume that similar results would be obtained using *fem-1(lf)* and *fem-2(lf)* mutations.] Because of these results, we have not examined mutant combinations with the upstream genes that regulate both sex determination and dosage compensation (VILLENEUVE and MEYER 1990).

The complementary experiments in which *XX* animals are transformed into phenotypic males were done using mutant alleles of *tra-1*, *tra-2* and *tra-3*. Null and strong *lf* mutations in each of the *tra* genes cause masculinization of both the soma and germline of *XX* animals. However, none of these mutations completely masculinize all *XX* tissues (Table 1). For example, although *tra-2(lf) XX* animals make only sperm, the soma is incompletely male. For *tra-1(lf)*, the soma is fully male, but the germline often makes oocytes. Nonetheless, mutations in each of the *tra* genes were found to be fully [*tra-2(lf)*] or partially [*tra-1(lf)* and *tra-3(lf)*] epistatic to *gld-1(Tum)* alleles (Table 1). In the case of *tra-2*, both *tra-2(lf)* and *gld-1(Tum);tra-2(lf) XX* animals have a male germline that never forms a tumor. As *XX tra-2(lf)* animals have a completely normal male germline and somatic gonad, this result is consistent with the observation that *gld-1(null) X0* males are unaffected (FRANCIS *et al.* 1995). Because the normal function of *tra-2* is to negatively regulate the *fem* genes, the suppression of the *gld-1(Tum)* phenotype in a *tra-2(lf)* background should be dependent on *fem* gene activity. This expectation was confirmed by constructing a *XX gld-1(Tum);tra-2(lf);fem-3(lf)* triple mutant. *XX* animals of this genotype have a female soma and a tumorous germline, showing that *fem-3* activity is required for suppression of tumor formation in a *tra-2(lf)* background.

Although *tra-1(lf)* and *tra-3(lf)* alleles can also suppress tumor formation in *XX gld-1(Tum)* animals, these mutations are only partially epistatic to a *gld-1(Tum)* allele. A male germline is found in 43% of *gld-1(Tum);tra-3(lf)* and in 29% of *gld-1(Tum);tra-1(lf) XX* animals. The remaining animals make sperm but also have ectopically proliferating germ cells (Table 1). This phenotype most likely reflects the fact that *tra-1(lf)* and *tra-3(lf)* do not completely eliminate oogenesis in the *XX* germline (HODGKIN 1987; SCHEDL *et al.* 1989). Because of this, some *gld-1(Tum);tra-1(lf)* or *tra-3(lf)* germ cells may fail to adopt the male fate and so proliferate ectopically.

Suppression of tumor formation by germline masculinizing mutations: The above described results show that tumor formation is correlated with female development but do not address whether it is germline or somatic sex that is important. We therefore examined whether certain mutations that masculinize only the germline (Mog phenotype) are epistatic to *gld-1(Tum)* alleles. The Mog mutations used (Table 1) were (1) a *fem-3(gf)* allele that makes germline *fem-3* activity insensitive to negative regulation (BARTON *et al.* 1987; AHRINGER and KIMBLE 1991); (2) a *lf* mutation in *mog-1*, a gene that may function in repressing the terminal *fem/fog* genes (GRAHAM and KIMBLE 1993) and (3) a germline-specific *tra-3(lf)* allele *bn75* [designated *tra-3(Mog)*] (B. CAPOWSKI, B. BRENDZA and S. STROME, personal communication). The corresponding double mutants with *gld-1(Tum)* display a masculinized germline phenotype [shown for *gld-1(Tum);fem-3(gf)* in Figure 1a] similar to that of each Mog single mutant. The germline makes excess sperm and usually displays no evidence of ectopic proliferation. Thus *gld-1(Tum) XX* germ cells that are forced to adopt the male identity differentiate as sperm rather than forming tumors. The tumorous phenotype is therefore correlated with germline but not somatic sex. For all three double mutants, however, a small fraction of gonad arms (<10%) have ectopically proliferating germ cells in the most proximal region of the germline. As *gld-1(Tum)* alleles cause a partial feminization of the *XX* germline (FRANCIS *et al.* 1995), we believe that the ectopic proliferation that sometimes occurs in these double mutants arises because some *fem-3(gf)*, *mog-1(lf)* and *tra-3(Mog)* germ cells fail to adopt a male fate in a *gld-1(Tum)* background. Consistent with this idea, we found that *gld-1(q485* or *q365)* can dominantly suppress the Mog phenotype of *fem-3(gf)*. In particular, unlike *fem-3(gf)* animals, which make only sperm at 25°, some *gld-1(Tum)/+;fem-3(gf) XX* animals make both sperm and oocytes and are self-fertile (data not shown). Partial suppression of *fem-3(gf)* in *gld-1(Tum)* homozygotes may account for why some germ cells in a low percentage of animals proliferate instead of differentiate as sperm.

Tumor formation is independent of somatic sexual environment: As a final test of the sex and tissue specificity of tumor formation, we asked whether tumors form in *X0* animals that have a female germline and a male soma. Mutations in the genes *fog-1* and *fog-3* feminize only the germline (BARTON and KIMBLE 1990; ELLIS and KIMBLE 1995). As a result, *fog-1(lf)* and *fog-3(lf) X0* animals have a normal male soma but possess a germline that only makes oocytes. As described above, *fog-1(lf) gld-1(null)*, *gld-1(null) fog-3(lf)* and *fog-1(lf) gld-1(null) fog-3(lf) XX* animals are somatic females that form germline tumors. The corresponding *X0* double

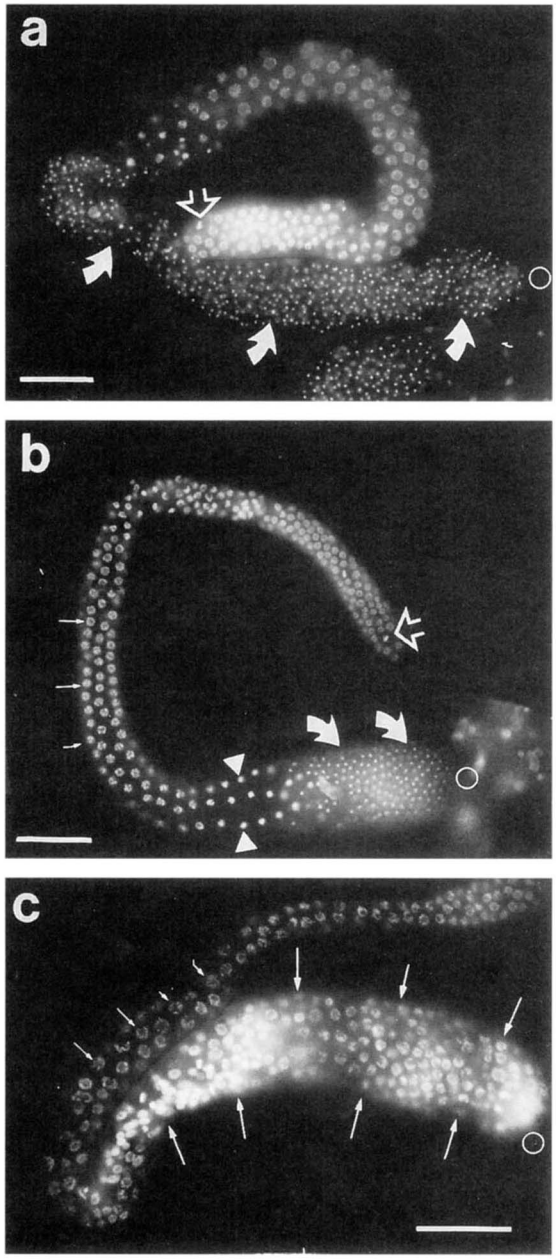

FIGURE 1.—Tumor formation is correlated with the female germline fate. Each panel shows a dissected gonad stained with DAPI to visualize germ cell nuclei. An open circle indicates the proximal end of each gonad. (a) The germline from an *XX gld-1(q485);fem-3(q95gf)* animal. Like the *XX fem-3(gf)* single mutant, this double mutant usually has a masculinized germline that makes only sperm (small nuclei in the regions indicated by curved arrows). The distal half of the germline contains proliferating germ cells (open arrow points to a mitotic cell), followed by cells in meiotic prophase. (b and c) Comparison of germline development in gonad arms dissected from *X0* males of the genotypes *gld-1(q485)* (b) and *fog-1(lf) gld-1(q485)* (c). The *gld-1(q485)* male germline appears normal in all respects: distal mitotic germ cells (open arrow) are followed first by germ cells in meiotic prophase (short arrows) and then by primary spermatocytes (triangles) and sperm (curved arrows). In *fog-1(lf) gld-1(q485)*, the organization of the distal half of the germline appears normal, but a tumor of mitotically active cells forms proximally (in the region indicated by large arrows). Scale bars, 10 μm.

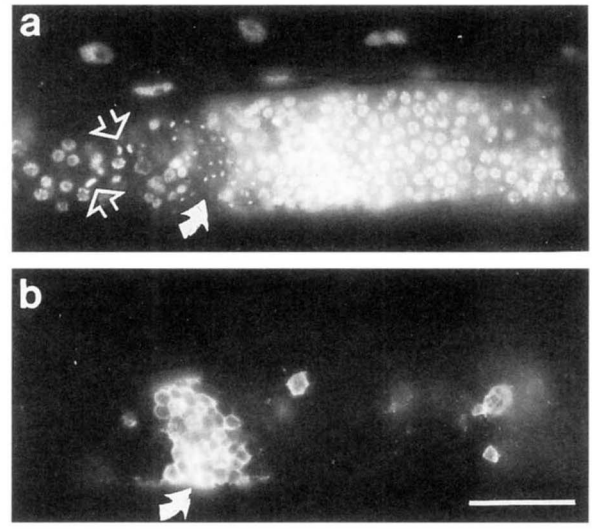

FIGURE 2.— *X0 fog-1(lf) gld-1(q485)* males sometimes make sperm. The proximal region of a *X0 fog-1(q180) gld-1(q485)* male gonad is shown stained with DAPI (a) and with SP56 (b), an antibody directed against a sperm-specific antigen. Sperm are identified by their small nuclear size (in the region of curved arrow in a) and positive staining for SP56 antigen (in b). About 80% of adult *X0 fog-1(q180) gld-1(Tum)* males make some sperm, but the number of sperm made is highly variable. Metaphase and telophase figures are indicated (open arrows). These are mitotic, rather than meiotic figures, as male germ cells undergoing meiotic divisions stain with SP56.

and triple mutants have a male soma, but these animals also develop germline tumors [Table 1, and shown in Figure 1 for *fog-1(lf) gld-1(Tum) X0* males]. Examination of DAPI-stained animals indicates that tumors form in these males by the same mechanism as in *XX gld-1(Tum)* germlines. In all cases, germ cells in meiotic prophase are first observed proximally in the L4 stage; these cells then appear to exit meiotic prophase and return to mitotic proliferation. In adults, more distal germ cells continue to enter meiotic prophase and may contribute to tumor growth by later returning to mitotic proliferation. Similar germline phenotypes were observed by ELLIS and KIMBLE (1995) for *X0 fog-1 gld-1(Tum)* and *gld-1(Tum) fog-3(lf)* double mutants. Based on these results, we conclude that tumor formation occurs independently of somatic sexual environment as long as the germline sex determination pathway is set in the female mode.

In the course of these experiments, we observed a surprising result: *fog-1(lf) gld-1(Tum) X0* males usually make some sperm. As shown in Figure 2, these males have a tumorous germline containing sperm that can be recognized by their compact nuclei and their expression of a sperm-specific antigen (WARD *et al.* 1986). This is not an allele-specific interaction, as spermatogenesis was observed in mutant combinations of two *fog-1* alleles (*q180* or *q187*) with three different *gld-1*

alleles (*q485*, *q268* or *q365*) (see MATERIALS AND METHODS). Although the two *fog-1* alleles examined are not known to be null, both are strong *lf* mutations (BARTON and KIMBLE 1990; ELLIS and KIMBLE 1995). Therefore it appears that in the absence of *gld-1* function, *X0* germ cells can develop as sperm by a pathway that requires little or no *fog-1* activity. In contrast, sperm were never observed in *XX fog-1(lf) gld-1(Tum)* worms or in *gld-1(Tum) fog-3(lf)* and *fog-1(lf) gld-1(Tum) fog-3(lf)* animals of either chromosomal sex.

Summary of interactions between the sex determination genes and *gld-1(Tum)* mutations: These experiments demonstrate that the *gld-1(null)* tumorous phenotype is dependent on germline sexual identity but is not dependent on the chromosomal sex of the animal or on somatic sexual identity. For the germline, the ultimate effect of the sex determination gene mutations is to alter the activity of the terminal *fem/fog* genes. Because the *gld-1(null)* tumorous phenotype is reponsive to the activity state of the terminal *fem/fog* genes, then *gld-1(+)* must act downstream of these genes on the branch that directs oogenesis (see DISCUSSION and Figure 9).

Investigation of the role of *gld-1* in promoting hermaphrodite spermatogenesis using two types of *gld-1 gf* mutations

The existence of a *gld-1(+)* activity that promotes hermaphrodite spermatogenesis was previously inferred from a *gld-1* haplo-insufficiency phenotype: *XX gld-1(null)/+* and *Df(gld-1)/+* germlines make fewer sperm than normal (FRANCIS *et al.* 1995). This *gld-1* function may also account for the existence of two classes of *gld-1(gf)* alleles that cause transformations in germline sexual fates. Alleles of the first type, the *gld-1(Fog)* mutations, feminize the germline of both sexes, so that *XX* animals usually make only oocytes and *X0* males make both sperm and oocytes. Alleles of the second type, the *gld-1(Mog)* mutations, masculinize the *XX* germline, so that hermaphrodites make an excess of sperm. To learn how these *gf* alleles affect the sex determination pathway, we constructed double mutants between *gld-1(Fog)* or *gld-1(Mog)* and mutations in the major sex determination loci (results summarized in Table 2).

***gld-1(Fog)* alleles suppress germline masculinizing mutations:** Genetic criteria indicate the *gld-1(Fog)* allele *q126* produces a poisonous *gld-1* product that acts to partially feminize both *XX* and *X0* germlines. Because *gld-1(null)* males are unaffected, the *q126* mutant product must interfere with another gene product involved in germline sex determination (FRANCIS *et al.* 1995). As indicated in Table 2, *q126* is able to partially suppress the masculinized germline phenotypes conferred by a *fem-3(gf)* allele or by *lf* alleles of each of the *tra* genes. Whereas each of these mutants makes only sperm [or some animals make sperm and then oocytes in the case of *tra-1(lf)* and *tra-3(lf)* mutants], all *XX* double mutants with *q126* make sperm and then oocytes. The suppression of *tra-2(lf)* and *tra-3(lf)* indicates that the *q126* product cannot feminize the germline by affecting *tra-2(+)* and *tra-3(+)* activity. Instead *gld-1(q126)* is likely to interfere with a gene product that acts downstream or independently of *tra-2* and *tra-3*.

gld-1(q126);fem-3(gf) XX animals are always self-fertile hermaphrodites, demonstrating that *gld-1(q126)* and *fem-3(gf)* mutually suppress one another. Mutual suppression has previously been reported for other combinations of *gf* mutations that affect germline sex determination and most likely represents a balance between the opposite effects of masculinizing and feminizing mutations (BARTON *et al.* 1987; SCHEDL and KIMBLE 1988).

Effects of *gld-1(Mog)* alleles on sex determination: *gld-1(Mog)* alleles that fall into three subclasses (C1, C2 and C3) were originally isolated as dominant suppressors of certain mutations that feminize the *XX* germline (FRANCIS *et al.* 1995). The C3 allele, *oz10*, retains *gld-1* functions that are required for oogenesis. Homozygous *oz10 XX* animals make excess sperm but also can make oocytes late in adulthood and become self-fertile. In contrast, C1 and C2 *gld-1(Mog)* homozygotes never make oocytes and, as indicated by complementation data, these alleles disrupt *gld-1* functions required for oogenesis (FRANCIS *et al.* 1995).

To determine whether *gld-1(Mog)* alleles can bypass the need for the *fem* genes or *fog-1* and *-3* in directing spermatogenesis, we constructed the *XX* double mutants described in Table 2. *XX* animals homozygous for the C3 allele *oz10* and either a *fem-3(lf)* or *fog-1(lf)* mutation only make oocytes; *fem-3(lf)* and *fog-1(lf)* are thus epistatic to *oz10* with regard to germline sex determination. Similarly, no sperm are made in double mutants between C1 or C2 *gld-1(Mog)* and mutations in the terminal *fem/fog* genes. Thus, like *oz10*, the C1 and C2 alleles do not bypass the need for the terminal *fem/fog* genes in directing spermatogenesis. However, unlike *oz10*, *XX* double mutants containing a C1 or C2 allele show no cytological signs of oogenesis. Instead, the proximal germline of each of the C1 and C2 *gld-1(Mog)* double mutants described in Table 2 fills with undifferentiated germ cells that have a nuclear morphology characteristic of germ cells at the pachytene stage of meiotic prophase (Figure 3). In all the double mutants analyzed, germ cells in adult animals usually remain arrested in meiotic prophase. In double mutants of certain genotypes, however, the germline occasionally becomes tumorous, probably because germ cells exit meiotic prophase and return to mitotic proliferation. These phenotypes are similar to the undifferentiated pachytene arrest phenotype conferred by class B *gld-1* alleles (FRANCIS *et al.* 1995).

The absence of spermatogenesis in C1 and C2 double mutants indicates that mutations in the *fem* genes and

TABLE 2

Interactions of *gld-1* Fog and Mog alleles with sex determination gene mutations

Genotype[a]		Phenotype[b] Soma	Germline
gld-1(Fog)			
gld-1(q126)	*XX*	Female	Oocytes (76%) Sperm, then oocytes (24%) (self-fertile)
gld-1(q126)	*X0*	Male	Sperm, then oocytes
q126; tra-2(lf)	*XX*	Incomplete male	Sperm, then oocytes
q126; tra-3(lf)	*XX*	Incomplete male	Sperm, then oocytes
q126; tra-1(lf)	*XX*	Male	Sperm, then oocytes
q126; fem-3(gf)	*XX*	Female	Sperm, then oocytes (self-fertile)
gld-1(Mog)			
C1			
gld-1(q93)	*XX*	Female	Sperm
q93;fem-1(lf)	*XX*	Female	Germ cells arrested in meiotic prophase
q93;fem-2(lf)	*XX*	Female	Germ cells arrested in meiotic prophase
q93;fem-3(lf)	*XX*	Female	Germ cells arrested in meiotic prophase[c]
q93;fog-1(lf)	*XX*	Female	Germ cells arrested in meiotic prophase[c]
q93;fog-1(lf)	*X0*	Male	Germ cells arrested in meiotic prophase
q93/+;fog-2(lf)	*XX*	Female	Sperm, then oocytes[d] (self-fertile)
q93;fog-2(lf)	*XX*	Female	Sperm
q93;tra-1(gf)/+	*XX*	Female	Sperm, then germ cells arrested in meiotic prophase
q93/+;tra-2(gf)/+	*XX*	Female	Sperm, then oocytes[d] (self-fertile)
q93;tra-2(gf)	*XX*	Female	Sperm, then germ cells arrested in meiotic prophase
C2			
gld-1(oz30)	*XX*	Female	Sperm
oz30;fem-3(lf)	*XX*	Female	Germ cells arrested in meiotic prophase (98%) Tumorous (2%)
oz30;fog-1(lf)	*XX*	Female	Germ cells arrested in meiotic prophase (99%) Tumorous (1%)
C3			
gld-1(oz10)	*XX*	Female	Sperm (72%) Excess sperm, then oocytes (28%)
oz10;fem-3(lf)	*XX*	Female	Oocytes
oz10;fog-1(lf)	*XX*	Female	Oocytes
oz10;fog-1(lf)	*X0*	Male	Oocytes

[a] See MATERIALS AND METHODS for details. Refer to Table 1 for the phenotype of sex determination single mutants. Animals were grown at 20° and scored 1 and 2 days after L4 by Nomarski optics and in some cases also by DAPI staining. For *tra-3, fem-1, -2* and *-3*, genotype was m(−/−) z(−/−).

[b] Phenotype is completely penetrant unless indicated. $n > 100$.

[c] Tumorous germ lines were observed in <1% of animals with these genotypes.

[d] Oogenesis continues as in wild type (see FRANCIS *et al.* 1995).

fog-1 and *-3* are epistatic to C1 and C2 alleles with regard to promotion of hermaphrodite spermatogenesis. This suggests that the germ cells in the double mutant develop along the female pathway. If so, germ cell arrest at the pachytene stage of meiotic prophase would simply reflect a disruption by C1 and C2 alleles of *gld-1* functions that are required for oogenesis and progression through meiotic prophase (FRANCIS *et al.* 1995). We favor this hypothesis based on several findings. First, the C3 allele *oz10* does not have a defect in oogenesis, and the *fog-1(lf) gld-1(oz10)* and *gld-1(oz10);fem-3(lf)* double mutants make functional oocytes. Second, complementation data show that C1 and C2 alleles are defective for oogenesis and that these alleles can cause germ cells to arrest at pachytene in certain combinations of *gld-1 trans*-heterozygotes (FRANCIS *et al.* 1995). Third, the pachytene arrested germ cells are not overtly intersexual as they do not express the sperm-specific antigens detected by the SP56 monoclonal antibody (data not shown) (WARD *et al.* 1986). Fourth, there is a parallel between the pachytene arrest phenotype of the C1 and C2 *gld-1(Mog)* double mutants and what

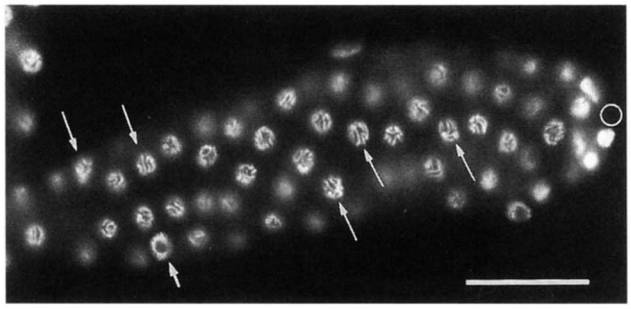

FIGURE 3.—Germ cells in *XX gld-1(Mog);fem-3(lf)* animals arrest at the pachytene stage of meiotic prophase. The proximal region of a *gld-1(q93);fem-3(lf)* hermaphrodite gonad arm is shown stained with DAPI. All proximal germ cells arrest in meiotic prophase and display a pachytene stage nuclear morphology (representative nuclei are indicated with long arrows). The nucleus of one somatic sheath cell (short arrow) is also visible. Scale bar, 10 μm.

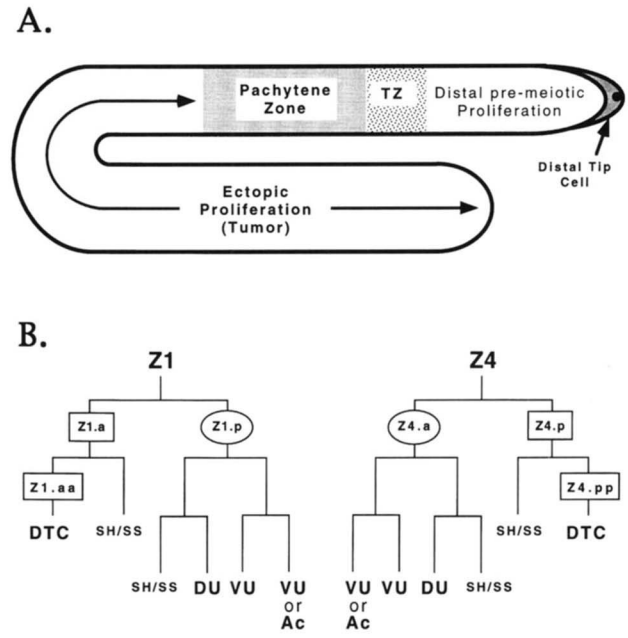

FIGURE 4.—(A) Diagram of an adult *gld-1* tumorous gonad arm, illustrating the two populations of proliferating germ cells. The distal portion of the *gld-1(Tum)* germline appears similar to wild type. A mitotic stem cell population is followed more proximally by a transition zone (TZ) where germ cells enter meiotic prophase and, by in turn, a region of pachytene germ cells. Proximal to the pachytene zone, the germline consists of ectopically proliferating cells that have exited meiotic prophase and returned to mitosis. (B) Partial cell lineage of the hermaphrodite somatic gonad. Depicted are the first three cell divisions (in L1 and young L2 larvae) of the somatic gonad precursor cells Z1 and Z4. Z1 and Z4 undergo mirror-image symmetrical lineages to produce a DTC and the following additional cells: two sheath/spermathecal blast cells (SH/SS), a dorsal uterine blast cell (DU), ventral uterine blast cells (VU) and the anchor cell (AC). Adapted from KIMBLE and HIRSH (1979).

occurs in *gld-1(Tum)* germlines. In either genetic background, germ cells that do not develop as male are unable to progress beyond pachytene to later stages of female meiotic prophase. However, although germ cells return to mitosis in *gld-1(Tum)* single mutants, germ cells in the C1 and C2 double mutants for the most part remain arrested in meiotic prophase. This difference suggests C1 and C2 *gld-1(Mog)* alleles retain *gld-1(+)* functions that prevent germ cells from exiting meiotic prophase but cannot supply the *gld-1(+)* functions required for further meiotic progression and oogenesis.

Dependence of tumor formation and premeiotic proliferation on the *glp-1*-mediated signaling pathway

In wild type, proliferating germ cells are found throughout the gonad of early larvae but are limited to the distal region in late larvae and adults as more proximal germ cells have entered the meiotic pathway. Proliferation of these germline stem cells is promoted by the *glp-1*-mediated signaling pathway. When the distal tip cell (DTC) of the somatic gonad is ablated with a laser microbeam, distal germ cells divide a few times, then enter meiosis and subsequently produce gametes (KIMBLE and WHITE 1981). The germline receptor for the DTC signal is a transmembrane protein, encoded by the *glp-1* gene, that belongs to the *lin-12/Notch* family of receptor molecules (AUSTIN and KIMBLE 1987, 1989; YOCHEM and GREENWALD 1989; CRITTENDEN *et al.* 1994).

In *gld-1(Tum)* hermaphrodites, germline proliferation in early larvae is apparently identical to wild type, and the proximal germ cells enter meiotic prophase at the normal time (FRANCIS *et al.* 1995). However, *gld-1(Tum)* germ cells exit meiotic prophase, return to the mitotic cell cycle and proliferate ectopically. The distal region of the late larval and adult *gld-1(Tum)* germline appears similar to wild type; a mitotic stem cell population is followed more proximally by a transition zone where germ cells enter meiotic prophase, which is in turn followed by a region of pachytene germ cells. In the mutant, the germline proximal to the pachytene zone is made up of ectopically proliferating cells that have exited meiotic prophase and returned to mitosis. As a result, *gld-1(Tum)* hermaphrodite gonads have two distinct populations of mitotically active germ cells: a distal premeiotic population, corresponding to the stem cell population in wild type, and a proximal population comprised of germ cells undergoing ectopic proliferation (Figure 4A). To distinguish between the two populations, we refer to mitotic germ cells in early larvae and in the distal region of late larvae and adults as "premeiotic germ cells" and to the ectopically proliferating germ cells that are the result of exit from meiotic prophase as a "germline tumor" (Figure 4A). In the second half of this section, we investigate the role of the *glp-1* signaling pathway in the control of premeiotic germ cell proliferation and tumor formation in *gld-1(Tum)* mutants.

Premeiotic proliferation is dependent on the

DTC: If the distal premeiotic germ cells in *XX gld-1(Tum)* mutants are similar to their wild-type counterparts, then their proliferation should depend on DTC signaling. To test this idea, we ablated the DTC in wild-type and *gld-1(Tum)* larvae (late L3/young L4 stage). Operated animals were allowed to develop for 24–48 hr, after which germlines were released by dissection and stained with DAPI to visualize nuclear morphology.

In ablated wild-type gonads, all distal germ cells enter meiotic prophase and reach the pachytene stage within 28–32 hr after DTC elimination; these cells later differentiate as oocytes (KIMBLE and WHITE 1981) (data not shown). Similarly, distal germ cells in operated *gld-1(Tum)* gonads also enter meiosis; by 28–32 hr all distal nuclei display a pachytene morphology (Figure 5, a and b). Therefore distal premeiotic proliferation in *gld-1(Tum)* hermaphrodites is signal dependent, indicating that it is under the same controls as in wild type. In contrast, proximal germ cells in the ablated gonads still form a tumor (Figure 5a), indicating that ectopic proliferation is not dependent on the DTC.

Further analysis of operated *gld-1(Tum)* gonads revealed that the distal meiotic germ cells fail to complete meiotic prophase and instead appear to return to mitosis. This is indicated by the later appearance (within 32–35 hr of DTC elimination) of dividing cells intermixed among the distal pachytene-stage germ cells (Table 3, Figure 5c). Because dividing cells display both a spindle pole morphology (data not shown) and metaphase plate configuration typical of mitotic germ cells, they appear to have returned to a mitotic cell cycle. Based on these results, we conclude the *gld-1(Tum)* germ cells enter the meiotic pathway in response to DTC ablation but then return to mitosis. This behavior is analogous to the *gld-1(Tum)* return to mitosis phenotype that occurs more proximally in the unoperated hermaphrodite gonad (FRANCIS *et al.* 1995).

Dependence of tumor formation on the somatic gonad: To further examine the dependence of premeiotic proliferation in *gld-1(Tum)* germlines on the somatic gonad, several additional ablation experiments were performed. As shown in Figure 4B, *XX* early L1 larvae contain two somatic precursor cells, Z1 and Z4, which give rise to the entire hermaphrodite somatic gonad (KIMBLE and HIRSH 1979). When Z1 and Z4 are ablated in wild type, the germ cell precursors, Z2 and Z3, divide one or two times but then fail to divide further or to form gametes (KIMBLE and WHITE 1981). The same result is observed when the daughters of Z1 and Z4 (Z1.a, Z1.p, Z4.a and Z4.p) are killed in wild type (data not shown). Similarly, when Z1 or Z4 (or their four daughters) are ablated in *XX gld-1(Tum)* L1 larvae, germ cells undergo one or two divisions but then cease dividing (data not shown). In a few cases, some germ cells in ablated *gld-1(Tum)* animals appeared to enter meiotic prophase, as judged from the appearance of pachytene-stage nuclei in DAPI-stained preparations, but a tumor was never formed. These results indicate that, as in wild-type, the initial proliferation in *gld-1(Tum)* early larvae is dependent on the somatic gonad.

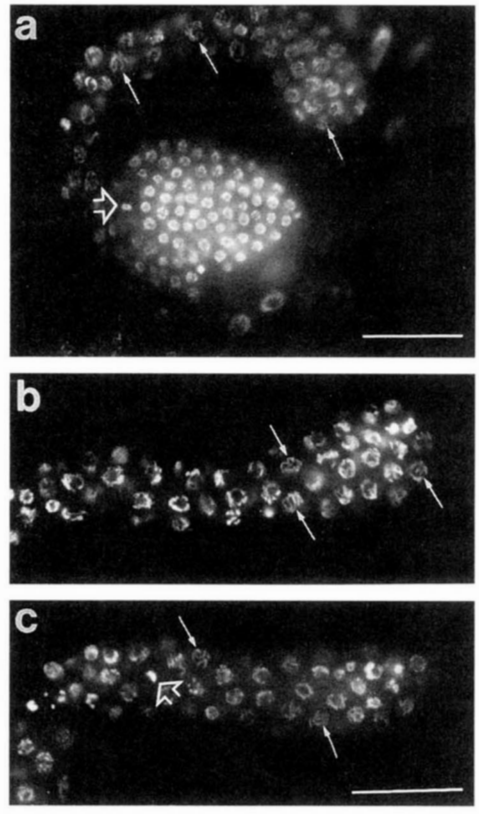

FIGURE 5.—Distal mitotic cells in *gld-1(Tum)* germlines enter the meiotic pathway after ablation of the somatic DTC but then return to mitosis. The DTC was ablated in *gld-1(Tum); unc-32* larvae at the late L3 larval stage, and operated animals were grown for an additional 28 (a and b) or 32 (c) hr. Animals were then dissected, fixed and stained with DAPI. By 28 hr (a and b), all distal cells display a pachytene morphology (representative nuclei in the plane of focus are marked with long arrows), indicating entry into meiotic prophase in response to DTC ablation. Proximal germ cells in the same gonads proliferate ectopically (open arrow in a). (Proximal germ cells entered meiotic prophase normally, at about the time of DTC ablation, whereas distal germ cells divided about two times before entering meiosis.) By 32 hr (c), mitotic figures (open arrow) begin to appear among the pachytene nuclei in the distal region, indicating a return to mitosis by the distal meiotic germ cells. Scale bar, 10 μm.

We next attempted to determine whether specific lineages giving rise to different parts of the somatic gonad are important for promoting tumor formation by *gld-1(Tum) XX* germ cells. In wild-type L1 larvae, ablation of Z1.a and Z4.p—the precursor cells that give rise to two DTCs and parts of the sheath and spermatheca (refer to Figure 4B)—results in germ cells undergoing several rounds of division and then differentiating as sperm at the normal time during L4 (KIMBLE and WHITE 1981). When the same ablation is done in *gld-1(Tum)* hermaphrodites, some germ cells differentiate as sperm but most go on to form a tumor (Table

TABLE 3

Ablations of somatic gonad cells in *gld-1(Tum)* hermaphrodites

Genotype	Larval stage	Precursor cell(s) ablated	Descendants of ablated precursors cell(s)	No. of animals	Adult germline phenotype
gld-1(q268 or q485); unc-32	L3/L4	Z1.aa or Z4.pp	DTC	>100	Enter meiosis, then return to mitosis[a]
	L1	Z1.a and Z4.p	DTCs, one half of SH/SP	12	Ectopic proliferation[b]
	L1	Z1.p and Z4.a	AC, uterus, one half of SH/SP	9	Ectopic proliferation[b]
	L2	Z1.aa and Z4.pp	DTCs	11	Ectopic proliferation[b]
	L2	Z1.pp and Z4.aa	AC, ventral uterus	12	Ectopic proliferation[b]
	L2	Z1.ppp and Z4.aaa	AC, one half of ventral uterus	10	Ectopic proliferation[b]
	L2	Z1.aa, Z1.pp, Z4.pp and Z4.aa	DTCs, AC, ventral uterus	3	Ectopic proliferation[b]
gld-1(q268); unc-32 glp-1(q172)	L3/L4	Z1.aa or Z4.pp	DTC	9	Enter meiosis, then return to mitosis[a]
gld-1(q485); unc-32 glp-1(q172); fem-3(q20gf)	L3/L4	Z1.aa or Z4.pp	DTC	8	Enter meiosis ⇒ all form sperm
gld-1(q268); unc-32 glp-1(q415)	L3/L4	Z1.aa or Z4.pp	DTC	8	Enter meiosis, then return to mitosis[a]

DTC, distal tip cell; SH/SP, sheath and spermatheca lineage; AC, anchor cell.

[a] Distal premeiotic germ cells enter meiotic prophase after DTC ablation but then return to mitosis (see Figure 7, b and c and text). There is no obvious effect on ectopic proliferation.

[b] Proximal ectopic proliferation was unaffected by the ablation. Distal premeiotic proliferation was affected only when the DTC was eliminated; however, these distal germ cells were not examined for the return to mitosis phenotype.

3, also see MATERIALS AND METHODS). Tumors also form after ablation of Z1.p and Z4.a, the precursor cells that give rise to the anchor cell, uterus and part of the sheath/spermatheca lineage (Table 3). Finally, ablation of the two distal tip cells (Z1.aa and Z4.pp) and the precursors to the anchor cell (Z1.pp and Z4.aa) does not prevent tumor formation. Taken together, these results indicate that somatic factors that support ectopic proliferation (tumor formation) can be supplied both by descendants of Z1.a and Z4.p and by descendants of Z1.p and Z4.a.

Dependence of the *gld-1(Tum)* phenotype on *glp-1* function

Genetic and cytological evidence indicate that the *glp-1* protein acts as the germline receptor for signaling by the DTC (AUSTIN and KIMBLE 1987; CRITTENDEN *et al.* 1994) and potentially by other somatic gonad cells (SEYDOUX *et al.* 1990). Because somatic signaling is required for continued premeiotic proliferation in *gld-1(Tum)* germlines, it appeared likely that premeiotic proliferation would also be dependent on *glp-1*(+) activity. This idea was tested by constructing a series of double mutants between *gld-1(Tum)* alleles and two *glp-1* molecular null alleles (KODOYIANNI *et al.* 1992) [designated *glp-1(0)*].

Continued premeiotic proliferation is dependent on *glp-1*(+) function: The *glp-1(0)* molecular null alleles *q46* and *q175* are each associated with a nonsense mutation in the amino-terminal half of the *glp-1* coding region (KODOYIANNI *et al.* 1992) that abolishes accumulation of stable *glp-1* protein (CRITTENDEN *et al.* 1994). In each single mutant, the germline precursors Z2 and Z3 divide about two times to produce two to four germ cells in each hermaphrodite gonad arm and four to eight germ cells in the single male gonad arm (AUSTIN and KIMBLE 1987). In both sexes, these germ cells enter meiosis prematurely and differentiate as sperm by the L3 stage. Table 4 lists the double mutants constructed with *glp-1(q46* or *q175)* and each of three *gld-1(Tum)* alleles (*q485*, *q268* or *q365*). All double mutants, both as *XX* hermaphrodites and *X0* males, have a *glp-1(lf)* phenotype; germ cells divide several times but then enter meiotic prophase prematurely and form sperm. This suggests that premeiotic proliferation by *gld-1(Tum)* germ cells remains largely dependent on *glp-1*(+) activity (also see below).

Tumor formation is independent of *glp-1*(+) function: Because the results presented above indicate that

TABLE 4

Germline phenotypes of *gld-1(Tum); glp-1(null)* animals

Genotype	Phenotype and number of germ cells/gonad arm
Single mutants	
XX unc-32 glp-1(q175 or *q46)*[a]	~2–4 Pachytene germ cells ⇒ ~16 sperm[b]
X0 unc-32 glp-1(q175 or *q46)*	~4–8 Pachytene germ cells ⇒ ~32 sperm[b]
Double mutants	
XX gld-1(q485);unc-32 glp-1(q175 or *q46)*[c]	~16 Pachytene germ cells[d] ⇒ ~64 sperm[e]
XX gld-1(q268);unc-32 glp-1(q175 or *q46)*[c]	~16 Pachytene germ cells[d] ⇒ ~64 sperm[e]
XX gld-1(q365);unc-32 glp-1(q175 or *q46)*[c]	~16 Pachytene germ cells[d] ⇒ ~64 sperm[e]
X0 gld-1(q268 or *q365);unc-32 glp-1(q46)*	~32 Pachytene germ cells[d] ⇒ ~128 sperm[e]
Triple mutants (feminized double mutants)	
XX gld-1(q485);unc-32 glp-1(q175);fem-3[f]	~16 Pachytene germ cells[d] ⇒ ectopic proliferation
XX gld-1(q485);unc-32 glp-1(q175);fem-1[g]	~16 Pachytene germ cells[d] ⇒ ectopic proliferation
XX fog-1 gld-1(q485);unc-32 glp-1(q175 or *q46)*[h]	~16 Pachytene germ cells[d] ⇒ ectopic proliferation
XX gld-1(q268);unc-32 glp-1(q175);fem-3[f]	~16 pachytene germ cells[d] ⇒ ectopic proliferation
XX gld-1(q268 or *q365);unc-32 glp-1(q175);fem-1*[g]	~16 pachytene germ cells[d] ⇒ ectopic proliferation
XX fog-1 gld-1(q268);unc-32 glp-1(q175 or *q46)*[h]	~16 pachytene germ cells[d] ⇒ ectopic proliferation

[a] Self-progeny of *unc-32 glp-1(q175* or *q46)/hT2* hermaphrodites.
[b] Described by AUSTIN and KIMBLE (1987).
[c] Self-progeny of *gld-1(Tum)/hT2;unc-32 glp-1(q175* or *q46)/hT2* hermaphrodites.
[d] Number of pachytene-stage germ cells/gonad arm counted in DAPI-stained *XX* hermaphrodites at the mid-L3 larval stage. Most gonad arms contained 15–18 pachytene germ cells (average 16), but a few contained as many as 20.
[e] Determined by counting sperm in DAPI-stained L4 animals.
[f] Self-progeny of *gld-1(Tum)/hT2;unc-32 glp-1(q175)/hT2;fem-3(e1996)/dpy-20 fem-3(q20gf) unc-24* hermaphrodites.
[g] Self-progeny of *gld-1(Tum)/hT2;unc-32 glp-1(q175)/hT2;fem-1(e2003ts)/dpy-13 unc-5* hermaphrodites.
[h] Self-progeny of *fog-1(q180) gld-1(Tum)/hT2;unc-32 glp-1(q175* or *q46)/hT2* hermaphrodites.

gld-1 tumors only form when the sex determination pathway is set in the female mode (terminal *fem/fog* genes inactive), *gld-1(Tum);glp-1(0)* animals may fail to form tumors because all germ cells develop in the male mode. To overcome this situation, we constructed a series of *gld-1(Tum);glp-1(0)* strains in which the male germline fate is eliminated by a *lf* mutation in one of three genes: *fem-1*, *fem-3* or *fog-1*. For simplicity, we refer to these triple mutants as feminized *gld-1(Tum);glp-1(0)* animals or feminized triple mutants. As controls, *fog-1(lf);glp-1(0)* and *glp-1(0);fem-1(lf)* hermaphrodites were examined. These double mutants show the *glp-1(0)* germ cell proliferation defect, but germ cells differentiate as small oocyte-like cells rather than sperm. The same phenotype was observed previously for a *fog-1(ts);glp-1(ts)* double mutant (BARTON and KIMBLE 1990).

Table 4 describes nine different feminized *gld-1(Tum);glp-1(0)* triple mutants that were constructed. These triple mutants differ in their *gld-1(q485, q268* or *q365)* and *glp-1(q175* or *q46)* genotypes, and in the particular feminizing mutation they carry, but all display the same tumorous germline phenotype. Although the tumors are generally smaller than those in *gld-1(Tum)* single mutants, germ cells continue to proliferate throughout adulthood (Figures 6 and 7a). More detailed examination of one feminized triple mutant [*fog-1(q180) gld-1(q485);glp-1(q175)*] revealed that *all* germ cells in these animals enter meiotic prophase at the same time as do germ cells in *gld-1(Tum);glp-1(0)* double mutants (data not shown—see MATERIALS AND METHODS). However, instead of forming sperm as in unfeminized *gld-1(Tum);glp-1(0)* double mutants, many of these germ cells appear to exit meiotic prophase and return to mitosis. These results indicate that neither exit from meiotic prophase nor ectopic proliferation are absolutely dependent on *glp-1(+)* activity. Therefore tumor formation cannot result solely from a failure to downregulate *glp-1* activity as germ cells progress through meiotic prophase. The finding that only feminized *gld-1(Tum);glp-1(0)* animals form tumors also reinforces the idea that the sex determination pathway must be set in the female mode for tumors to form.

Several properties of feminized *gld-1(Tum);glp-1(0)* triple mutants indicate that *glp-1(+)* activity, although not essential for tumor formation, is important for maximal tumor growth. First, as shown in Figure 6, tumors grow more slowly and variably in the triple mutants than in the corresponding *gld-1(Tum)* strains that are *glp-1(+)*. Second, unlike tumors formed in a *glp-1(+)* background, which are comprised mainly of small germ cells with compact nuclei of relatively homogeneous morphology, those formed in feminized *gld-1(Tum);glp-1(0)* triple mutants display a wide range of nuclear morphologies. Some germ cells display a pachytene morphology, others stain intensely with DAPI and may

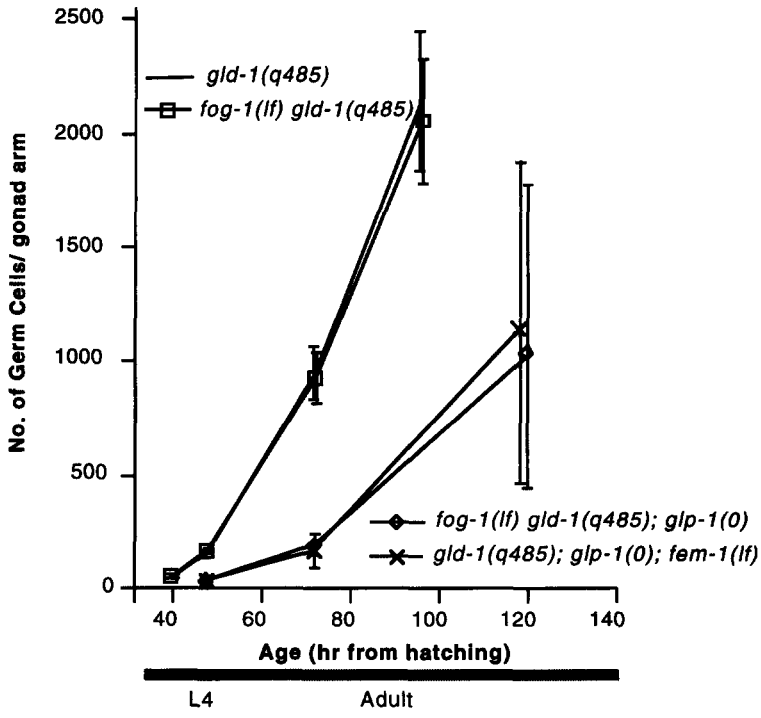

FIGURE 6.—Comparison of germline proliferation in the *gld-1(q485) XX* single mutant, *fog-1(q180) gld-1(q485) XX* double mutants and feminized *gld-1(q485);glp-1(0) XX* triple mutants. Each data point represents the mean number of germ cells counted in a minimum of 10 gonad arms. Plus or minus one standard deviation is indicated with error bars. Each of the feminized *gld-1(Tum);glp-1(0)* triple mutants describe in Table 4 forms a germline tumor. However, as shown here for two feminized triple mutants [using *glp-1(q175)* with either *fog-1(q180)* or *fem-1(e2003)*], these tumors grow more slowly and variably than when *glp-1(+)* activity is present.

be polyploid and still others have 12 condensed chromosomes (possibly representing early mitotic prophase). These features suggest that an absence of *glp-1* activity in *gld-1(Tum)* germlines sometimes results in aberrant cell cycles and occasional arrest of germ cells in pachytene. Third, the germline in these strains lacks any evident distal/proximal polarity, as all germ cells appear to enter the meiotic pathway at about the same time during larval growth (data not shown). These results indicating that *glp-1(+)* activity helps promote tumor formation are consistent with the finding of CRITTENDEN *et al.* (1994) that *gld-1* tumors express *glp-1* protein at high levels.

***gld-1(Tum)* mutations also affect premeiotic germline proliferation:** Because germ cells in *XX gld-1(Tum)* animals enter the meiotic pathway normally (FRANCIS *et al.* 1995), there was no *a priori* reason to suspect that *gld-1* regulates premeiotic germline proliferation. However, close examination of *gld-1(Tum);glp-1(0)* double and feminized triple mutants revealed a weak effect of *gld-1(Tum)* alleles on the proliferation of premeiotic germ cells. In *glp-1(0)* single mutants, the germline precursor cells Z2 and Z3 divide about two times and then differentiate as sperm (AUSTIN and KIMBLE 1987). In double mutants and feminized triple mutants, however, Z2 and Z3 undergo four premeiotic divisions to produce ~16 germ cells per hermaphrodite gonad arm and 32 cells in the single male gonad arm (Table 4). In both double and feminized triple mutants, germ cells enter meiosis in relative synchrony, so that all nuclei exhibit pachytene morphology by the mid-L3 stage (Figure 7, b and c). Increased premeiotic proliferation, in contrast to tumor formation, is not dependent on germline sexual fate; it occurs in animals that produce sperm [*gld-1(Tum);glp-1(0) XX* or *X0*] and in feminized triple mutants where the male fate is inactivated.

The small increase in premeiotic proliferation in the double and feminized triple mutants argues that *gld-1(+)* inhibits this aspect of germline development. However, because *gld-1(null)* mutations do not noticeably increase premeiotic germ cell proliferation in *glp-1(+)* animals (FRANCIS *et al.* 1995), the role of *gld-1* in inhibiting premeiotic germline cell division must be relatively small. Because early larval germ cells do not appear to contain maternally supplied *glp-1(+)* RNA or protein (EVANS *et al.* 1994; CRITTENDEN *et al.* 1994; SEYDOUX and FIRE 1994), the elimination of *gld-1* function in *glp-1(0)* mutants cannot affect *glp-1* activity. Therefore *gld-1* may repress germline mitosis (or promote entry into the meiotic pathway) by a mechanism that is at least partially independent of *glp-1* activity.

***gld-1(Tum)* alleles are epistatic to certain *glp-1(lf)* alleles:** If *gld-1* negatively regulates premeiotic proliferation (either by regulating a molecule other than *glp-1* or by regulating *glp-1* and a *glp-1*-independent molecule), then elimination of *gld-1(+)* activity might render germ cells more responsive to the *glp-1*-dependent mitotic signal. To test this idea, we examined whether *gld-1(null)* can fully or partially suppress nonnull *glp-1(lf)* mutations. Double mutants of *gld-1(q485)* were constructed with two classes of *glp-1(lf)* alleles. The first class includes three strong nonconditional *glp-1(lf)* mutations that affect the extracellular domain of the *glp-1* protein (GLP-1) and the second class includes three

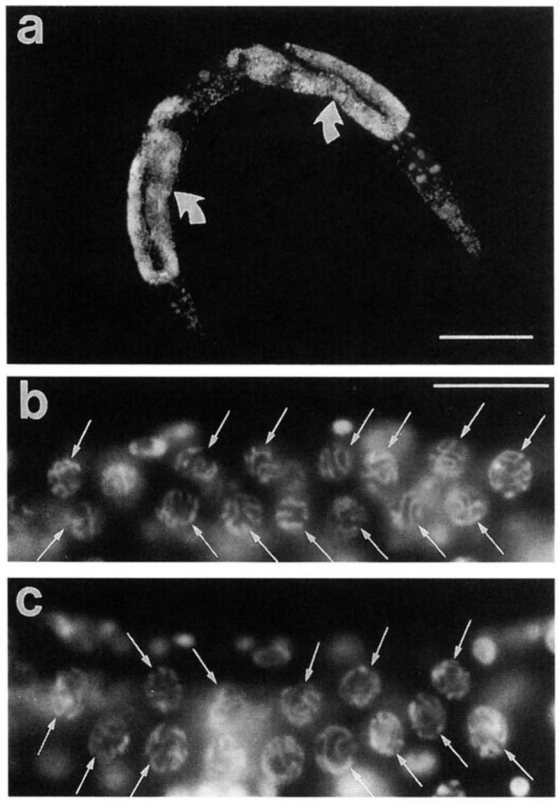

FIGURE 7.—Tumor formation in feminized *gld-1(Tum);glp-1(0)* XX germlines. (a) A *fog-1(q180) gld-1(q268);glp-1(q175)* adult hermaphrodite stained with DAPI to visualize germline tumors. Each proximal gonad arm is indicated by a curved arrow. Scale bar, 50 μm. (b and c) Comparison of the larval germline phenotypes of (b) *gld-1(q268);glp-1(q175)* and (c) *fog-1(q180) gld-1(q485);glp-1(q175) XX* animals. Each panel shows DAPI-stained germ cells in one gonad arm of a mid-L3 larva. At this stage, animals of each genotype contain 15–18 germ cells in pachytene of meiotic prophase (arrows, a few pachytene cells are in a different focal plane). Whereas pachytene germ cells in the *gld-1(Tum);glp-1(0)* double mutant differentiate as sperm, those in the feminized triple mutant exit meiosis and give rise to a tumor (Table 4). Scale bar, 10 μm.

temperature-sensitive *glp-1* alleles (diagrammed in Figure 8C).

Each nonconditional *glp-1* mutation confers a strong *glp-1(lf)* phenotype similar to that of *glp-1(0)* alleles (AUSTIN and KIMBLE 1987). *q172* is an in-frame deletion that removes most of the three extracellular *lin-12/Notch* (LN) repeats of GLP-1, *q158* is a missense mutation in the second LN repeat and *oz25* is a missense mutation in the seventh epidermal growth factor-like motif (KODOYIANNI *et al.* 1992). Germline tumors form in all three *XX gld-1(q485);glp-1(lf)* double mutants. Germ cells in *gld-1(q485);glp-1(q172)* mutants proliferate at essentially the same rate as those in the *gld-1(q485)* single mutant, whereas proliferation in double mutants with *glp-1(q158* or *oz25)* is somewhat reduced and shows more variability between animals (Figure 8A). Thus *gld-1(q485)* partially or completely suppresses the germline proliferation defects of these *glp-1(lf)* alleles. The *gld-1(q485);glp-1(q172)* double mutant was chosen for more detailed analysis because robust proliferation was observed in every animal.

As was previously shown, *glp-1(+)* activity is required for continuous premeiotic proliferation in *gld-1(Tum)* germlines but is dispensible for ectopic tumorous germ cell proliferation. However, because *gld-1(q485)* is completely epistatic to *glp-1(q172)*, we suspected that premeiotic proliferation in the double mutant may be close to normal. If correct, this idea would suggest that *glp-1(q172)* is able to function essentially like a *glp-1(+)* allele when in a *gld-1(Tum)* background. Several observations indicate that this is the case. First, proximal germ cells in *XX gld-1(q485);glp-1(q172)* mutants enter meiotic prophase at the same time as those in *gld-1(null);glp-1(+)* and wild-type hermaphrodites (data not shown). Second, the premeiotic *glp-1(q172)* proliferative defect can be suppressed in germlines that show no ectopic tumorous proliferation. *X0 gld-1(q485);glp-1(q172)* males, for example, have a male germline that appears normal in all respects; in contrast, *glp-1(q172) X0* males have a Glp germline that makes only 20–32 sperm (AUSTIN and KIMBLE 1987). *gld-1(Tum)* mutations also suppress the *glp-1(q172)* proliferative defect in *XX* germlines that are exclusively undergoing male development. *XX gld-1(q485);glp-1(q172);fem-3(gf)* hermaphrodites, like the *XX gld-1(q485);fem-3(gf)* double mutant (shown in Figure 1), exhibit a Mog phenotype. Third, we used ablation experiments to show that the premeiotic proliferation occurring in *gld-1(q485);glp-1(q172)* germlines depends on signaling by the DTC. When the DTC is ablated in the *XX gld-1(null);glp-1(q172)* double mutant or the *gld-1(null);glp-1(q172);fem-3(gf)* triple mutant, distal germ cells stop proliferating and enter meiosis (Table 3). This indicates that the *glp-1(q172)* product supports signal-dependent proliferation in *gld-1(Tum)* germlines and therefore must retain some *glp-1(+)* activity. The premeiotic proliferation behavior of *glp-1(q172)* in the *gld-1(null)* background does not result from a novel property of the *glp-1(q172)* allele; *glp-1(q415)* is also strongly suppressed by *gld-1(null)* (Table 3; Figure 8B). That *glp-1(q172)* can support continued premeiotic proliferation in a *gld-1(null)* but not a *gld-1(+)* background is consistent with the proposal that *gld-1(+)* has a role in the negative regulation of premeiotic proliferation. This function of *gld-1*, unlike that which prevents exit from meiotic prophase and a return to mitosis, is not sex-specific.

A second set of experiments was aimed at examining the role of the intracellular domain of the *glp-1* protein in expression of the *gld-1(Tum)* phenotype. Recent work indicates that the GLP-1 intracellular domain, which contains six ankyrin repeats, is necessary and possibly sufficient for *glp-1*-mediated signaling (KODOYIANNI *et al.* 1992; ROEHL and KIMBLE 1993). Double mu-

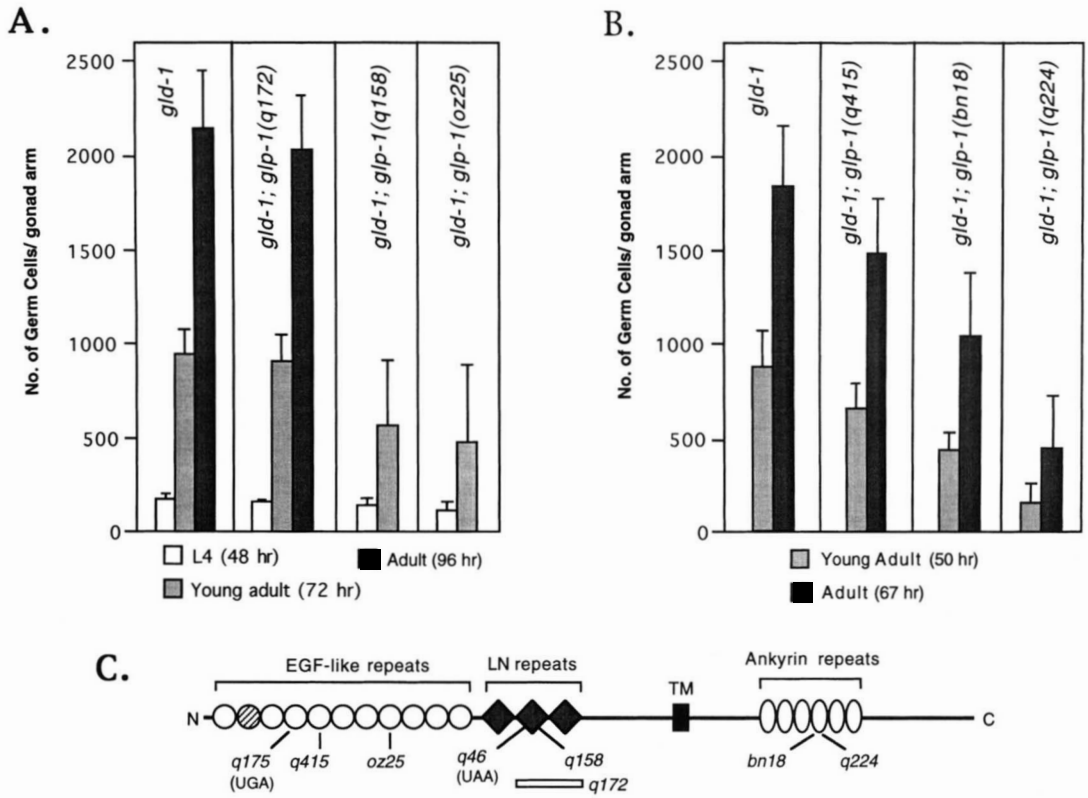

FIGURE 8.—Germline proliferation in selected *gld-1(q485);glp-1(lf)* hermaphrodites. The number of germ cells was determined in 10 or more gonad arms from animals grown for the indicated times after hatching. The mean number of germ cells per hermaphrodite gonad arm is shown with plus or minus one standard deviation represented by an error bar. (A) Comparison of proliferation in double mutants with a nonconditional *glp-1(lf)* allele. *glp-1(q172)* supports tumor growth as well as *glp-1(+)*, whereas *q158* and *oz25* result in decreased proliferation. In a *gld-1(+)* background, these *glp-1(lf)* alleles confer a germline phenotype similar to a *glp-1(0)* allele, about two to four germ cells per gonad arm (AUSTIN and KIMBLE 1987). (B) Comparison of proliferation in *gld-1(q485);glp-1(ts)* double mutants grown at grown at 25° (the restrictive temperature for these *glp-1* alleles). The two *glp-1* alleles (*q224* and *bn18*), which contain substitutions in an intracellular ankyrin repeat (KODOYIANNI *et al.* 1992), have a much stronger effect on proliferation than does *q415*, a missense mutation in a conserved extracellular residue of GLP-1. (C) The positions of 8 *glp-1(lf)* mutations examined in double mutants are shown on a schematic representation of the *glp-1* protein. The extracellular GLP-1 domain contains nine EGF-like repeats (open circles), one pseudo-EGF like sequence (hatched circle) and three LN repeats. The intracellular portion contains six ankyrin repeats (also called CDC10/SWI6 repeats). See KODOYIANNI *et al.* (1992) for discussion of each of the *glp-1(lf)* mutations shown. All are point mutations except *gld-1(q172)*, which is an in-frame deletion of 87 residues. Adapted from KODOYIANNI *et al.* (1992) and YOCHEM and GREENWALD (1989).

tants were constructed with two temperature sensitive (ts) *glp-1(lf)* mutations that affect an ankyrin repeat: *glp-1(q224)*, a missense mutation in ankyrin repeat 4 that results in a strong Glp-1 phenotype (AUSTIN and KIMBLE 1987), and *glp-1(bn18)*, a missense mutation at a different site in repeat 4 that results in a weak Glp-1 phenotype (KODOYIANNI *et al.* 1992). For comparison, we also constructed a double mutant with *glp-1(q415)*, a ts mutation in the extracellular domain of GLP-1 that alters a conserved cysteine residue (Figure 8C). Of the three *gld-1(null);glp-1(ts)* double mutants, the greatest effect on tumor growth was observed with *glp-1(q224)*, the ankyrin repeat mutant with a strong Glp-1 phenotype (Figure 8B). In *gld-1(null);glp-1(q224)* hermaphrodites, some gonad arms fail to form a tumor (all germ cells differentiated as sperm by late L4), whereas others form tumors that grow more slowly and variably than in the other two double mutants. Thus *glp-1(q224)* has the strongest effect on tumor formation and growth of any nonnull *glp-1(lf)* allele examined. This suggests that normal functioning of the intracellular ankyrin repeats is important for supporting both premeiotic proliferation in *gld-1(Tum)* germlines and subsequent tumor growth. Consistent with this idea, Figure 8C also shows that the other ankyrin repeat mutant, *glp-1(bn18)*, promotes tumor growth to a lower level than does the extracellular domain mutant *glp-1(q415)*. Because *glp-1(bn18)* confers a weaker Glp-1 phenotype than *glp-1(q415)* in a *gld-1(+)* background (KODOYIANNI *et al.* 1992), this difference suggests the intracellular ankyrin repeats play an important role in promoting ectopic proliferation by *gld-1(null)* germ cells.

In summary, the analysis of *gld-1(null);glp-1(0* or *lf)* double mutants shows that *glp-1(+)* activity is important for two aspects of the *gld-1(Tum)* phenotype. First, it is required to maintain continued premeiotic

proliferation by *gld-1(Tum)* germ cells and second, it promotes a maximal level of ectopic proliferation among germ cells that have returned to mitosis. Non-null *glp-1(lf)* alleles can differ significantly in their ability to promote ectopic proliferation. In general, ectopic proliferation is promoted more strongly by mutant *glp-1* receptors with an altered extracellular domain than by receptors with an altered intracellular domain.

DISCUSSION

The present work extends our understanding of the roles of *gld-1* in germline development. We used epistasis analysis with *gld-1(Tum)* alleles to show that the *gld-1* function necessary for oocyte development acts downstream of (and in response to) the germline sex determination pathway. Therefore tumors are likely to result from a defect in oogenesis. These results do not distinguish between whether *gld-1* functions to specify the oocyte fate or instead act to execute an early essential step in oocyte differentiation. We also address the role of *gld-1* in promoting hermaphrodite spermatogenesis. Results from epistasis analysis using *gld-1(Fog)* and *gld-1(Mog)* alleles are consistent with the proposal that *gld-1(+)* promotes hermaphrodite spermatogenesis by assisting the activities of the terminal *fem/fog* genes. Below, we consider a model for how *gld-1* activity might accomplish the seemingly contradictory tasks of directing oogenesis and promoting hermaphrodite spermatogenesis. Finally, we address the role of the somatic gonad and the *glp-1* signaling pathway in promoting tumor formation in *gld-1* mutants.

The germline sex determination pathway controls the *gld-1* function that directs oocyte development: Sexual fates in the *C. elegans* germline are determined through the control of five terminal regulators: *fem-1*, *fem-2*, *fem-3*, *fog-1* and *fog-3* (Figure 9) (for recent reviews see KUWABARA and KIMBLE 1992; CLIFFORD *et al.* 1994). When the sex determination pathway is set in the male mode (terminal *fem/fog* genes active), oogenesis is repressed and germ cells are directed to adopt the male fate and differentiate as sperm. When the pathway is set in the female mode (by inactivation of at least one of the terminal *fem/fog* genes), germ cells adopt the female fate and differentiate as oocytes. The male mode is thought to be initially set by the *fog-2* (*XX* hermaphrodite larvae) and *her-1* (*X0* males) genes being active, whereas the female mode is initially set by both genes being inactive (*XX* adults). Previous work has not identified a gene(s) that acts downstream of the *fem/fog* genes to specify the female germline fate.

Based on the epistasis results with a null *gld-1(Tum)* allele, Figure 9 shows *gld-1(+)* acting downstream of the sex determination loci as part of the pathway for oogenesis. As this position implies, the behavior of *gld-1* tumorous germ cells was found to depend on the activity state of the terminal *fem/fog* genes. When the terminal *fem/fog* genes are active, as occurs in the germlines of *X0* males and certain *XX* mutants [*tra-2(lf)*, *tra-3(lf)*, *tra-3(Mog)*, *mog-1(lf)* and *fem-3(gf)*], *gld-1(Tum)* germ cells differentiate as sperm just as wild-type germ cells do. Conversely, when any one of the terminal *fem/fog* genes is inactivated by mutation, *gld-1(null)* germ cells form a tumor regardless of either somatic sexual phenotype or *X* chromosome dosage. Feminized *X0 gld-1(null)* germ cells, for example, form tumors when the soma is female, as in a *fem-3(lf)* background, and when the soma is male, as in a *fog-1(lf)* or *fog-3(lf)* background. Because *X0 gld-1* tumors are observed in multiple mutant backgrounds, it appears unlikely that they arise from gene- or allele-specific interactions between *gld-1* and genes in the sex determination pathway. Therefore tumor formation in *gld-1(Tum)* animals appears to only require that the germline sex determination pathway is set in the female mode via inactivation of the terminal *fem/fog* genes. We place *gld-1(+)* on a branch that directs oocyte development because it does not negatively regulate spermatogenesis.

Functioning at the end of the sex determination pathway, does *gld-1(+)* direct oocyte development by acting to specify the oocyte (female) fate or by acting at an early step in the differentiation of a cell that is already specified as an oocyte (also see FRANCIS *et al.* 1995)? Knowledge of the sexual identity of *gld-1* tumorous germ cells is key to distinguishing between these two models: if the tumorous germ cells are sexually uncommitted, then *gld-1(+)* is likely to specify the oocyte fate, but if they are already specified as female, *gld-1(+)* would function in oocyte differentiation. A third possibility, that *gld-1* tumorous germ cells are intersexual, is unlikely. As elimination of any one of the terminal *fem/fog* genes has no effect on the *XX* tumorous phenotype, intersexuality of *gld-1(Tum)* germ cells would have to result from the inappropriate activation of an as yet unidentified gene that acts downstream of the terminal *fem/fog* genes to promote male germ cell development. The genetic epistasis experiments presented here do not distinguish between the first two models as they only show that *gld-1*'s role in oocyte development depends on the upstream sex determination genes being set in the female mode (terminal *fem/fog* genes inactive). The uncertainty of the role of *gld-1* in oocyte development is indicated in Figure 9 by placing *gld-1* in a shaded box that represents both specification of the oocyte fate and oocyte differentiation.

Analysis of the *gld-1* function that promotes hermaphrodite spermatogenesis: Experiments discussed in the accompanying paper indicate that *gld-1(+)* has a function in promoting hermaphrodite spermatogenesis. To determine the likely position of this activity in the germline sex determination pathway, we have investigated epistatic relationships between *gf* Fog and Mog *gld-1* alleles and mutations in sex determination loci. Interpretation of epistasis data using *gf* mutations is less

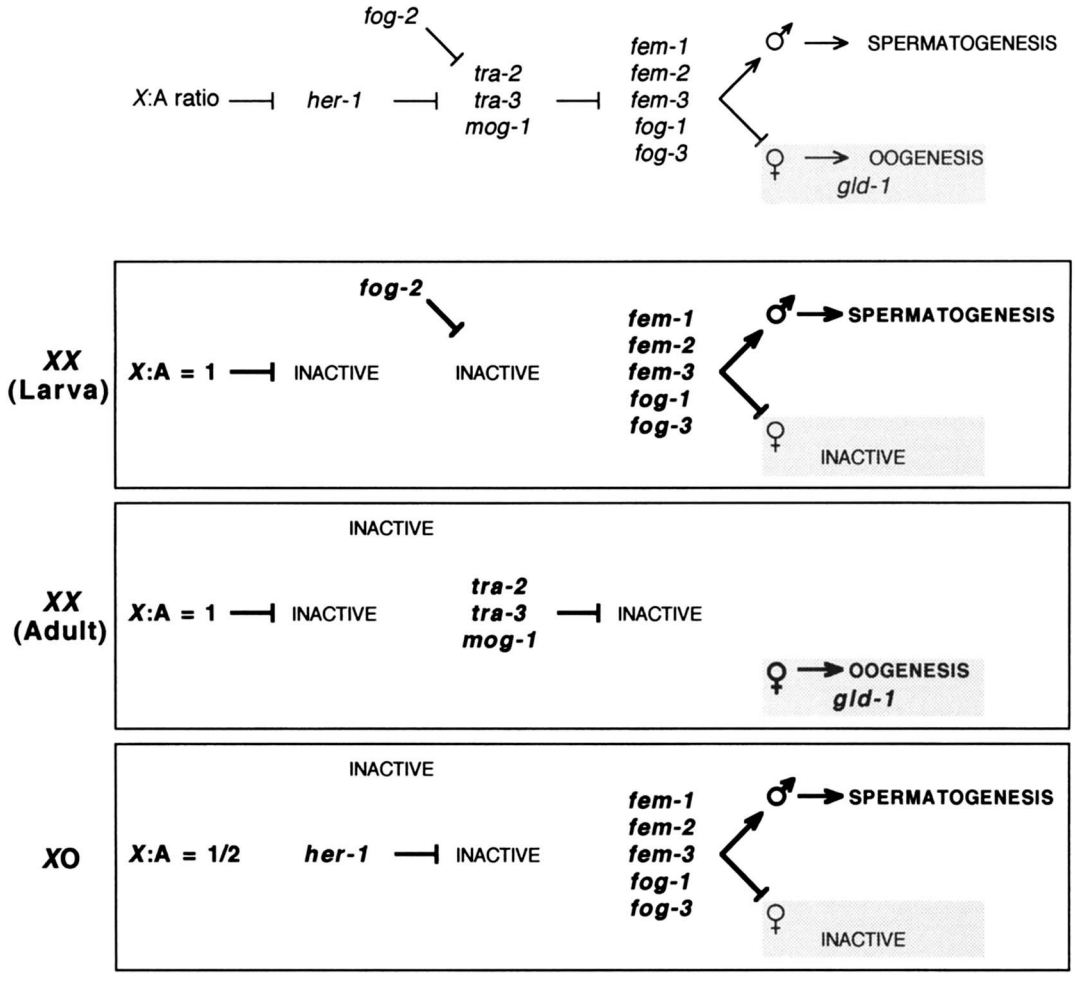

FIGURE 9.— *gld-1* acts at the end of the sex determination cascade, on the branch that directs oogenesis. Genetic pathway for germline sex determination in *C. elegans*. Negative regulation is indicated by a bar and positive regulation by an arrow. Not shown are *tra-1*, whose role in germline sex determination is uncertain, and several upstream genes that act to transduce the *X*:A ratio and control the activity of the *her-1* gene (reviewed by VILLENEUVE and MEYER 1990). In hermaphrodite (*XX*) larvae, the terminal *fem/fog* genes are free to direct spermatogenesis because *tra-2* (and possibly *tra-3* and *mog-1*) is negatively regulated by the *fog-2* gene. In the *XX* adult, *tra-2* is active and functions with *tra-3* and *mog-1* to negatively regulate the *fem/fog* genes, resulting in the switch to oogenesis. In males (*X0*), *her-1* is transcriptionally active, and *her-1* protein acts to negatively regulate *tra-2*. This relieves repression of the terminal *fem/fog* genes, which act together to inhibit oogenesis and direct spermatogenesis (reviewed by KUWABARA and KIMBLE 1992; CLIFFORD *et al.* 1994). In the absence of terminal *fem/fog* activity, *gld-1*(+) directs oocyte development. *gld-1* is shown in a shaded box to indicate that it may specify the oocyte fate or act at an early step in oocyte differentiation. See text for details.

straightforward than with null mutations because of uncertainties about the exact relationship between the *gf* activity and wild-type activity. However, we were not able to use *gld-1(null)* mutations because the tumorous phenotype precludes us from determining the sexual identity of the germ cells.

Based on genetic studies, the *gf gld-1(Fog)* alleles are likely to produce an abnormal gene product that interferes with specification of the male germline fate (FRANCIS *et al.* 1995). In double mutants, *gld-1(Fog)* was found to be epistatic to putative null alleles of *tra-2* and *tra-3*. Because the *gf gld-1(Fog)* mutant product interferes with hermaphrodite spermatogenesis even in the absence of the *tra-2* and *-3* products, it must act on or poison the product of another gene that functions downstream or independently of *tra-2* and *-3*. By extension, *gld-1*(+) is likely to act downstream or independently of *tra-2* and *-3*. Any of the terminal *fem/fog* gene products are possible candidates for activities that might be poisoned by *gf gld-1(Fog)* mutant products; poisoning of terminal *fem/fog* gene activity would explain why *gld-1(Fog)* alleles feminize the *X0* male germline whereas the *gld-1(null)* mutation does not.

gld-1(Mog) alleles masculinize the hermaphrodite germline by disrupting the switch from spermatogenesis to oogenesis (FRANCIS *et al.* 1995). The *gf gld-1(Mog)* mutations may masculinize the germline by interfering with negative regulation of the terminal *fem/fog* genes. This hypothesis predicts that spermatogenesis in *gld-1(Mog)* mutants should be dependent on the terminal

fem/fog genes. Double mutant analysis shows that male germline development in *gld-1(Mog)* mutants does require the activity of the terminal *fem/fog* genes (Table 2; also see RESULTS).

How does *gld-1* both promote spermatogenesis and provide functions essential for oogenesis? One speculative model to accommodate both functions is based on the idea that the *gld-1* product may exist in two forms, one that promotes spermatogenesis and another that is essential for oogenesis. When the terminal *fem/fog* genes are active, *gld-1* product/activity assumes a state that promotes hermaphrodite spermatogenesis. The altered *gld-1* product/activity might assist or enhance the terminal *fem/fog* genes, for example, and thereby form part of a positive feedback loop that promotes spermatogenesis. However, because spermatogenesis can occur in *gld-1(null)* hermaphrodites and males (Table 1) (FRANCIS *et al.* 1995), the altered *gld-1* product/activity is not essential for male germline development. When the terminal *fem/fog* genes are inactive, *gld-1* product exists in a state that directs oogenesis. The central feature of this model is that the activity state of terminal *fem/fog* genes determines whether *gld-1* functions to promote hermaphrodite spermatogenesis or to direct oocyte development. There is ample precedent from other systems for regulatory molecules that exist in distinct forms to perform different functions. For example, protein–protein interactions allow the yeast *alpha2-a1* complex to repress haploid-specific genes, whereas *alpha2* alone represses only **a** cell type specific genes (HERSKOWITZ *et al.* 1992). Similarly, chemical modification (phosphorylation) converts the *Escherichia coli OmpR* protein from a transcriptional activator to a transcriptional repressor (RAMPERSAUD *et al.* 1994).

Although it may seem paradoxical that *gld-1* has distinct functions in female and male germline development, a precedent is provided by the *C. elegans* sex determination gene *xol-1*, which has distinct functions in the two sexes (MILLER *et al.* 1988). *xol-1* has a major function in directing the male modes of sex determination and dosage compensation in *X0* animals and a minor function in promoting female development in *XX* animals.

Etiology of tumor formation and role of the *glp-1* gene: The *gld-1(Tum)* adult hermaphrodite contains two distinct populations of mitotic germline cells. The first, located at the distal end of the gonad, corresponds to the stem cell population in wild type; the second, which fills the proximal gonad, consists of ectopically dividing tumorous cells. Germ cells that have entered meiotic prophase separate the two populations of proliferative cells (Figure 4A). To distinguish the two groups of mitotically active cells, we refer to the normal mitotic population as "premeiotic germ cells" and the ectopically proliferating population as a "germline tumor" (see RESULTS).

Our previous studies of *gld-1(Tum)* hermaphrodites suggested that tumors arise from germ cells that exit meiotic prophase and return to mitosis (FRANCIS *et al.* 1995). Several results presented here further support this idea, while also demonstrating that *gld-1(Tum)* alleles have no major effect on the controls that govern premeiotic proliferation. Two lines of investigation indicate that premeiotic proliferation in *gld-1(Tum)* hermaphrodites is, for the most part, dependent on the *glp-1*-mediated signaling pathway. First, when the DTC is killed in *XX gld-1(Tum)* larvae, distal germ cells stop proliferating and enter meiotic prophase (Figure 5). Therefore, as in wild type (KIMBLE and WHITE 1980), premeiotic proliferation by *gld-1(Tum)* germ cells is dependent on the signaling molecule produced by the DTC. Second, the *glp-1* gene, which encodes the likely receptor for the DTC signal, is also required for premeiotic proliferation in *gld-1(Tum)* mutants. In *XX gld-1(Tum)* larvae homozygous for a *glp-1* molecular null mutation [designated *glp-1(0)*] (KODOYIANNI *et al.* 1992; CRITTENDEN *et al.* 1994), germ cells divide several times but then prematurely enter meiosis and form sperm (Table 4 and Figure 7b). Taken together, these results demonstrate that *gld-1(Tum)* alleles do not override the normal controls that make premeiotic proliferation dependent on the *glp-1* signaling pathway.

In contrast to its role in premeiotic proliferation, the *glp-1* signaling pathway is not required for the ectopic proliferation that accounts for tumor formation in *gld-1(Tum)* hermaphrodites. After DTC ablation in L3/L4 larvae, distal germ cells enter meiotic prophase but then return to mitosis (Table 3 and Figure 4). Thus, in the absence of the DTC signal, germ cells can return to mitosis. Investigation of the role of *glp-1* activity in the return to mitosis phenotype is complicated by the fact that all germ cells in *gld-1(Tum);glp-1(0)* animals differentiate as sperm during larval growth. Therefore we examined *XX gld-1(Tum);glp-1(0)* triple mutants whose germline had been feminized by a *lf* mutation in one of three genes (*fem-1*, *fem-3* or *fog-1*). As in the double mutants, all germ cells in the feminized triple mutants enter meiosis prematurely: by mid-L3, each hermaphrodite gonad arm contains only 15–18 germ cells, all of which reach pachytene at approximately the same time (Table 4 and Figure 7c). Because the germline sex determination pathway is set in the female mode, triple mutant germ cells do not form sperm but instead return to mitosis and give rise to a tumor (Figure 7a). The observation that all germ cells in the feminized triple mutants enter pachytene provides further evidence that tumors derive from meiotic prophase germ cells that return to mitosis. As tumor formation occurs in the absence of *glp-1(+)* activity, neither the initial exit from pachytene nor the subsequent ectopic proliferation can result solely from a failure to negatively regulate *glp-1(+)* activity during meiotic prophase.

Although *glp-1* activity is not essential for tumor for-

mation in *gld-1(Tum)* animals, its elimination has quantitative and qualitative effects on tumor growth. Tumors grow more slowly and variably in the absence of *glp-1* activity (Figure 6), and many tumorous germ cells exhibit abnormal nuclear morphologies that are not seen in a *glp-1(+)* background. These differences suggest *glp-1* protein may be important for promoting the maximal level of cell cycling by germ cells undergoing ectopic proliferation. Indeed, CRITTENDEN *et al.* (1994) show that ectopically proliferating cells in *gld-1(Tum)* germlines express *glp-1* protein at high levels. Because *glp-1* protein is not required for tumors to form, its expression by tumorous germ cells is likely to represent a secondary consequence of proliferation; in particular, ectopic proliferation may lead to a general upregulation of factors that drive mitosis, including *glp-1*. Because it is unclear whether proximal germ cells are exposed to *glp-1* ligand, it is possible that ligand is not necessary for the promotion of ectopic proliferation by *glp-1*. The intracellular ankyrin repeats of *glp-1*, which mediate signaling (KODOYIANNI *et al.* 1992; ROEHL and KIMBLE 1993), do seem to play a critical role in promoting *gld-1(Tum)* ectopic proliferation, as *glp-1* mutations affecting these repeats more severely diminish tumor growth than do mutations affecting the extracellular domain of the protein (see RESULTS).

***gld-1* functions as a sex nonspecific negative regulator of premeiotic proliferation:** An unexpected finding to emerge from these studies is that *gld-1(Tum)* alleles can have a small effect on premeiotic proliferation. Elimination of *gld-1(+)* function clearly does not override the need for the *glp-1* signaling pathway because all germ cells in *gld-1(Tum);glp-1(0)* double mutants (both *XX* and *XO*) and feminized triple mutants enter meiosis prematurely. However, compared with *glp-1(0)* single mutants, germ cells in the double and triple mutants undergo two extra rounds of division before entering meiosis (Table 4). Although slight, this effect suggests that *gld-1* may play a nonessential role in either negatively regulating premeiotic proliferation or promoting entry into meiotic prophase. Because added premeiotic divisions occur in both double mutants (which produce sperm) and feminized triple mutants (which produce tumors), this effect of *gld-1(Tum)* alleles is not dependent on germline sexual fate. This suggests that the *gld-1* function regulating premeiotic proliferation is distinct from the sex-specific *gld-1* function required for oogenesis.

Because *gld-1(Tum)* and *glp-1(lf)* mutations have opposite effects on premeiotic proliferation, it is possible that *gld-1(+)* might promote entry into meiotic prophase by acting as a negative regulator of *glp-1(+)* activity. However, if this is the case, *gld-1* cannot be the only negative regulator of *glp-1*, as germ cells enter meiotic prophase normally in the *gld-1(null);glp-1(+)* single mutant. Moreover, negative regulation of a molecule distinct from *glp-1* must play a role in the inhibition of mitosis by *gld-1*. The two extra rounds of proliferation and entry into meiotic prophase observed in the *gld-1(null);glp-1(0)* double and feminized triple mutants are not dependent on *glp-1* activity. Two lines of evidence argue that germlines of these animals completely lack *glp-1* product. First, the two *glp-1(0)* alleles used in this study make no detectable *glp-1* protein (KODOYIANNI *et al.* 1992; CRITTENDEN *et al.* 1994). Second, antibody staining and RNA *in situ* hybridization data (EVANS *et al.* 1993; CRITTENDEN *et al.* 1994; SEYDOUX and FIRE 1994) argue that *glp-1(0)* mutant larval germ cells are unlikely to contain maternally derived *glp-1(+)* RNA (or protein made from maternal RNA).

If *gld-1(+)* inhibits premeiotic germ cell proliferation (either by negatively regulating a factor distinct from *glp-1* or by negatively regulating *glp-1* and a second molecule), we reasoned that an absence of *gld-1(+)* function might lead to suppression of the proliferation defects found in nonnull *glp-1(lf)* mutants. In fact, *gld-1(null)* is partially or completely epistatic to several *glp-1* mutations that alter residues in the extracellular *glp-1* domain (Figure 8). Because full epistasis was observed with *glp-1(q172)*, a strong *lf* mutation, the combination of *gld-1(Tum)* and *glp-1(q172)* was examined in most detail. The strong epistasis of *gld-1(null)* to *glp-1(q172)* is the result of two effects. First, even though *q172* is a strong *glp-1(lf)* allele, it appears to promote ectopic proliferation in tumors to approximately the same extent as does a *glp-1(+)* allele (Figure 8A). Second, premeiotic proliferation in this double mutant also appears normal. This was initially indicated by examination of *X0 gld-1(Tum);glp-1(q172)* animals, which have a normal male germline, and confirmed by ablation results that show that the DTC is essential for premeiotic proliferation in the double mutant (Table 3). The DTC ablations indicate that *glp-1(q172)* product must retain some residual *glp-1(+)* receptor function. Apparently, *gld-1(null)* mutants either increase residual *glp-1* activity in the *glp-1(lf)* mutants or allow premeiotic proliferation to occur at a level of *glp-1* activity that is not sufficient in a *gld-1(+)* background.

Certain other *glp-1(lf)* mutations (*e.g.*, *q415* and *q158*; Figure 8) that affect the extracellular domain of *glp-1* also show substantial suppression of the *glp-1(lf)* proliferation defect in a *gld-1(null)* background. Like *q172*, these alleles probably retain some *glp-1(+)* function. In contrast, *glp-1(q224)*, a mutation in one of the intracellular ankyrin repeats of *glp-1*, is only weakly suppressed by *gld-1(null)*.

Taken together, the various combinations of *gld-1* and *glp-1* mutations suggest that *gld-1(+)* acts to negatively regulate germ cell proliferation before entry into the meiotic pathway. Because this *gld-1(+)* function is not dependent on sexual fate, it must be distinct from the *gld-1* function required for oogenesis. Because premeiotic *gld-1* function is clearly not essential for the negative regulation of proliferation required for entry

into meiotic prophase, it is possible that this *gld-1*(+) function is redundant with that of another gene(s). These results thus suggest that *gld-1* acts to negatively regulate mitosis at two distinct stages of germline development: premeiotically, in both sexes, and during the pachytene stage of meiotic prophase, when the upstream sex determination cascade is set in the female mode.

***gld-1* is a cell-type specific tumor suppressor gene:** Tumor formation in animals lacking *gld-1* activity is not only restricted to a single tissue (the germline) but is also dependent on sexual identity. A consequence of this sex specificity is that expression of the tumorous phenotype can be manipulated by changes in cell identity. In *gld-1(Tum)* hermaphrodites, *XX* germ cells that would normally form a tumor can instead differentiate as sperm when the germline is masculinized by a mutation in a second gene [*e.g.*, *fem-3(gf)*]. Thus differentiation along an alternative pathway (spermatogenesis) has the effect of suppressing tumor formation. *X0 gld-1(Tum)* males are unaffected, as mutant germ cells adopt the male fate and execute spermatogenesis normally. However, when the male germline fate is inactivated by a mutation in a second gene [*fog-1(lf)* or *fog-3(lf)*], *X0 gld-1(Tum)* germ cells form a tumor by the same mechanism as do mutant *XX* germ cells. Because *fog-1* and *fog-3* mutations by themselves cause *X0* germ cells to develop as oocytes, the likely role of *fog-1* and *fog-3* mutations in generating male germline tumors is that they set the germline sex determination pathway in the female mode. This change then generates a cell type that responds to the absence of *gld-1* activity by exiting meiotic prophase and proliferating ectopically.

Could changes in cell identity play a role in multistep tumorigenesis in mammals? One example where an alteration in cell type may be important for tumor formation is the acute lymphoblastic leukemia caused by the t(10;14)(q24q11) translocation (HATANO *et al.* 1991). The translocation causes T cells to ectopically express the homeobox gene *SPX* (also called *Hox11*), a locus required for spleen development (HATANO *et al.* 1992; ROBERTS *et al.* 1994). Ectopic expression of SPX presumably results in the expression of genes that are inappropriate for T cell development and may potentially generate a hybrid cell type that is susceptible to additional events leading to tumor formation.

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LITERATURE CITED

AHRINGER, J., and J. KIMBLE, 1991 Control of the sperm-oocyte switch in *Caenorhabditis elegans* hermaphrodites by the *fem-3* 3' untranslated region. Nature **349:** 346–348.

AHRINGER, J., T. A. ROSENQUIST, D. N. LAWSON and J. KIMBLE, 1992 The *C. elegans* gene *fem-3* is regulated post-transcriptionally. EMBO J. **11:** 2303–2310.

AUSTIN, J., and J. KIMBLE, 1987 *glp-1* is required in the germ line for regulation of the decision between mitosis and meiosis in *C. elegans*. Cell **51:** 589–599.

AUSTIN, J., and J. KIMBLE, 1989 Transcript analysis of *glp-1* and *lin-12*, homologous genes required for cell interactions during development of *Caenorhabditis elegans*. Cell **58:** 565–571.

AVERY, L., and H. HORVITZ, 1987 A cell that dies during wild-type *C. elegans* development can function as a neuron in a *ced-3* mutant. Cell **51:** 1071–1078.

BAE, E., K. R. COOK, P. K. GEYER and R. N. NAGOSHI, 1994 Molecular characterization of ovarian tumors in Drosophila. Mech. Dev. **47:** 151–164.

BARTON, M. K., and J. KIMBLE, 1990 *fog-1*, a regulatory gene required for specification of spermatogenesis in the germ line of *Caenorhabditis elegans*. Genetics **125:** 29–39.

BARTON, M. K., T. B. SCHEDL and J. KIMBLE, 1987 Gain-of-function mutations of *fem-3*, a sex-determination gene in *Caenorhabditis elegans*. Genetics **115:** 107–119.

BRENNER, S., 1974 The genetics of *Caenorhabditis elegans*. Genetics **77:** 71–94.

CLIFFORD, R., R. FRANCIS and T. SCHEDL, 1994 Somatic control of germ cell development in *Caenorhabditis elegans*. Semin. Dev. Biol. **5:** 21–30.

CRITTENDEN, S. L., E. R. TROEMEL, T. C. EVANS and J. KIMBLE, 1994 GLP-1 is localized to the mitotic region of the *C. elegans* germ line. Development **120:** 2901–2911.

DONIACH, T., 1986 Activity of the sex-determining gene *tra-2* is modulated to allow spermatogenesis in the *C. elegans* hermaphrodite. Genetics **114:** 53–76.

DONIACH, T., and J. HODGKIN, 1984 A sex-determining gene, *fem-1*, required for both male and hermaphrodite development in *Caenorhabditis elegans*. Dev. Biol. **106:** 223–235.

ELLIS, R. E., and J. KIMBLE, 1995 The *fog-3* gene and regulation of cell fate in the germline of *Caenorhabditis elegans*. Genetics **139**(2) (in press).

EVANS, T. C., S. L. CRITTENDEN, V. KODOYIANNI and J. KIMBLE, 1994 Translational control of maternal *glp-1* mRNA establishes an asymmetry in the *C. elegans* embryo. Cell **77:** 183–194.

FRANCIS, R., M. K. BARTON, J. KIMBLE and T. SCHEDL, 1994 *gld-1*, a tumor suppressor gene required for oocyte development in *C. elegans*. Genetics **139:** 579–606.

GOODWIN, E. B., P. G. OKKEMA, T. C. EVANS and J. KIMBLE, 1993 Translational regulation of *tra-2* by its 3' untranslated region controls sexual identity in *C. elegans*. Cell **75:** 329–339.

GRAHAM, P., and J. KIMBLE, 1993 The *mog-1* gene is required for the switch from spermatogenesis to oogenesis in *Caenorhabditis elegans*. Genetics **133:** 919–931.

HATANO, M., C. W. M. ROBERTS, M. MINDEN, W. M. CRIST and S. J. KORSMEYER, 1991 Deregulation of a homeobox gene *HOX11*, by the t(10;14) in T cell leukemia. Science **253:** 79–82.

HATANO, M., C. W. M. ROBERTS, T. KAWABE, J. SHUTTER and S. KORSMEYER, 1992 Cell cycle progression, cell death, and T cell lymphoma in *Hox11* transgenic mice. Blood **80** (Suppl.): 80a.

HERSKOWITZ, I., J. RINE and J. STRATHERN, 1992 Mating-type determination and mating-type interconversion in *Saccharomyces cerevisiae*, pp 583–656 in *The Molecular and Cellular Biology of the Yeast Saccharomyces: Gene Expression*, edited by E. W. JONES, J. R. PRINGLE and J. R. BROACH. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

HODGKIN, J., 1980 More sex-determination mutants of *Caenorhabditis elegans*. Genetics **96:** 649–664.

HODGKIN, J., 1986 Sex determination in the nematode *Caenorhabditis elegans:* analysis of *tra-3* suppressors and characterization of the *fem* genes. Genetics **114:** 15–52.

HODGKIN, J., 1987 A genetic analysis of the sex-determining gene *tra-1* in the nematode *Caenorhabditis elegans*. Genes Dev. **1:** 731–745.

HODGKIN, J., M. EDGLEY, D. RIDDLE and D. ALBERTSON, 1988 Appendix 4, Genetics, pp 491–584 in *The Nematode Caenorhabditis elegans*, edited by W. B. WOOD. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

HORVITZ, H. R., S. BRENNER, J. HODGKIN and R. K. HERMAN, 1979 A uniform genetic nomenclature for the nematode *Caenorhabditis elegans*. Mol. Gen. Genet. **175:** 129–133.

KIMBLE, J., and D. HIRSH, 1979 Postembryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditis elegans*. Dev. Biol. **70:** 396–417.

KIMBLE, J. E., and J. G. WHITE, 1981 On the control of germ cell development in *Caenorhabditis elegans*. Dev. Biol. **81:** 208–219.

KODOYIANNI, V., E. MAINE and J. KIMBLE, 1992 Molecular basis of loss-of-function mutations in the *glp-1* gene of *Caenorhabditis elegans*. Mol. Biol. Cell **3:** 1199–1213.

KUWABARA, P. E., and J. KIMBLE, 1992 Molecular genetics of sex determination in *C. elegans*. Trends Genet. **8:** 164–168.

KUWABARA, P. E., P. G. OKKEMA and J. KIMBLE, 1992 *tra-2* encodes a membrane protein and may mediate cell communication in the *Caenorhabditis elegans* sex determination pathway. Mol. Biol. Cell **3:** 461–473.

MCKIM, K. S., T. STARR and A. M. ROSE, 1992 Genetic and molecular analysis of the *dpy-14* region in *Caenorhabditis elegans*. Mol. Gen. Genet. **233:** 241–251

MILLER, L. M., J. D. PLENEFISCH, L. P. CASSON and B. J. MEYER, 1988 *xol-1*: a gene that controls the male modes of both sex determination and *X* chromosome dosage compensation in *C. elegans*. **55:** 167–183.

RAMPERSAUD, A., S. L. HARLOCKER and M. INOUYE, 1994 The OmpR protein of *Escherichia coli* binds to sites in the *ompF* promoter region in a hierarchical manner determined by its degree of phosphorylation. J. Biol. Chem. **269:** 12559–12566.

ROBERTS, C. W. M., J. R. SHUTTER and S. J. KORSMEYER, 1994 *Hox11* controls the genesis of the spleen. Nature **368:** 747–749.

ROEHL, H., and J. KIMBLE, 1993 Control of cell fate in *C. elegans* by a *glp-1* peptide consisting primarily of ankyrin repeats. Nature **364:** 632–635.

SCHEDL, T., and J. KIMBLE, 1988 *fog-2*, a germ-line-specific sex determination gene required for hermaphrodite spermatogenesis in *Caenorhabditis elegans*. Genetics **119:** 43–61.

SCHEDL, T., P. L. GRAHAM, M. K. BARTON and J. KIMBLE, 1989 Analysis of the role of *tra-1* in germline sex determination in the nematode *Caenorhabditis elegans*. Genetics **123:** 755–769.

SEYDOUX, G., and A. FIRE, 1994 Soma-germline asymmetry in the distributions of embryonic RNAs in *Caenorhabditis elegans*. Development **120:** 2823–2834.

SEYDOUX, G., T. SCHEDL and I. GREENWALD, 1990 Cell-cell interactions prevent a potential inductive interaction between soma and germ line in *Caenorhabditis elegans*. Cell **61:** 939–951.

SULSTON, J., and J. HODGKIN, 1988 Methods, pp 587–606 in *The Nematode Caenorhabditis elegans*, edited by W. B. WOOD. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

TRENT, C., B. PURNELL, S. GAVINSKI, J. HAGEMAN, C. CHAMBLIN *et al.*, 1991 Sex-specific transcriptional regulation of the *C. elegans* sex-determining gene *her-1*. Mechan. Dev. **34:** 43–56.

VILLENEUVE, A. M., and B. J. MEYER, 1990 The regulatory hierarchy controlling sex determination and dosage compensation in *Caenorhabditis elegans*. Adv. Genet. **27:** 117–188.

WARD, S., T. M. ROBERTS, S. STROME, F. M. PAVALKO and E. HOGAN, 1986 Monoclonal antibodies that recognize a polypeptide antigenic determinant shared by multiple sperm specific proteins. J. Cell Biol. **102:** 1778–1786.

YOCHEM, J., and I. GREENWALD, 1989 *glp-1* and *lin-12*, genes implicated in distinct cell-cell interactions in *Caenorhabditis elegans*, encode similar transmembrane proteins. Cell **58:** 553–563.

ZARKOWER, D., and J. HODGKIN, 1992 Molecular analysis of the *C. elegans* sex-determining gene *tra-1*: a gene encoding two zinc finger proteins. Cell **70:** 237–249.

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