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Lissemore, James L.; Currie, Peter D.; Turk, Christine M.; and Maine, Eleanor M., "Intragenic Dominant Suppressors of *glp-1*, a Gene Essential for Cell-Signaling in *Caenorhabditis elegans*, Support a Role for *cdcl O/SWZ6/Ankyrin* Motifs in GLP-1 Function" (1993). *Biology*. 17.

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Intragenic Dominant Suppressors of *glp-1*, a Gene Essential for Cell-Signaling in *Caenorhabditis elegans*, Support a Role for *cdc10/SWI6*/Ankyrin Motifs in GLP-1 Function

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Manuscript received June 7, 1993

Accepted for publication August 28, 1993

ABSTRACT

The *glp-1* gene product mediates cell-cell interactions required for cell fate specification during development in *Caenorhabditis elegans*. To identify genes that interact with *glp-1*, we screened for dominant suppressors of two temperature-sensitive *glp-1* alleles and recovered 18 mutations that suppress both germline and embryonic *glp-1* phenotypes. These dominant suppressors are tightly linked to *glp-1* and do not bypass the requirement for a distal tip cell, which is thought to be the source of a signal that is received and transduced by the GLP-1 protein. Using single-strand conformation polymorphism (SSCP) analysis and DNA sequencing, we found that at least 17 suppressors are second-site intragenic revertants. The suppressors, like the original *glp-1(ts)* mutations, are all located in the *cdc10/SWI6*/ankyrin domain of GLP-1. *cdc10/SWI6*/ankyrin motifs have been shown to mediate specific protein-protein interactions in other polypeptides. We propose that the *glp-1(ts)* mutations disrupt contact between GLP-1 and an as yet unidentified target protein(s) and that the dominant suppressor mutations restore appropriate protein-protein interactions.

CELL-cell signaling is a major mechanism for determining cell fate during development in multicellular organisms (GREENWALD and RUBIN 1992; GURDON 1992; HORVITZ and HERSKOWITZ 1992). In the nematode *Caenorhabditis elegans*, the *glp-1* gene is essential for at least two developmentally important cell-cell interactions: maternal *glp-1* activity is required for induction of the anterior pharynx during embryogenesis and zygotic *glp-1* activity is required for mitotic proliferation of the germ line throughout larval and adult stages (AUSTIN and KIMBLE 1987; PRIESS, SCHNABEL and SCHNABEL 1987). In addition, *glp-1* activity is needed for normal formation of the hypodermis and, in the absence of functional *lin-12* gene product, for generation of several structures required for larval viability (PRIESS, SCHNABEL and SCHNABEL 1987; LAMBIE and KIMBLE 1991b).

glp-1 encodes a putative transmembrane protein with multiple epidermal growth factor (EGF)-like and *lin-12/Notch/glp-1* (LNG) motifs in the extracellular domain and seven *cdc10/SWI6*/ankyrin motifs (hereafter referred to as *cdc10/SWI6* motifs) in the intracellular domain (YOCHER and GREENWALD 1989). Several *glp-1*-related genes have been found in both vertebrates and invertebrates: *lin-12* in *C. elegans* (YOCHER, WESTON and GREENWALD 1988), *Notch* in *Drosophila* (WHARTON *et al.* 1985), *Xotch* in *Xenopus*

(COFFMAN, HARRIS and KINTNER 1990), *int-3* and *Motch* in mice (DEL AMO *et al.* 1992; ROBBINS *et al.* 1992; REAUME *et al.* 1992), *Notch1* and *Notch2* in rats (WEINMASTER, ROBERTS and LEMKE 1991, 1992), and *TAN-1* in humans (ELISEN *et al.* 1991). While *glp-1*, *lin-12* and *Notch* are known to mediate cell-cell interactions required for specification of cell fate (LAMBIE and KIMBLE 1991a; ARTAVANIS-TSAKONAS and SIMPSON 1991), the function of the vertebrate genes has not been determined.

Genetic, molecular and immunolocalization data are consistent with GLP-1 protein being a plasma membrane receptor (AUSTIN and KIMBLE 1987, 1989; YOCHER and GREENWALD 1989; S. CRITTENDEN and J. KIMBLE, unpublished data). Very little is known, however, about the postulated signal transduction pathway mediated by GLP-1. To identify additional components of this signalling pathway, numerous recessive suppressors of *glp-1* missense mutations have been isolated; these suppressors define 22 genes. Mutations in nine *sog* genes (suppressor of *glp-1*) lack an obvious phenotype other than suppression of *glp-1* and little is known about their function (MAINE and KIMBLE 1993; A.-M. HOWELL and J. PRIESS, unpublished data). Mutations in four *sel* genes (suppressor and enhancer of *lin-12*) suppress the maternal effect lethality caused by a partial loss-of-function *glp-1* allele but have no apparent phenotype beyond their interactions with *glp-1* and *lin-12* (SUNDARAM and GREENWALD 1993). In contrast, mutations in eight suppres-

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genes, previously identified as *dpy* and *sqt* genes, alter body shape (MAINE and KIMBLE 1989). At least four of them (*dpy-2*, *dpy-7*, *dpy-10* and *sqt-1*) encode collagen (KRAMER *et al.* 1988; JOHNSTONE, SHAFI and BARRY 1992; J. KRAMER, unpublished data), which suggests that GLP-1 interacts with the extracellular matrix. Finally, an allele of *sog-10* that suppresses only the germline phenotype of *glp-1* also has a conditional feminized germline phenotype (MAINE and KIMBLE 1993).

As a step toward further elucidation of the mechanism of GLP-1 mediated cell-signalling, we have isolated 18 dominant suppressors of two *glp-1* temperature sensitive (*ts*) mutations: *glp-1(q224)* and *glp-1(q231)*. Both *glp-1(ts)* mutations have single amino acid substitutions in the fourth *cdc10/SWI6* repeat of the intracellular domain of GLP-1 (KODOYIANNI, MAINE and KIMBLE 1992). *cdc10/SWI6* domains mediate specific protein-protein interactions in many other systems (BLANK, KOURILSKY and ISRAEL 1992; MICHAELY and BENNETT 1992) and presumably play the same role in GLP-1. All 18 dominant suppressors are tightly linked to *glp-1*. Molecular analysis revealed that at least 17 of these suppressors are second-site intragenic revertants with single amino acid substitutions in the *cdc10/SWI6* domain. The location of these suppressors supports the notion that *cdc10/SWI6* repeats are important for GLP-1 function and that they mediate interaction with a yet to be identified target protein(s).

MATERIALS AND METHODS

Strains and culture methods: Worms were maintained on agar plates as described (BRENNER 1974). The wild-type strain *C. elegans* var. Bristol (N2) and most mutants are described in HODGKIN *et al.* (1988) except where indicated. Nomenclature follows the guidelines of HORVITZ *et al.* (1979).

Mutations used in this study were *dpy* (*dumpy*), *glp* (germline proliferation defective), *him* (high incidence of males), *sma* (*small*) and *unc* (*uncoordinated*):

linkage group III: *dpy-18(e364)*, *dpy-19(e1259ts)*, *glp-1(q224ts)*, *q231ts*, *sma-2(e502)*, *unc-32(e189)*, *unc-36(e251)*, *unc-69(e587)*;

linkage group V: *him-5(e1467)*.

Isolation of dominant suppressors of *glp-1(ts)*: Fourth larval stage (L4) hermaphrodites carrying *glp-1(ts)* and a closely linked marker mutation, *unc-32*, were raised at permissive temperature [12° for *glp-1(q224)* and 15° for *glp-1(q231)*], mutagenized with 10 mM ethyl methanesulfonate (EMS) as described (BARTON and KIMBLE 1990) and returned to plates at permissive temperature.

One strategy used for isolation of suppressor mutations employed a temperature regime that required suppression of both germline and embryonic phenotypes. Using this strategy, three mutations (*q240*, *q246* and *q252*) were isolated as dominant suppressors of *glp-1(q224)*, and 10 mutations (*q277*, *q278*, *q279*, *q280*, *q281*, *q282*, *q283*, *q284*, *q285* and *q286*) were isolated as dominant suppressors of *glp-1(q231)* (Table 1). All but one of these suppressors (*i.e.*, *q252*) were isolated from animals grown on plates as follows. Mutagenized L4s were placed on 100 × 15 mm plates (10

TABLE 1

Dominant suppressors of *glp-1(ts)*

<i>glp-1(ts)</i>	Selection temperature	Alleles	Mutation frequency ^a
<i>glp-1(q224)</i>	25°	<i>q240</i> , <i>q333</i> , <i>q336</i> , <i>q337</i>	1/400,000
	22°	<i>q334</i> , <i>q335</i>	ND ^b
	20°	<i>q246</i> , <i>q252</i>	1/400,000 ^c
<i>glp-1(q231)</i>	25°	None	<1/44,000
	20°	<i>q277</i> , <i>q278</i> , <i>q279</i> , <i>q280</i> , <i>q281</i> , <i>q282</i> , <i>q283</i> , <i>q284</i> , <i>q285</i> , <i>q286</i>	1/31,000

ND, not determined.

^a Calculations for frequency of dominant suppressors assumes ~100 progeny from each *glp-1(ts)* hermaphrodite (see MATERIALS AND METHODS). Frequency is given per haploid genome.

^b Mutations were derived from liquid culture, and therefore mutation frequency cannot be easily estimated.

^c *q252* was recovered from liquid culture and *q246* from plates (see MATERIALS AND METHODS). Frequency estimate is for the latter allele only.

hermaphrodites per plate) and grown at 15° to ensure proper embryonic development of the F₁; when the oldest F₁ animals reached L4, plates were shifted to restrictive temperature (20° or 25°). Plates were screened visually for the oldest

F₁ animals reached L4, plates were shifted to restrictive temperature (20° or 25°). Plates were screened visually for viable F₂ progeny. From any given plate, we kept only one fertile animal to ensure independence of the induced mutation. One mutation, *q252*, was isolated from liquid culture as follows. Mutagenized animals were placed in 100-ml liquid cultures [S-basal medium with *Escherichia coli* (SULTON and BRENNER 1974)] at 15°. Cultures were shifted to 20° after 5 days (age of F₁ progeny were visually monitored to ensure they were shifted before reaching adulthood), and grown for 2–3 days. Animals were harvested and then bleached (WOOD 1988), a process that kills adults but allows embryos to develop properly. Eggs from each culture flask were placed on separate Petri dishes and grown at restrictive temperature.

Five suppressors of *glp-1(q224)* (*q333*, *q334*, *q335*, *q336* and *q337*) were recovered using a second strategy designed to isolate suppressors of the *glp-1* germline phenotype. First, *glp-1(q224)* animals were bleached to obtain a synchronized population of embryos for mutagenesis. After treatment with EMS, animals were maintained at 15° only until L1, shifted to 22° or 25° until adulthood, and then shifted back down to 15°. Using this temperature regimen, germline development takes place at restrictive temperature and embryonic development occurs at permissive temperature. Therefore, a suppressor might rescue the germline phenotype but not the embryonic one. This strategy was carried out both on plates and in liquid culture. Upon testing, each of the five suppressors recovered using this scheme rescued both embryonic and germline defects.

The mutation frequency for isolation of dominant suppressors was estimated only for those selections done with animals grown on plates. To determine this number, we counted broods of several mutagenized animals and found that these hermaphrodites produced an average of ~100 F₁ progeny. Five suppressors of *glp-1(q224)* were recovered from ~1,000,000 F₁ progeny, while 10 suppressors of *glp-1(q231)* were isolated from ~150,000 F₁ animals.

Dominance tests: To remove extraneous mutations from the genome, suppressed lines were outcrossed to wild-type (N2) and fertile *unc-32 glp-1(ts) sup(x)* animals were re-

TABLE 2
Fine structure mapping of dominant suppressor *q240*

Parental genotype	Recombinant phenotype	Recombinant genotype	No. of recombinants ^a
<i>sma-2 unc-69/unc-32 glp-1 q240</i>	Sma	<i>sma-2 unc-32 glp-1 q240/sma-2 unc-69</i>	11/263
		<i>sma-2 glp-1 q240/sma-2 unc-69^b</i>	15/263
		<i>sma-2 glp-1/sma-2 unc-69</i>	0/263
		<i>sma-2/sma-2 unc-69</i>	237/263
<i>dpy-19 unc-69/sma-2 glp-1 q240</i>	Unc	<i>sma-2 glp-1 q240 unc-69/dpy-19 unc-69</i> or <i>sma-2 unc-69/dpy-19 unc-69</i>	131/131
		<i>sma-2 glp-1 unc-69/dpy-19 unc-69</i>	0/131

^a Number of recombinants of a particular class as a proportion of total number of recombinants picked.

^b Distinguished from *sma-2/sma-2 unc-69* by testing Sma recombinants at 25° where *q240* is not 100% penetrant.

covered in the F₂. Here, suppressors are designated *sup(x)*. For each suppressor, rescue of *glp-1(ts)* always segregated with *unc-32*. To test whether a given suppressor was dominant, heterozygous *unc-32 glp-1(ts) sup(x)/sma-2 glp-1(q231)* males were mated to *sma-2 glp-1(q231)* hermaphrodites and their progeny were raised at 20°. Non-Sma cross-progeny [*unc-32 glp-1(ts) sup(x)/sma-2 glp-1(q231)*] were picked and their percent fertility, brood sizes and percent viable progeny determined. To test whether dominant suppression had a maternal component, eight suppressors (*q240*, *q246*, *q252*, *q277*, *q279*, *q280*, *q333* and *q335*) were tested as follows: *glp-1(ts); him-5* males were mated to *unc-32 glp-1(ts) sup(x)* hermaphrodites and their progeny were raised at 20°. Non-Unc cross-progeny were picked and their brood sizes and percent viable progeny were determined.

Genetic mapping: Mapping was done using standard tests. Twelve dominant suppressors (*q240*, *q246*, *q252*, *q277*, *q279*, *q280*, *q282*, *q284*, *q285*, *q333*, *q335* and *q336*) were roughly positioned by three-factor mapping: Dpy non-Unc-36 and Unc-36 non-Dpy recombinants were obtained from *unc-36 dpy-18/unc-32 glp-1(ts)* animals carrying a dominant suppressor. One suppressor, *q240*, was positioned more accurately by three-factor mapping (Table 2). Sma non-Unc-69 recombinants from *sma-2 unc-69/unc-32 glp-1(q224) q240* animals and Unc-69 non-Dpy recombinants from *dpy-19 unc-69/sma-2 glp-1(q224) q240* animals were examined. In no case was the dominant suppressor separated from *glp-1*. *sma-2 glp-1 q240/sma-2 unc-69* were distinguished from *sma-2/sma-2 unc-69* by testing Sma recombinants at 25.5° where *q240* is not 100% penetrant. Similarly, a subset of the Unc recombinants were tested at 25.5° to distinguish *sma-2 glp-1 q240/dpy-19 unc-69* from *sma-2 unc-69/dpy-19 unc-69*. Since only a subset were retested, all Unc recombinants are listed in one category in Table 2.

Single-strand conformation polymorphism (SSCP) analysis: PCR-SSCP (polymerase chain reaction followed by SSCP analysis) was modified from ORITA *et al.* (1989) and IWAHANA, YOSHIMOTO and ITAKURA (1992). Genomic DNA was isolated (WOOD 1988) from strains homozygous for each dominant suppressor mutation except for *q280*. Instead of genomic DNA, a *glp-1* genomic clone from strain *glp-1(q231) q280* was used as a template for PCR. PCRs were performed with the primer pairs listed in Table 3 using 0.25 μm each primer, 10–30 ng *C. elegans* genomic DNA or 100 pg genomic clone DNA, 70 μm each dNTP, 1.5 mM MgCl₂, 2% formamide, 10 μCi [α-³²P]dATP (3000 Ci/mmol, 10 μCi/μl, ICN Biomedicals, Inc.), 50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25°), 0.1% Triton X-100 and 2.5 U *Taq* polymerase (Promega) in 100 μl. Thermal cycler conditions were optimized for each set of primers. Following PCR, radiolabeled DNAs (2.5 μl) were digested with various restriction endonucleases in 5-μl reactions in order to generate

100–500 bp fragments for SSCP analysis. After digestion, 0.5 μl of each sample were mixed with 4.5 μl of 95% formamide, 10 mM NaOH, 20 mM EDTA, 0.25% each xylene cyanol and bromphenol blue, denatured by boiling for 7 min, and cooled on ice. Samples were electrophoresed on non-denaturing 6% polyacrylamide/10% glycerol gels (28 cm × 18 cm × 0.4 mm) containing 1X TBE (89 mM Tris, 89 mM boric acid, 2 mM EDTA) at room temperature. Some samples were also analyzed on 6% polyacrylamide/10% glycerol gels at 4° and on 6% polyacrylamide gels without glycerol at room temperature and at 4°. Gels were electrophoresed at 300–500 V in 1X TBE running buffer. After electrophoresis, gels were transferred to filter paper and subjected to autoradiography without drying.

DNA sequencing: Unlabeled PCR products amplified from genomic DNA (or cloned DNA for *q280*) were directly sequenced after purification with GeneClean (Bio 101) either by thermal cycle sequencing (MURRAY 1989) using a *fmol* DNA sequencing kit (Promega) or with a Sequenase Version 2.0 kit (U.S. Biochemical). Thermal cycle sequencing was performed according to manufacturer's instructions. The Sequenase procedure was modified using a protocol from B. HALL (University of Rochester) provided by R. YOKOYAMA (Syracuse University). Annealing reactions combined 0.5–1.0 μg of PCR product with 0.5 pmol of sequencing primer and 2.0 μl of 5X Sequenase buffer in a total volume of 10 μl. Samples were placed in a boiling water bath for 5 min, quench cooled in an ethanol/dry ice bath for 5 min and thawed at room temperature for 5 min. Sequencing reactions were conducted according to the manufacturer's protocol except that extension and termination reactions were each carried out for 30 sec instead of 5 min. In all cases where SSCPs were found, the entire shifted fragment was sequenced. All mutations were confirmed by sequencing both DNA strands. At least one dominant suppressor of each type was sequenced to confirm the presence of the original *glp-1(ts)* mutation, which also confirmed that suppressor types 2, 4 and 5 suppress both *glp-1(q224)* and *glp-1(q231)* (Table 4).

Determination of brood size and percent hatching: L4 hermaphrodites homozygous for both *glp-1(ts)* and a dominant suppressor were picked from stocks grown at the appropriate temperature (15°, 20° or 25°), placed individually on Petri dishes, and transferred every ~24 hr to a fresh plate. The total number of embryos produced by each hermaphrodite was counted; embryos were scored for viability ~36 hr after the hermaphrodite had been transferred. Hatched progeny were counted once they had achieved at least the L3 stage of development. As controls, brood sizes and percent hatching of *glp-1(q231)*, *glp-1(q224)* and *glp-1(q224)/glp-1(q231)* animals were determined. Dominant suppressors were tested as heterozygotes with the suppressor

TABLE 3
Primers used for PCR-SSCP analysis of *glp-1* dominant suppressor mutations

Region	Size (bp)	Primer	Primer location ^a	Primer sequence
1	1135	EM-7	2456–2475	5'-CACCAAGAGCTGCTCTAACA-3'
		VK-8	3574–3590	5'-TAGATGCACTTCTCGCC-3'
2	1157	EM-8	3435–3454	5'-CCCACCACTGGCCACAGTC-3'
		EM-14	4572–4591	5'-GGGAGACTTGGATCTCGCGC-3'
3	1258	EM-9	4222–4241	5'-GTTACACAGACAGCCATGAG-3'
		VK-5	5463–5479	5'-CCCGAATCCTGAAGGAC-3'
4	721	VK-4	5381–5397	5'-GGATACTGTGCCCATGA-3'
		VK-17	6085–6101	5'-CTTACAAGTACCTCCG-3'
5	1214	EM-11	6016–6035	5'-GCTCAAGATTACTAGGCAGC-3'
		VK-1	7213–7229	5'-GTGACAACAGCTTGCCG-3'
6	1451	EM-4	6935–6952	5'-GTGGTTTTGACGGTGGAG-3'
		EM-2A	8367–8386	5'-GGCAGCAAGCCAGTGAGAA-3'
7	1222	EM-3	8200–8217	5'-CTGATCTACCAGCCGAGC-3'
		VK-12	9406–9422	5'-CATTGACCGGTGGTGC-3'
8	786	SC-4	9201–9220	5'-CAACAAGTGCAGCATCGTCT-3'
		EM-1	9969–9986	5'-TCGGATCGAAATGAGGAG-3'

^a Nucleotide positions from *glp-1* genomic sequence reported by YOCHER and GREENWALD (1989). Amino acid coding sequence begins at nucleotide 2513 and ends at 9529.

TABLE 4
Molecular changes in intragenic dominant suppressors of *glp-1(ts)*^a

Type	Nucleotide change ^b	Codon change	Amino acid change ^b	<i>glp-1(ts)</i> allele	Suppressor allele
1	G 8582 → A	AGU → AAU	Ser 997 → Asn	<i>q231</i>	<i>q278, q282, q283, q285</i>
2	G 8991 → A	AGU → AAU	Ser 1133 → Asn	<i>q224</i>	<i>q335</i>
3	G 8996 → A	GGU → AGU	Gly 1135 → Ser	<i>q231</i>	<i>q284</i>
				<i>q231</i>	<i>q280</i>
4	G 8997 → A	GGU → GAU	Gly 1135 → Asp	<i>q224</i>	<i>q246</i>
				<i>q231</i>	<i>q277, q279</i>
5	G 9032 → A	GUU → AUU	Val 1147 → Ile	<i>q224</i>	<i>q240, q252, q333, q334, q336, q337</i>
				<i>q231</i>	<i>q286</i>

^a See also Figures 3 and 4.

^b Nucleotide and amino acid positions from YOCHER and GREENWALD (1989).

provided by the mother or the father as described above in DOMINANCE TESTS.

Distal tip cell ablations: Distal tip cell ablations were performed as described (MAINE and KIMBLE 1993).

RESULTS

Isolation of *glp-1(ts)* dominant suppressors: The germ line of wild-type hermaphrodites first produces ~300 sperm and subsequently makes a variable but much greater number of oocytes. Thus, an unmated hermaphrodite produces ~300 offspring. In contrast, worms homozygous for strong loss-of-function *glp-1* alleles have severely underproliferative germ lines (AUSTIN and KIMBLE 1987). As a result, *glp-1* hermaphrodites make only a few sperm and no oocytes and are therefore sterile (AUSTIN and KIMBLE 1987). Depending on the temperature, the germ line of *glp-1(ts)* mutants can have an intermediate amount of proliferation and produce some embryos, but these embryos are often inviable. The *glp-1(ts)* mutants used in this study, *glp-1(q224)* and *glp-1(q231)*, have reduced

brood sizes: *glp-1(q224)* animals produce no embryos at 20° or 25° (AUSTIN and KIMBLE 1987; Table 6); *glp-1(q231)* animals produce no embryos at 25° and an average of 27 inviable embryos at 20° (AUSTIN and KIMBLE 1987; MAINE and KIMBLE 1989; Table 6). Both mutations encode single amino acid substitutions (Gly → Glu) in the fourth *cdc10/SWI6* repeat (Figures 3 and 4; KODOYIANNI, MAINE and KIMBLE 1992).

Two mutagenesis strategies were used to isolate the 18 dominant suppressors reported here in an attempt to isolate mutations that would suppress both germline and embryonic phenotypes and those that would suppress the germline phenotype alone (see MATERIALS AND METHODS). With each strategy, some suppressors were selected at 22° and 25° in order to isolate mutations that might bypass the requirement for *glp-1* function altogether while others were selected at 20° in an effort to find a wide constellation of suppressor types.

Thirteen dominant suppressors (*q240, q246, q252,*

TABLE 5
Tests for dominant suppression of *glp-1(ts)* at 20°

Type	Suppressor	Genotype: ^a maternal chromosome/ paternal chromosome	Brood size (n) ^b	% Viable progeny ^c
—	—	<i>glp-1(q224)</i>	0	NA
—	—	<i>glp-1(q231)^d</i>	27 ± 4.5	0
—	—	<i>glp-1(q231)/glp-1(q224)</i>	6 ± 2 (15)	0
—	<i>q281</i>	<i>glp-1(q231)/glp-1(q231 q281)</i>	308 ± 25 (4)	>99
1	<i>q278</i>	<i>glp-1(q231)/glp-1(q231 q278)</i>	282 ± 19 (3)	99
	<i>q282</i>	<i>glp-1(q231)/glp-1(q231 q282)</i>	282 ± 16 (4)	>99
	<i>q283</i>	<i>glp-1(q231)/glp-1(q231 q283)</i>	208 ± 24 (2)	99
	<i>q285</i>	<i>glp-1(q231)/glp-1(q231 q285)</i>	263 ± 2 (2)	99
2	<i>q335</i>	<i>glp-1(q231)/glp-1(q224 q335)</i>	212 ± 20 (4)	92
		<i>glp-1(q224 q335)/glp-1(q224)</i>	164 ± 59 (3)	88
	<i>q284</i>	<i>glp-1(q231)/glp-1(q231 q284)</i>	258 ± 17 (4)	98
3	<i>q280</i>	<i>glp-1(q231)/glp-1(q231 q280)</i>	213 ± 22 (3)	88
		<i>glp-1(q231 q280)/glp-1(q231)</i>	185 ± 19 (5)	84
4	<i>q246</i>	<i>glp-1(q231)/glp-1(q224 q246)</i>	174 ± 32 (2)	98
		<i>glp-1(q224 q246)/glp-1(q224)</i>	217 ± 10 (5)	>99
	<i>q277</i>	<i>glp-1(q231)/glp-1(q231 q277)</i>	210 ± 28 (4)	99
		<i>glp-1(q231 q277)/glp-1(q231)</i>	296 ± 20 (5)	>99
	<i>q279</i>	<i>glp-1(q231)/glp-1(q231 q279)</i>	252 ± 15 (4)	99
		<i>glp-1(q231 q279)/glp-1(q231)</i>	263 ± 14 (5)	>99
5	<i>q240</i>	<i>glp-1(q231)/glp-1(q224 q240)</i>	265 ± 8 (3)	99
		<i>glp-1(q224 q240)/glp-1(q224)</i>	255 ± 12 (3)	99
	<i>q252</i>	<i>glp-1(q231)/glp-1(q224 q252)</i>	236 ± 39 (3)	98
		<i>glp-1(q224 q252)/glp-1(q224)</i>	286 ± 9 (5)	99
	<i>q333</i>	<i>glp-1(q231)/glp-1(q224 q333)</i>	235 ± 19 (3)	>99
		<i>glp-1(q224 q333)/glp-1(q224)</i>	244 ± 36 (5)	98
	<i>q334</i>	<i>glp-1(q231)/glp-1(q224 q334)</i>	282 ± 20 (4)	99
	<i>q336</i>	<i>glp-1(q231)/glp-1(q224 q336)</i>	265 ± 14 (3)	99
	<i>q337</i>	<i>glp-1(q231)/glp-1(q224 q337)</i>	258 ± 14 (2)	99
	<i>q286</i>	<i>glp-1(q231)/glp-1(q231 q286)</i>	202 ± 13 (3)	99

NA, not applicable.

^a Heterozygotes were generated as described in MATERIALS AND METHODS. Only a subset of alleles were tested for a maternal effect.

^b n, number of hermaphrodites whose progeny were counted. Standard errors are given.

^c Viable progeny are those that survive at least until L3 stage.

^d From MAINE and KIMBLE (1989).

q277, *q278*, *q279*, *q280*, *q281*, *q282*, *q283*, *q284*, *q285* and *q286*) were isolated in selections for fertile hermaphrodites and five dominant suppressors (*q333*, *q334*, *q335*, *q336* and *q337*) were isolated in screens for hermaphrodites with proliferative germlines. Upon retesting, suppressors in the latter group also rescued both the germline and embryonic *glp-1* phenotypes (Tables 5 and 6) and all suppressors remained dominant upon retesting (Table 5). We have not been able to detect a novel visible phenotype for any of them in a *glp-1(ts)* background, even at 15° where the *glp-1(ts)* phenotype is less severe (Table 6 and data not shown).

Frequencies with which suppressors were isolated:

Dominant suppressors of *glp-1(q224)* were recovered at a frequency of ~1/400,000 haploid genomes and dominant suppressors of *glp-1(q231)* were recovered at a frequency of ~1/31,000 haploid genomes (Table 1). The typical frequency for loss of function mutations in *C. elegans* under our conditions is 3–4 × 10⁻⁴ mutations/haploid genome. The low mutation fre-

quency of the dominant suppressors suggests that a simple loss of gene function is not associated with dominant suppression of either *glp-1(ts)* allele.

Dominant suppressors are tightly linked to *glp-1*:

Each of the 18 dominant suppressors is linked to the original *glp-1(ts)* mutation on LG III (data not shown). No unlinked dominant suppressors were recovered. Twelve dominant suppressors representing a range of suppressor efficiencies (see below and Tables 5 and 6) were selected for three-factor mapping using *unc-36* and *dpy-18*. All 12 suppressors map between *unc-36* and *dpy-18*, in a region extending ~0.9 map units (mu) to the left and ~7.8 mu to the right of *glp-1* (data not shown). In addition, fine structure mapping of one allele, *q240*, places it within the ~2.2 mu region defined by *sma-2* and *unc-69* (Table 2). None of 26 recombinants in the 0.25 mu between *sma-2* and *glp-1* separated *q240* from *glp-1(q224)*; neither did any of the estimated 119 recombinants in the 2.0 mu between *glp-1* and *unc-69*. (This number is estimated based on 131 recombinants in the 2.2 mu between

TABLE 6
Suppression of *glp-1(ts)* by homozygous dominant suppressors at 15°, 20°, 25°

Type	Suppressor	Genotype ^a	15°		20°		25°	
			Brood size ^b	% Viable progeny ^c	Brood size ^d	% Viable progeny ^c	Brood size ^e	% Viable progeny ^c
—	—	<i>glp-1(q224)</i>	163 ± 14	78	0	0	0	NA
—	—	<i>glp-1(q231)</i>	214 ± 22	87	27 ± 4.5 ^f	0 ^f	0	NA
1	<i>q281</i>	<i>glp-1(q231 q281)</i>	ND	ND	146 ± 12	69	44 ± 4	0
	<i>q278</i>	<i>glp-1(q231 q278)</i>	ND	ND	271 ± 27	>99	56 ± 22	70
	<i>q282</i>	<i>glp-1(q231 q282)</i>	271 ± 15	>99	264 ± 27	>99	92 ± 4	90
	<i>q283</i>	<i>glp-1(q231 q283)</i>	ND	ND	171 ± 15	83	90 ± 17	89
	<i>q285</i>	<i>glp-1(q231 q285)</i>	ND	ND	239 ± 70	89	69 ± 13	87
2	<i>q335</i>	<i>glp-1(q224 q335)</i>	226 ± 11	98	244 ± 11	>99	0	NA
	<i>q284</i>	<i>glp-1(q231 q284)</i>	185 ± 18	>99	229 ± 17	98	29 ± 28	14
3	<i>q280</i>	<i>glp-1(q231 q280)</i>	ND	ND	185 ± 19	84	0	NA
4	<i>q246</i>	<i>glp-1(q224 q246)</i>	255 ± 4	>99	243 ± 17	98	1 ± 0.7	71
	<i>q277</i>	<i>glp-1(q231 q277)</i>	328 ± 11	>99	265 ± 13	>99	123 ± 5	91
	<i>q279</i>	<i>glp-1(q231 q279)</i>	ND	ND	270 ± 12	98	144 ± 12	95
5	<i>q240</i>	<i>glp-1(q224 q240)</i>	ND	ND	180 ± 11	98	139 ± 7	97
	<i>q252</i>	<i>glp-1(q224 q252)</i>	213 ± 10	>99	262 ± 13	>99	43 ± 10	88
	<i>q333</i>	<i>glp-1(q224 q333)</i>	ND	ND	236 ± 25	>99	46 ± 26	89
	<i>q334</i>	<i>glp-1(q224 q334)</i>	ND	ND	223 ± 7	>99	31 ± 3	84
	<i>q336</i>	<i>glp-1(q224 q336)</i>	ND	ND	237 ± 6	>99	46 ± 16	91
	<i>q337</i>	<i>glp-1(q224 q337)</i>	ND	ND	237 ± 25	99	40 ± 21	86
	<i>q286</i>	<i>glp-1(q231 q286)</i>	286 ± 11	>99	279 ± 9	>99	136 ± 6	96

NA, not applicable; ND, not done.

^a Each strain is marked with *unc-32(e189)*.

^b The progeny of five hermaphrodites of each genotype were counted, with the following exceptions: *q284*, 4 broods; *q335*, 3 broods. Standard errors are given.

^c Percentage of progeny to develop to at least L3.

^d The progeny of three hermaphrodites of each genotype were counted with the exception of two broods of *q335*.

^e The progeny of three hermaphrodites of each genotype were counted with the following exceptions: *q240*, 4 broods; *q246*, 25 broods; *q284*, 5 broods; *q335*, >20 broods; *q337*, 4 broods.

^f MAINE and KIMBLE (1989).

sma-2 and *unc-69*. See MATERIALS AND METHODS.) These map data suggested that the dominant suppressors might be intragenic revertants. In no case was the original *glp-1(ts)* mutant lesion corrected, however, because each suppressor strain contains at least occasional Glp animals if raised at a sufficiently high temperature (data not shown). Because these dominant suppressors were later shown to be intragenic revertants (see below), we did not assign them new gene names.

Molecular localization of *glp-1(ts)* dominant suppressor mutations: If the dominant suppressors are indeed intragenic revertants of *glp-1(ts)*, then molecular characterization of the mutations should provide information on the structure and function of GLP-1. Toward this end, we used single-strand confirmation polymorphism (SSCP) analysis (ORITA *et al.* 1989; IWAHANA, YOSHIMOTO and ITAKURA 1992) of the *glp-1* gene to localize dominant suppressor mutations that might be present in *glp-1*. We used DNA sequencing to determine the precise molecular nature of the mutations.

Genomic DNA isolated from homozygous strains of each dominant suppressor mutation, except for *q280*,

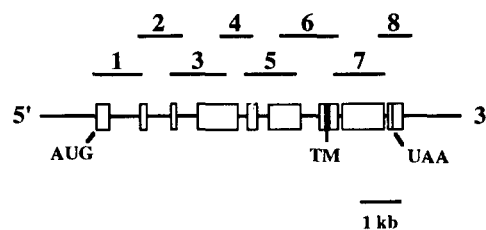


FIGURE 1.—Regions of *glp-1* amplified for SSCP analysis. Bars above the gene structure diagram indicate approximate endpoints and sizes of PCR products amplified from genomic DNA from homozygous *glp-1* dominant suppressor strains. Open boxes, exons; AUG and UAA, start and stop codons, respectively; TM, transmembrane domain.

was used as the template for PCR. For *q280*, a genomic clone of *glp-1* from *glp-1(q231) q280* was used for PCR. For each dominant suppressor, eight overlapping segments spanning the *glp-1* gene (Figure 1) were amplified by PCR in the presence of [α -³²P]dATP. Radiolabeled PCR products were digested with a variety of restriction endonucleases to generate 100–500 bp fragments suitable for SSCP analysis. Every portion of the *glp-1* gene was represented at least once on a fragment in this size range. Restriction fragments were denatured and electrophoresed on nondenatur-

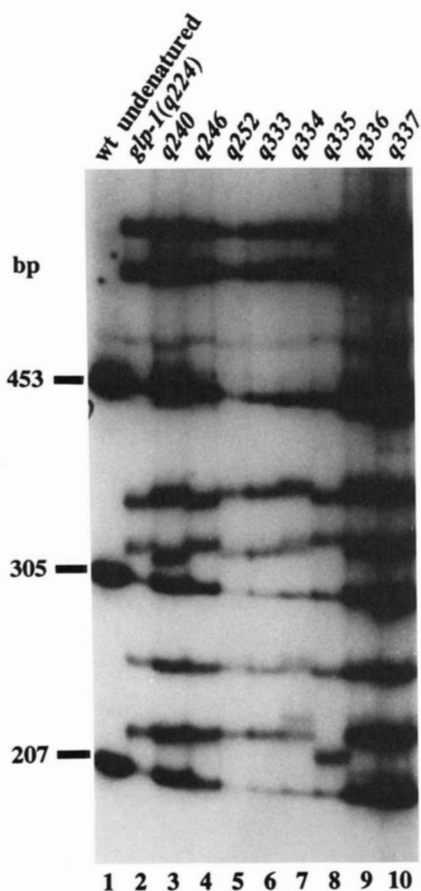


FIGURE 2.—SSCP analysis of dominant suppressors of *glp-1(q224)*. Radiolabeled PCR products from region 7 of the *glp-1* gene (see Figure 1) were generated from the indicated strains. Region 7 contains the entire *cdc10/SWI6* domain of *glp-1*. PCR products were digested with *HinfI* and subjected to SSCP analysis as described in MATERIALS AND METHODS. Samples were denatured prior to electrophoresis, except for that in lane 1, and were separated on a 6% polyacrylamide/10% glycerol gel at room temperature. bp, base pairs; wt, wild type.

ing polyacrylamide gels with or without glycerol. Under these conditions, single nucleotide changes may alter the electrophoretic mobility of one or both single-stranded molecules from a given DNA fragment.

An example of SSCP analysis of region 7 from the eight dominant suppressors of *glp-1(q224)* is shown in Figure 2. SSCPs were detected in the 305 nt *HinfI* fragment (Figure 2; lanes 3, 5, 6, 7, 9 and 10) and in the 207 nt *HinfI* fragment (Figure 2; lane 8). DNA sequencing of regions corresponding to the shifted fragments revealed a single nucleotide substitution that would cause a single amino acid substitution in each case (Table 4).

Further SSCP analysis and limited DNA sequencing uncovered single nucleotide substitutions within *glp-1* in all but one of the remaining suppressor strains (Table 4); we were unable to locate the mutation in *q281*. All of the base substitutions found are G-C → A-T transitions of the sort typically produced by EMS mutagenesis. Several independently isolated suppres-

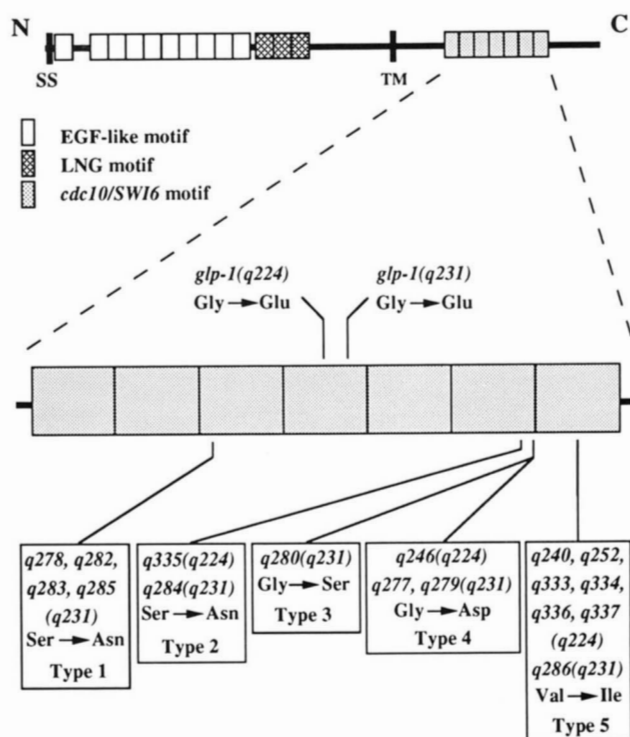


FIGURE 3.—Location of dominant suppressor mutations of *glp-1(q224)* and *glp-1(q231)* in the predicted GLP-1 protein. Schematic diagram of the GLP-1 protein, oriented with the N-terminus to the left, indicates repeated amino acid sequence motifs (boxes), a signal sequence (SS) and a transmembrane domain (TM). Locations of amino acid substitutions for *glp-1(ts)* mutations (KODOYIANNI, MAINE and KIMBLE 1992) and *glp-1(ts)* dominant suppressor mutations are indicated above and below the blown-up *cdc10/SWI6* region, respectively. See also Table 4 and Figure 4.

sor lines carry the same second-site mutation in addition to the original *glp-1(ts)* mutation (Table 4). Thus, each of the 17 molecularly characterized suppressors contains one of only five different base substitutions, which we have designated types 1–5 (Table 4). These base substitutions result in five different amino acid substitutions at four different codons. The amino acid substitutions range from very conservative (Val 1147 → Ile) to very dissimilar (Gly 1135 → Asp) (Table 4). Type 2, 4 and 5 mutations were recovered as suppressors of both *glp-1(q231)* and *glp-1(q224)* and were detected by SSCP analysis. Type 1 and 3 mutations were recovered as suppressors of *glp-1(q231)* alone and did not show SSCPs under a variety of electrophoresis conditions. Type 1 and 3 were discovered by sequencing the region in the vicinity of the other three classes. We did not detect any SSCPs for *q281* and partial sequencing of *q281* demonstrated that this suppressor does not carry any of the five nucleotide substitutions found in the other dominant suppressors. Therefore, the *q281* mutation must lie elsewhere in the *glp-1* gene or in a nearby gene.

The most striking feature of the dominant suppressors is that they are all located within the *cdc10/SWI6* domain (Figures 3 and 4). In addition, the location of

the type 5 suppressor indicated the presence of a degenerate seventh *cdc10/SWI6* repeat that had not been reported earlier (Figure 4). Inspection of the *lin-12* amino acid sequence revealed that it too contains a loosely conserved seventh *cdc10/SWI6* repeat [amino acids 1267–1299 (YOCHEM, WESTON and GREENWALD 1988)]. In general, the intragenic revertants do not seem to make their respective repeats more like the fourth repeat, where the two *glp-1(ts)* mutations are found. The lone exception is the type 4 substitution; Asp (D) is present at this location in the fourth repeat (Figure 4). Four of the amino acid substitutions occur within a stretch of 15 amino acids spanning the sixth and seventh *cdc10/SWI6* repeats; a fifth substitution is located in the third repeat (Figures 3 and 4). Interestingly, the type 2 suppressor places Asn (N) in the sixth repeat at the corresponding position also occupied by Asn in the second and fifth repeats (Figure 4). Likewise, the type 4 suppressor places Asp (D) in the sixth repeat in the corresponding position also occupied by Asp in the fourth and fifth repeats (Figure 4).

Further characterization of *glp-1* suppression: Is there a correlation between suppressor strength and the type of substitution? To determine the strength of each suppressor, we quantified the average brood size of *glp-1(ts)* hermaphrodites carrying each suppressor. Brood size is an indirect measure of the extent of germline proliferation. In addition, we assayed the degree of embryonic viability by determining the percent of progeny that hatch and reach adulthood.

*Efficiency of suppression of the *glp-1(ts)* germline phenotype:* Originally, each suppressor was tested for dominance at 20° in heterozygotes where the suppressor was donated by the father (Table 5). To determine whether there was a maternal component to the suppression, a subset of eight dominant suppressors representing the range of suppressor efficiencies also were characterized as heterozygotes with the suppressor donated by the mother. No substantial maternal effect was observed (Table 5). Subsequently, strains homozygous for both *glp-1(ts)* and a dominant suppressor were characterized at 15°, 20° and 25° (Table 6).

Germline proliferation varies slightly (<2-fold) in *glp-1(ts)* strains that are heterozygous for different dominant suppressors regardless of which parent contributes the suppressor. At 20°, some heterozygous suppressor strains (*e.g.*, *q277*) produce a brood close to wild type (~300 progeny) while other heterozygous strains (*e.g.*, *q335*) produce a brood that is ~50% of wild type (Table 5). However, dominant suppressors share some common characteristics. First, each one is incompletely penetrant; some Glp animals are observed in each dominant suppressor strain if raised at sufficiently high temperature (data not shown). Second, each dominant suppressor is less effective at the

more stringent 25° than at an intermediate temperature of 20° (Table 6). Third, those suppressors that do not restore brood sizes to approximately wild-type levels as heterozygotes at 20° are more effective when present in two copies (Tables 5 and 6).

*Efficiency of suppression of the *glp-1(ts)* embryonic phenotype:* Progeny viability varies widely at 25° in strains carrying different dominant suppressors (Table 6). Viability is always lower at 25° than at 20°, where hatching generally is restored to at least 98%. Among inviable progeny, most (~80%) die as embryos, but some (~20%) die as newly hatched L1s (data not shown). In general, the extent of suppression of the embryonic lethality by any given dominant suppressor correlates with the extent of suppression of the germline defect.

Comparison of suppression strength with suppressor type: The five different suppressor types vary <2-fold with respect to their dominant suppression of both *glp-1(ts)* alleles at 20° (Table 5). Furthermore, even when homozygous the various suppressor types are very similar in their effectiveness at 15° and 20° (Table 6).

The situation at 25°, however, is quite different. For example, the type 5 substitution is a much more effective suppressor of the germline phenotype of a given *glp-1(ts)* allele than are types 2 and 3 (Table 6). In addition, there are substantial differences in suppression of the germline phenotype of the two *glp-1(ts)* alleles by a given dominant suppressor, *e.g.*, type 4 suppresses *glp-1(q231)* much better than *glp-1(q224)* (Table 6).

Do *glp-1* intragenic revertants bypass the requirement for a distal tip cell? Mitotic proliferation of the germ line in wild-type nematodes requires the distal tip cell, a somatic cell located at the distal end of the gonad (KIMBLE and WHITE 1981). One possible model for control of germline proliferation is that a signal produced by the distal tip cell is received and transduced by GLP-1 to stimulate mitosis and/or to inhibit meiosis in the distal region of the germ line. Intragenic revertants that cause constitutive activity of GLP-1 (*i.e.*, gain-of-function mutation) might render the putative distal tip cell signal unnecessary. To test whether any of the intragenic revertants have this effect, the distal tip cell was ablated in one gonad arm of developing hermaphrodite larvae, and subsequent germline development was observed. The unoperated gonad arm in each animal served as an internal control.

We ablated distal tip cells in animals carrying one of 14 intragenic revertants (all but *q246*, *q278*, *q334* and *q337*). Because they could not be separated from *glp-1(ts)*, the dominant suppressors were examined in a *glp-1(ts)* background. Germline proliferation was assayed by counting the number of germline nuclei

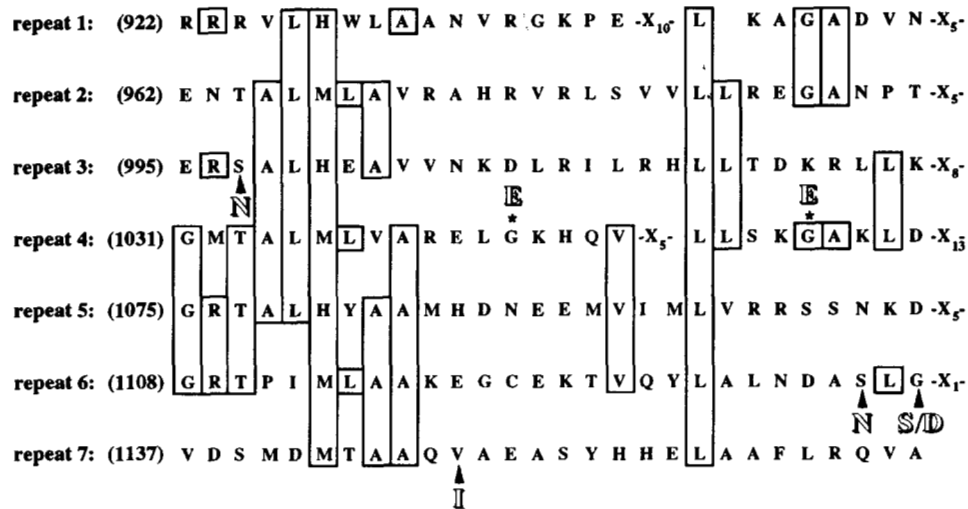


FIGURE 4.—Alignment of *cdc10/SWI6* repeats in GLP-1 and location of *glp-1(ts)* dominant suppressor mutations. The six previously described *cdc10/SWI6* repeats and a loosely conserved seventh repeat are aligned as in KODOYIANNI, MAINE and KIMBLE (1992). Numbers in parentheses to the left of each repeat refer to the location of the first amino acid in the repeat within GLP-1 [amino acid positions from YOCHAM and GREENWALD (1989)]. Boxed amino acids are found at a given position in at least three repeats. Amino acid substitutions are indicated by shadowed letters: *glp-1(ts)* mutations (KODOYIANNI, MAINE and KIMBLE 1992) are indicated with asterisks above the wild-type amino acids and dominant suppressor mutations are indicated with arrowheads below the wild-type amino acids. See also Table 4 and Figure 3.

before and after distal tip cell ablation. In each case, germline proliferation in the operated gonad arm stopped after ablation of its distal tip cell while proliferation continued in the intact gonad arm (data not shown). Hence, none of the tested intragenic revertants bypasses the requirement for a distal tip cell in the process of germline mitosis.

DISCUSSION

We have described 17 intragenic dominant suppressors of two temperature-sensitive *glp-1* alleles. Remarkably, all of the suppressors are single amino acid substitutions within the *cdc10/SWI6* region of the putative intracellular domain of GLP-1 (Table 4; Figures 3 and 4). Thirteen suppressor mutations are tightly clustered at three sites within a 15-amino acid region spanning the sixth and seventh *cdc10/SWI6* repeats; four other suppressor mutations are located at a single site in the third repeat. The observation that 17 dominant suppressors of *glp-1* are intragenic revertants in the *cdc10/SWI6* region combined with the presence of five *glp-1(ts)* missense mutations in this same domain (KODOYIANNI, MAINE and KIMBLE 1992) provides striking evidence of the importance of *cdc10/SWI6* repeats for GLP-1 function.

Though we do not know the function of the *cdc10/SWI6* repeats in GLP-1, data from other proteins carrying these repeats clearly suggest they play a role in highly specific protein-protein interactions. Deletion analysis and site-directed mutagenesis of *cdc10/SWI6* repeats from human erythrocyte ankyrin (DAVIS and BENNETT 1990; DAVIS, OTTO and BENNETT 1991), rat GABP β (THOMPSON, BROWN and MC-

KNIGHT 1991), and several members of the I κ B family [*i.e.*, chicken pp40/I κ B β (INOUE *et al.* 1992), human Bcl-3 (BOURS *et al.* 1993; WULCZYN, NAUMANN and SCHEIDEREIT 1992), human NF- κ B p105 and I κ B/MAD-3 (HATADA, NAUMANN and SCHEIDEREIT 1993), *Drosophila cactus* (KIDD 1992)] have shown that this motif mediates direct protein-protein contacts with distinct target proteins. These proteins perform a variety of functions: ankyrin is a cytoskeletal protein, GABP β is a subunit of a transcription factor and I κ B-related proteins prevent nuclear translocation of *rel*-related transcription factors. Several of the approximately 30 amino acids in each repeat are highly conserved within and between proteins; however, most of the residues are highly variable (MICHAELY and BENNETT 1992; BLANK, KOURILSKY and ISRAEL 1992). This variability suggests that the consensus amino acids in the *cdc10/SWI6* motif form a basic framework conducive to protein-protein contacts and that the variable residues confer distinct functional identities upon each repeat. For example, only two of the 22 repeats present in ankyrin are necessary, although not sufficient, for binding to the anion exchanger while tubulin is able to interact with many repeats that do not bind to the anion exchanger (DAVIS, OTTO and BENNETT 1991). While individual *cdc10/SWI6* repeats can interact with very specific target proteins, the repeats in general recognize a diverse group of target proteins that appears to lack a conserved recognition sequence (MICHAELY and BENNETT 1992). For example, the anion exchanger and GABP α , which binds to GABP β , share little sequence similarity and seem to have very different

recognition domains (MICHAELY and BENNETT 1992). In light of these findings from other systems, it is likely that the *cdc10/SWI6* domain in GLP-1 mediates interaction with an as yet unidentified protein (or proteins) that may be a component of the GLP-1 mediated signalling pathway.

There are several possible mechanisms by which *glp-1* dominant suppressors might restore GLP-1 function. First, the dominant suppressor mutations may change the functional identity of the *cdc10/SWI6* repeats in which they are located. The amino acid substitutions in *glp-1(q224)* and *glp-1(q231)* may disrupt contact between a specific repeat and a putative target protein. Such an effect has been demonstrated by site-directed mutagenesis of the *cdc10/SWI6* repeats in pp40/I κ B β which abolished direct association with the *rel* gene product (INOUE *et al.* 1992). The suppressor mutations located in the third, sixth and seventh repeats may allow these repeats to substitute for the normal function of the fourth repeat, *i.e.*, they may interact with a target protein that normally binds to the fourth repeat. Second, if the target protein interacts with more than one repeat [as the targets of ankyrin, GABP β , and I κ B-related proteins apparently do (INOUE *et al.* 1992; BOURS *et al.* 1993; WULCZYN, NAUMANN and SCHEIDEREIT 1992; KIDD 1992; HATADA, NAUMANN and SCHEIDEREIT 1993)], then repeats containing a suppressor mutation may have increased affinity for the target thereby stabilizing the interaction between the fourth repeat and the target. Third, the dominant suppressors may restore and stabilize proper folding of the *cdc10/SWI6* domain disrupted by the *glp-1(ts)* mutations. Such misfolding could directly inhibit GLP-1 function or could destabilize the protein so that it is more easily degraded. In ankyrin, deletion of some of the 22 repeats alters the structure of the remaining repeats as monitored by circular dichroism spectra (DAVIS, OTTO and BENNETT 1991) so interactions between *cdc10/SWI6* repeats within a protein appear to be necessary for correct folding. In addition, MICHAELY and BENNETT (1992) have recently proposed a structure for *cdc10/SWI6* repeats in which extensive contacts between repeats stabilize the structure of the domain. Intragenic suppression by restoration of structural stability has been proposed for mutations affecting *Staphylococcus aureus* nuclease (SHORTLE and LIN 1985) and bacteriophage P22 gene 9 tailspike protein (MAURIDES, SCHWARZ and BERGET 1990). Furthermore, it is possible that the various suppressor types do not act in the same fashion.

Can the intragenic revertants reported here suppress other *glp-1* mutations in the *cdc10/SWI6* region? Since at least three classes of revertants suppress two different *glp-1(ts)* lesions in this domain (Table 4) and since the revertants may suppress by stabilizing or

strengthening interactions with a target protein (see above), we think it is possible that they would suppress the other three known mutations in this region (KODOYIANNI, MAINE and KIMBLE 1992). This phenomenon of "global" suppression, in which a given intragenic revertant suppresses multiple different alleles, has been reported for mutations affecting *S. aureus* nuclease (SHORTLE and LIN 1985) and *E. coli trp* repressor (KLIG, OXENDER and YANOFSKY 1988). While the mechanism of global suppression in these cases is not known, it may involve increased conformational stability of the protein (SHORTLE and LIN 1985).

Although we have not genetically separated the suppressor and *glp-1(ts)* mutations, we can draw some tentative conclusions about the effect of the suppressors on *glp-1* function. Since germline proliferation in dominant suppressor strains is dependent upon the distal tip cell and since *glp-1(ts) sup(x)/++* strains generated during mapping of the suppressors lack an obvious phenotype (data not shown), the suppressors alone are unlikely to be strong gain-of-function mutations, assuming *glp-1* acts downstream of the distal tip cell. Nevertheless, the intragenic revertants could be weak gain-of-function alleles by themselves if they increase the strength of the interaction between GLP-1 and the putative target protein. For instance, amino acid substitutions found in intragenic revertants of *E. coli trp* repressor mutations cause increased repressor function when they are present in an otherwise wild-type protein (KLIG, OXENDER and YANOFSKY 1988). In addition, the intragenic *glp-1* suppressors are probably not strong loss-of-function mutations. At 15°, *glp-1(q224)* and *glp-1(q231)* have moderate levels of *glp-1* function, as measured by brood size and embryonic viability (Table 6). In general, strains carrying a dominant suppressor and *glp-1(ts)* have larger broods and higher embryonic viability than those with the *glp-1(ts)* mutations alone (Table 6). Therefore, the suppressor mutations do not exacerbate the *Glp* phenotype at 15°, but instead they continue to suppress it.

It is not obvious why dominant suppressors of *glp-1(q231)* were recovered 10 times more frequently than suppressors of *glp-1(q224)* (Table 2). While there are clearly not 10 times as many sites within *glp-1* that can be mutated to suppress *glp-1(q231)* as can suppress *glp-1(q224)*, all five suppressor types reported here were recovered as suppressors of *glp-1(q231)* while only three were recovered as *glp-1(q224)* suppressors (Table 4). The absence of type 1 and 3 suppressors of *glp-1(q224)* could simply be a statistical artifact. If, however, type 1 and type 3 suppressors are specific to *glp-1(q231)* and if these sites are mutagenic hotspots, then this combination of factors may explain the frequency difference. Moreover, we can rule out differ-

ences in suppression strength as a possible explanation because at 20° the extent of suppression by a given suppressor type is similar for both *glp-1(ts)* alleles (Table 5). If suppression strength were the crucial factor determining mutation frequency, we should have recovered suppressors of both *glp-1(ts)* alleles at similar frequencies at 20°.

Elucidation of the function of *cdc10/SWI6* repeats in GLP-1 and identification of the putative target protein(s) with which they interact will be crucial for understanding the GLP-1 mediated signalling pathway. Such information is also likely to be relevant for discerning the mechanism of action of the *C. elegans lin-12* gene because *glp-1* and *lin-12* are closely related (50–60% amino acid identity) (YOICHEM and GREENWALD 1989), they are partially functionally redundant (LAMBIE and KIMBLE 1991b), and the *cdc10/SWI6* region appears to be important for *lin-12* function (GREENWALD and SEYDOUX 1990). In addition, a detailed understanding of the function of *cdc10/SWI6* repeats in GLP-1 may yield insight into the mechanisms by which Notch and related vertebrate proteins function. Further support for the importance of *cdc10/SWI6* repeats in GLP-1 and related proteins is provided by the results of *in vivo* expression of genes encoding truncated forms of these proteins consisting of portions of the intracellular domain with or without the transmembrane domain and a few amino acids from the extracellular domain (ELLISEN *et al.* 1991; JHAPPAN *et al.* 1992; ROBBINS *et al.* 1992; COFFMAN *et al.* 1993; STRUHL, FITZGERALD and GREENWALD 1993; ROEHL and KIMBLE 1993; REBAY, FEHON and ARTAVANIS-TSAKONAS 1993). *glp-1*, *lin-12*, *Notch* and *Xotch* truncations alter a variety of cell fate decisions while *int-3* and *TAN-1* truncations are associated with mammary tumors and acute T cell lymphoblastic leukemia, respectively. Although the truncated genes encode flanking amino acids in addition to the *cdc10/SWI6* repeats, it seems reasonable to propose that the observed biological effects are mediated by the *cdc10/SWI6* repeats. These findings also suggest that apparent uncoupling of extracellular signalling from the cytoplasmic domain of these proteins, where *cdc10/SWI6* repeats are located, has profound effects on cell fate specification and cell growth control. In light of the role of *glp-1* in promoting proliferation of the germ line in *C. elegans*, the hyperproliferative defects associated with *int-3* and *TAN-1* are particularly intriguing.

We thank the following: SARAH CRITTENDEN, JUDITH KIMBLE, ANN-MARIE HOWELL, JIM PRIESS, JIM KRAMER, MEERA SUNDARAM and IVA GREENWALD for communicating unpublished results; ANN MEANY, RUTH YOKOYAMA and SHOZO YOKOYAMA for advice and assistance with PCR and sequencing; KEVIN VAN DOREN for helpful discussions and use of the thermal cycler; DICK LEVY for use of the sequencing apparatus; PEI SHU for excellent technical assistance; JUDITH KIMBLE, LI QIAO, PEI SHU and FRAN LISSEMORE for critically

reading the manuscript; and JUDITH KIMBLE for primers and helpful discussions. This work was initiated in JUDITH KIMBLE's laboratory (University of Wisconsin-Madison), where it was funded by U.S. Public Health Service Grant GM11569 (E.M.M.), U.S. Public Health Service Grant GM31816 (J.K.) and Research Career Development Award HD00630 (J.K.). Most of the work has been funded by National Science Foundation Grant DCB-9003912 (E.M.M.). Many nematode strains used in this study were provided by the *Caenorhabditis elegans* Genetics Center, which is supported by the NIH National Center for Research Resources.

LITERATURE CITED

- ARTAVANIS-TSAKONAS, S., and P. SIMPSON, 1991 Choosing a cell fate: a view from the *Notch* locus. *Trends Genet.* **7**: 403–408.
- 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 *C. elegans*. *Cell* **58**: 565–571.
- 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.
- BLANK, V., P. KOURILSKY and A. ISRAEL, 1992 NF- κ B and related proteins: Rel/dorsal homologies meet ankyrin-like repeats. *Trends Biochem. Sci.* **17**: 135–140.
- BOURS, V., G. FRANZOSO, V. AZARENKO, S. PARK, T. KANNO, *et al.*, 1993 The oncoprotein Bcl-3 directly transactivates through κ B motifs via association with DNA-binding p50B homodimers. *Cell* **72**: 729–739.
- BRENNER, S., 1974 The genetics of *Caenorhabditis elegans*. *Genetics* **77**: 71–94.
- COFFMAN, C., W. HARRIS and C. KINTNER, 1990 *Xotch*, the *Xenopus* homolog of *Drosophila Notch*. *Science* **249**: 1438–1441.
- COFFMAN, C., P. SKOGLUND, W. HARRIS and C. KINTNER, 1993 Expression of an extracellular deletion of *Xotch* diverts cell fate in *Xenopus* embryos. *Cell* **73**: 659–671.
- DAVIS, L. H., and V. BENNETT, 1990 Mapping the binding sites of human erythrocyte ankyrin for the anion exchanger and spectrin. *J. Biol. Chem.* **265**: 10589–10596.
- DAVIS, L. H., E. OTTO and V. BENNETT, 1991 Specific 33-residue repeat(s) of erythrocyte ankyrin associate with the anion exchanger. *J. Biol. Chem.* **266**: 11163–11169.
- DEL AMO, F. F., D. E. SMITH, P. J. SWIATEK, M. GENDRON-MAGUIRE, R. J. GREENSPAN, *et al.*, 1992 Expression pattern of *Motch*, a mouse homolog of *Drosophila Notch*, suggests an important role in early postimplantation mouse development. *Development* **115**: 737–744.
- ELLISEN, L. W., J. BIRD, D. C. WEST, A. L. SORENG, T. C. REYNOLDS, *et al.*, 1991 *TAN-1*, the human homolog of the *Drosophila Notch* gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. *Cell* **66**: 649–661.
- GREENWALD, I., and G. M. RUBIN, 1992 Making a difference: the role of cell-cell interactions in establishing separate identities for equivalent cells. *Cell* **68**: 271–281.
- GREENWALD, I., and G. SEYDOUX, 1990 Analysis of gain-of-function mutations of the *lin-12* gene of *Caenorhabditis elegans*. *Nature* **346**: 197–199.
- GURDON, J. B., 1992 The generation of diversity and pattern in animal development. *Cell* **68**: 185–199.
- HATADA, E. N., M. NAUMANN and C. SCHEIDEREIT, 1993 Common structural constituents confer I κ B activity to NF- κ B p105 and I κ B/MAD-3. *EMBO J.* **12**: 2781–2788.
- HODGKIN, J., M. EDGLEY, D. L. RIDDLE and D. G. ALBERTSON, 1988 Genetic nomenclature, list of mapped genes, genetic map, physical maps, pp. 491–584 in *The Nematode Caenorhab-*

- ditiis elegans*, edited by W. B. WOOD. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- HORVITZ, H. R., and I. HERSKOWITZ, 1992 Mechanisms of asymmetric cell division: two Bs or not two Bs, that is the question. *Cell* **68**: 237–255.
- 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.
- INOUE, J., L. D. KERR, D. RASHID, N. DAVIS, H. R. BOSE, JR. *et al.*, 1992 Direct association of pp40/I κ B β with rel/NF- κ B transcription factors: role of ankyrin repeats in the inhibition of DNA binding activity. *Proc. Natl. Acad. Sci. USA* **89**: 4333–4337.
- IWAHANA, H., K. YOSHIMOTO and M. ITAKURA, 1992 Detection of point mutations by SSCP of PCR-amplified DNA after endonuclease digestion. *Biotechniques* **12**: 64–66.
- JHAPPAN, C., D. GALLAHAN, C. STAHL, E. CHU, G. H. SMITH, *et al.*, 1992 Expression of an activated *Notch*-related *int-3* transgene interferes with cell differentiation and induces neoplastic transformation in mammary and salivary glands. *Genes Dev.* **6**: 345–355.
- JOHNSTONE, I. L., Y. SHAFI and J. D. BARRY, 1992 Molecular analysis of mutations in the *Caenorhabditis elegans* collagen gene *dpy-7*. *EMBO J.* **11**: 3857–3863.
- KIDD, S., 1992 Characterization of the *Drosophila cactus* locus and analysis of interactions between cactus and dorsal proteins. *Cell* **71**: 623–635.
- KIMBLE, J. E., and J. G. WHITE, 1981 On the control of germ cell development in *Caenorhabditis elegans*. *Dev. Biol.* **81**: 208–219.
- KLIG, L. S., D. L. OXENDER and C. YANOFSKY, 1988 Second-site revertants of *Escherichia coli trp* repressor mutants. *Genetics* **120**: 651–655.
- KODOYIANNI, V., E. M. MAINE and J. KIMBLE, 1992 The molecular basis of loss-of-function mutations in the *glp-1* gene of *Caenorhabditis elegans*. *Mol. Cell. Biol.* **3**: 1199–1213.
- KRAMER, J., J. J. JOHNSON, R. S. EDGAR, C. BASCH and S. ROBERTS, 1988 The *sqt-1* gene of *C. elegans* encodes a collagen critical for organismal morphogenesis. *Cell* **55**: 555–565.
- LAMBIE, E. J., and J. KIMBLE, 1991a Genetic control of cell interactions in nematode development. *Annu. Rev. Genet.* **25**: 411–436.
- LAMBIE, E. J., and J. KIMBLE, 1991b Two homologous regulatory genes, *lin-12* and *glp-1*, have overlapping functions. *Development* **112**: 231–240.
- MAINE, E. M., and J. KIMBLE, 1989 Identification of genes that interact with *glp-1*, a gene required for inductive cell interactions in *Caenorhabditis elegans*. *Development* **106**: 133–143.
- MAINE, E. M., and J. KIMBLE, 1993 Suppressors of *glp-1*, a gene required for cell communication during development in *Caenorhabditis elegans*, define a set of interacting genes. *Genetics* **135**: 1011–1022.
- MAURIDES, P. A., J. J. SCHWARZ and P. B. BERGET, 1990 Intragenic suppression of a capsid assembly-defective P22 tail-spike mutation. *Genetics* **125**: 673–681.
- MICHAELY, P., and V. BENNETT, 1992 The ANK repeat: a ubiquitous motif involved in macromolecular recognition. *Trends Cell. Biol.* **2**: 127–129.
- MURRAY, V., 1989 Improved double-stranded DNA sequencing using linear polymerase chain reaction. *Nucleic Acids Res.* **17**: 8889.
- ORITA, M., Y. SUZUKI, T. SEKIYA and K. HAYASHI, 1989 Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. *Genomics* **5**: 874–879.
- PRIESS, J. R., H. SCHNABEL and R. SCHNABEL, 1987 The *glp-1* locus and cellular interactions in early *C. elegans* embryos. *Cell* **51**: 601–611.
- REAUME, A. G., R. A. CONLON, R. ZIRNGIBL, T. P. YAMAGUCHI and J. ROSSANT, 1992 Expression analysis of a *Notch* homologue in the mouse embryo. *Dev. Biol.* **154**: 377–387.
- REBAY, I., R. G. FEHON and S. ARTAVANIS-TSAKONIS, 1993 Specific truncations of *Drosophila Notch* define dominant activated and dominant negative forms of the receptor. *Cell* **74**: 319–329.
- ROBBINS, J., B. J. BLONDEL, D. GALLAHAN and R. CALLAHAN, 1992 Mouse mammary tumor gene *int-3*: a member of the *Notch* gene family transforms mammary epithelial cells. *J. Virol.* **66**: 2594–2599.
- 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.
- SHORTLE, D., and B. LIN, 1985 Genetic analysis of staphylococcal nuclease: identification of three intragenic “global” suppressors of nuclease-minus mutations. *Genetics* **110**: 539–555.
- STRUHL, G., K. FITZGERALD and I. GREENWALD, 1993 Intrinsic activity of the *lin-12* and *Notch* intracellular domains *in vivo*. *Cell* **74**: 331–345.
- SULSTON, J. E., and S. BRENNER, 1974 The DNA of *Caenorhabditis elegans*. *Genetics* **77**: 95–104.
- SUNDARAM, M., and I. GREENWALD, 1993 Suppressors of a *lin-12* hypomorph define genes that interact with both *lin-12* and *glp-1* in *Caenorhabditis elegans*. *Genetics* (in press).
- THOMPSON, C. C., T. A. BROWN and S. L. MCKNIGHT, 1991 Convergence of Ets- and Notch-related structural motifs in a heteromeric DNA binding complex. *Science* **253**: 762–768.
- WEINMASTER, G., V. J. ROBERTS and G. LEMKE, 1991 A homolog of *Drosophila Notch* expressed during mammalian development. *Development* **113**: 199–205.
- WEINMASTER, G., V. J. ROBERTS and G. LEMKE, 1992 *Notch2*: a second mammalian *Notch* gene. *Development* **116**: 931–941.
- WHARTON, K. A., K. M. JOHANSEN, T. XU and S. ARTAVANIS-TSAKONAS, 1985 Nucleotide sequence from the neurogenic locus *Notch* implies a gene product that shares homology with proteins containing EGF-like repeats. *Cell* **43**: 567–581.
- WOOD, W. B. (EDITOR), 1988 *The Nematode Caenorhabditis elegans*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- WULCZYN, F. G., M. NAUMANN and C. SCHEIDEREIT, 1992 Candidate proto-oncogene *bcl-3* encodes a subunit-specific inhibitor of transcription factor NF- κ B. *Nature* **358**: 597–599.
- YOCHER, J., and I. GREENWALD, 1989 *glp-1* and *lin-12*, genes implicated in distinct cell-cell interactions in *C. elegans*, encode similar transmembrane proteins. *Cell* **58**: 553–563.
- YOCHER, J., K. WESTON and I. GREENWALD, 1988 The *Caenorhabditis elegans lin-12* gene encodes a transmembrane protein with overall similarity to *Drosophila Notch*. *Nature* **335**: 547–550.

Communicating editor: R. K. HERMAN