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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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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 (Yochem and Greenwald 1989). Several glp-1-related genes have been found in both vertebrates and invertebrates: lin-12 in C. elegans (Yochem, Weston and Greenwald 1988), Notch in Drosophila (Wharton et al. 1985), Notch in Xenopus (Coffman, Harris and Kintner 1990), int-3 and Mox 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 (Ellisen 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; Yochem 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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sor 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, Shaft 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 1995).

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/SW16 repeat of the intracellular domain of GLP-1 (Kodoyianni, Maine and Kimble 1992). cdc10/SW16 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/SW16 domain. The location of these suppressors supports the notion that cdc10/SW16 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 (germ line proliferation defective), him (high incidence of males), sma (small) and unc (uncoordinated):

linkage group III: dpy-18(e364), dpy-19(e1259ts), glp-1(q224ts), glp-1(q231ts), sma-2(e502), unc-32(e189), unc-36(e251), unc-69(e687);

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 x 15 mm plates (10 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 F1 animals reached L4, plates were shifted to restrictive temperature (20° or 25°). Plates were screened visually for viable F1 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 (Sutton 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 regim, 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 progeny.

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-
covered in the F2. Here, suppressors are designated sup(s).
For each suppressor, rescue of glp-l(ts) always segregated with unc-32. To test whether a given suppressor was dominant, heterozygous unc-32 glp-l(ts) sup(s)/sma-2 glp-l(q231) males were mated to sma-2 glp-l(q231) hermaphrodites and their progeny were raised at 20 °C. Non-Sma cross-progeny [unc-32 glp-l(ts), sup(s)/sma-2 glp-l(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, q282, q284, q285, q333 and q335) were tested as follows: glp-l(ts); him-5 males were mated to unc-32 glp-l(ts) sup(s) hermaphrodites and their progeny were raised at 20 °C. Non-Sma 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-l(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-l(q224) q240 animals and Unc-69 non-Dpy recombinants from dpy-19 unc-69/sma-2 glp-l(q224) q240 animals were examined. In no case was the dominant suppressor separated from glp-l. The unmapped suppressor was distinguished from sma-2/sma-2 unc-69 by testing Sma recombinants at 25 °C where q240 is not 100% penetrant. Similarly, a subset of the Unc recombinants were tested at 25.5 °C to distinguish sma-2 glp-l q240/dpy-19 unc-69 from sma-2 unc-69/dpy-19 unc-69. Since only a subset were tested, all Unc recombinants are listed in one category in Table 2.

Single-strand conformation polymorphism (SSCP): 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-l genomic clone from strain glp-l(q231) q280 was used as a template for PCR. PCRs were performed with the primer pairs listed in Table 3 using 0.25 μM of 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 μM dCTP. (Sequenase 2.0 kit (U.S. Biochemical). Thermal cycle sequencing was performed according to the 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 μl of sequencing primer and 2 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 SSCP’s 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-l(ts) mutation, which also confirmed that suppressor types 2, 4 and 5 suppress both glp-l(q224) and glp-l(q231) (Table 4).

Determination of brood size and percent hatching: L4 hermaphrodites homozygous for both glp-l(ts) and a dominant suppressor were picked from stocks grown at the appropriate temperature (15 °C, 20 °C or 25 °C), 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 transfused. Hatched progeny were counted once they had achieved at least the L3 stage of development. As controls, brood sizes and percent hatching of glp-l(q231), glp-l(q224) and glp-l(q231)/glp-l(q231) animals were determined. Dominant suppressors were tested as heterozygotes with the suppressor

---

**TABLE 2**

Fine structure mapping of dominant suppressor q240

<table>
<thead>
<tr>
<th>Parental genotype</th>
<th>Recombinant phenotype</th>
<th>Recombinant genotype</th>
<th>No. of recombinantsa</th>
</tr>
</thead>
<tbody>
<tr>
<td>sma-2 unc-69/unc-32 glp-l(q231)</td>
<td>Sma</td>
<td>sma-2 unc-32 glp-l(q231)/sma-2 unc-69</td>
<td>11/263</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sma-2 glp-l(q240)/sma-2 unc-69</td>
<td>15/263</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sma-2 glp-l(q240)/sma-2 unc-69</td>
<td>0/263</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sma-2 glp-l(q240)/sma-2 unc-69</td>
<td>257/263</td>
</tr>
<tr>
<td>dpy-19 unc-69/sma-2 glp-l(q231)</td>
<td>Unc</td>
<td>sma-2 glp-l(q240)/dpy-19 unc-69 or sma-2 unc-69/dpy-19 unc-69</td>
<td>131/151</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sma-2 glp-l(q240)/dpy-19 unc-69</td>
<td>0/151</td>
</tr>
</tbody>
</table>

---

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 °C where q240 is not 100% penetrant.
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(tsl) 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(tsl) mutants can have an intermediate amount of proliferation and produce some embryos, but these embryos are often inviable. The glp-1(tsl) 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,
Dominant Suppressors of glp-1

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

<table>
<thead>
<tr>
<th>Type</th>
<th>Suppressor</th>
<th>Genotype: maternal chromosome/paternal chromosome</th>
<th>Brood size (n)</th>
<th>% Viable progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>—</td>
<td>glp-1(q224)</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>1</td>
<td>glp-1(q224)</td>
<td>glp-1(q281)</td>
<td>27 ± 4.5</td>
<td>&gt;99</td>
</tr>
<tr>
<td>2</td>
<td>q281</td>
<td>glp-1(q231)/glp-1(q224)</td>
<td>308 ± 25 (4)</td>
<td>&gt;99</td>
</tr>
<tr>
<td>1</td>
<td>q278</td>
<td>glp-1(q231)/glp-1(q278)</td>
<td>282 ± 19 (3)</td>
<td>99</td>
</tr>
<tr>
<td>2</td>
<td>q282</td>
<td>glp-1(q231)/glp-1(q282)</td>
<td>282 ± 16 (4)</td>
<td>&gt;99</td>
</tr>
<tr>
<td>3</td>
<td>q283</td>
<td>glp-1(q231)/glp-1(q283)</td>
<td>208 ± 24 (2)</td>
<td>99</td>
</tr>
<tr>
<td>4</td>
<td>q285</td>
<td>glp-1(q231)/glp-1(q285)</td>
<td>263 ± 2 (2)</td>
<td>99</td>
</tr>
<tr>
<td>5</td>
<td>q286</td>
<td>glp-1(q231)/glp-1(q286)</td>
<td>212 ± 20 (4)</td>
<td>92</td>
</tr>
<tr>
<td>6</td>
<td>q287</td>
<td>glp-1(q231)/glp-1(q287)</td>
<td>164 ± 59 (3)</td>
<td>88</td>
</tr>
<tr>
<td>7</td>
<td>q288</td>
<td>glp-1(q231)/glp-1(q288)</td>
<td>258 ± 17 (4)</td>
<td>98</td>
</tr>
<tr>
<td>8</td>
<td>q333</td>
<td>glp-1(q231)/glp-1(q333)</td>
<td>213 ± 22 (3)</td>
<td>88</td>
</tr>
<tr>
<td>9</td>
<td>q334</td>
<td>glp-1(q231)/glp-1(q334)</td>
<td>185 ± 19 (5)</td>
<td>84</td>
</tr>
<tr>
<td>10</td>
<td>q335</td>
<td>glp-1(q231)/glp-1(q335)</td>
<td>174 ± 32 (2)</td>
<td>98</td>
</tr>
<tr>
<td>11</td>
<td>q336</td>
<td>glp-1(q231)/glp-1(q336)</td>
<td>217 ± 10 (5)</td>
<td>&gt;99</td>
</tr>
<tr>
<td>12</td>
<td>q337</td>
<td>glp-1(q231)/glp-1(q337)</td>
<td>296 ± 20 (5)</td>
<td>&gt;99</td>
</tr>
<tr>
<td>13</td>
<td>q338</td>
<td>glp-1(q231)/glp-1(q338)</td>
<td>252 ± 15 (4)</td>
<td>99</td>
</tr>
<tr>
<td>14</td>
<td>q339</td>
<td>glp-1(q231)/glp-1(q339)</td>
<td>263 ± 14 (5)</td>
<td>&gt;99</td>
</tr>
<tr>
<td>15</td>
<td>q340</td>
<td>glp-1(q231)/glp-1(q340)</td>
<td>295 ± 8 (3)</td>
<td>99</td>
</tr>
<tr>
<td>16</td>
<td>q341</td>
<td>glp-1(q231)/glp-1(q341)</td>
<td>255 ± 12 (3)</td>
<td>99</td>
</tr>
<tr>
<td>17</td>
<td>q342</td>
<td>glp-1(q231)/glp-1(q342)</td>
<td>236 ± 39 (3)</td>
<td>98</td>
</tr>
<tr>
<td>18</td>
<td>q343</td>
<td>glp-1(q231)/glp-1(q343)</td>
<td>286 ± 9 (5)</td>
<td>99</td>
</tr>
<tr>
<td>19</td>
<td>q344</td>
<td>glp-1(q231)/glp-1(q344)</td>
<td>235 ± 19 (3)</td>
<td>&gt;99</td>
</tr>
<tr>
<td>20</td>
<td>q345</td>
<td>glp-1(q231)/glp-1(q345)</td>
<td>244 ± 36 (5)</td>
<td>98</td>
</tr>
<tr>
<td>21</td>
<td>q346</td>
<td>glp-1(q231)/glp-1(q346)</td>
<td>282 ± 20 (4)</td>
<td>99</td>
</tr>
<tr>
<td>22</td>
<td>q347</td>
<td>glp-1(q231)/glp-1(q347)</td>
<td>265 ± 14 (3)</td>
<td>99</td>
</tr>
<tr>
<td>23</td>
<td>q348</td>
<td>glp-1(q231)/glp-1(q348)</td>
<td>258 ± 14 (2)</td>
<td>99</td>
</tr>
<tr>
<td>24</td>
<td>q349</td>
<td>glp-1(q231)/glp-1(q349)</td>
<td>202 ± 13 (3)</td>
<td>99</td>
</tr>
</tbody>
</table>

NA, not applicable.

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

<table>
<thead>
<tr>
<th>Brood size</th>
<th>% Viable progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td></td>
</tr>
</tbody>
</table>

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

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 frequency 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
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, was used as the template for PCR. For q280, a genomic clone of glp-1 from glp-l(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/SWJ6 domain of glp-1. PCR products were digested with Hinfl 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.

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. SSCP analysis of the 305 nt Hinfl fragment (Figure 2; lanes 3, 5, 6, 7, 9 and 10) and in the 207 nt Hinfl 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 suppressors 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 SSCP's 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 SSCP's 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/SW16 domain (Figures 3 and 4). In addition, the location of
the type 5 suppressor indicated the presence of a
degenerate seventh ced10/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 ced10/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-l(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 ced10/SWI6 repeats; a fifth substitution is
located in the third repeat (Figures 3 and 4). Interest-
ingly, 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). Like-
wise, 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-l suppression: Is
there a correlation between suppressor strength and
the type of substitution? To determine the strength
each suppressor, we quantified the average brood
size of glp-l(ts) hermaphrodites carrying each sup-
pressor. Brood size is an indirect measure of the extent
of germline proliferation. In addition, we assayed the
degree of embryonic viability by determining the per-
cent of progeny that hatch and reach adulthood.

Efficiency of suppression of the glp-l(ts) germline pheno-
type: Originally, each suppressor was tested for dom-
inance 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 suppres-
sor donated by the mother. No substantial maternal
effect was observed (Table 5). Subsequently, strains
homozygous for both glp-l(ts) and a dominant sup-
pressor were characterized at 15°, 20° and 25°
(Table 6).

Germline proliferation varies slightly (<2-fold) in
glp-l(ts) strains that are heterozygous for different
dominant suppressors regardless of which parent con-
tributes 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 ob-
served in each dominant suppressor strain if raised at
sufficiently high temperature (data not shown). Sec-
ond, each dominant suppressor is less effective at the
more stringent 25° than at an intermediate tempera-
ture 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-l(ts) embryonic phe-
notype: 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 ger-
mline 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-l(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-l(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-
l(ts) alleles by a given dominant suppressor, e.g.,
type 4 suppresses glp-l(q231) much better than glp-l(q224)
(Table 6).

Do glp-l intragenic revertants bypass the require-
ment 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 trans-
duced 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 pu-
tative 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
ergernline development was observed. The unoperated
gonad arm in each animal served as an internal con-

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-l(ts), the dominant suppressors were examined in
a glp-l(ts) background. Germline proliferation was
assayed by counting the number of germline nuclei
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 cde10/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 cde10/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 cde10/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 the same domain (KODOYIANNI, MAINE and KIMBLE 1992).

Though we do not know the function of the cde10/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 cde10/SWI6 repeats from human erythrocyte ankyrin (DAVIS and BENNETT 1990; DAVIS, OTTO and BENNETT 1991), rat GABPβ (THOMPSON, BROWN and Mc-KNIGHT 1991), and several members of the IκB family [i.e., chicken pp40/1κB (INOUE et al. 1992), human Bcl-3 (BOURS et al. 1993; WULCZYNSKI, NAUMANN and SCHEIDEREIT 1992), human NF-κB p105 and IκB/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, GABPβ is a subunit of a transcription factor and IκB-related proteins prevent nuclear translocation of 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 BENNET 1992; BLANK, KOURILSKY and ISRAEL 1992). This variability suggests that the consensus amino acids in the cde10/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 cde10/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 GABPa, which binds to GABPβ, share little sequence similarity and seem to have very different

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**FIGURE 4.—Alignment of cde10/SWI6 repeats in GLP-1 and location of glp-1(ts) dominant suppressor mutations.** The six previously described cde10/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 YOCHI 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. See also Table 4 and Figure 3.
recognition domains (Michael 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 pp40/1xB3 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, GABPP, and 1 KB-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, Michael 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 type 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-
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quences in suppression strength as a possible explanation because at 20° the extent of suppression by a given suppressor type is similar for both glp-l(ts) alleles (Table 5). If suppression strength were the crucial factor determining mutation frequency, we should have recovered suppressors of both glp-l(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) (Yochem 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 mammmary 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.

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