TABLE 1 Proliferation of the MGF-dependent mast cell line MC-6 on established cell lines and transfected CV-1 cells

<table>
<thead>
<tr>
<th>Cell line</th>
<th>thymidine incorporation (c.p.m.)</th>
</tr>
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<tbody>
<tr>
<td>STO</td>
<td>6,728 ± 2,413*</td>
</tr>
<tr>
<td>NH-3T3</td>
<td>9,849 ± 3,891*</td>
</tr>
<tr>
<td>CV-1</td>
<td>2,064 ± 138</td>
</tr>
<tr>
<td>CV-1 WT MGF</td>
<td>7,552 ± 575†</td>
</tr>
<tr>
<td>CV-1 ST* MGF</td>
<td>13,244 ± 1,917†</td>
</tr>
<tr>
<td>CV-1 + carrier</td>
<td>2,365 ± 492</td>
</tr>
</tbody>
</table>

Proliferation assays were essentially carried out as previously described. Briefly, 20,000 feeder cells were plated into microtiter wells and irradiated with 5,000 rads to induce quiescence. After 24 h, 10,000 MC-6 cells were added to each well and after a further 24 h the cultures were pulsed for 4–5 h with 1 μCi [3H]thymidine. Wells were collected on fibreglass filters with an automated collector and incorporated [3H]thymidine counted by liquid scintillation spectrometry. This experiment was repeated four times with six replicates per treatment.

* P ≈ 0.05 versus CV-1 cells or CV-1 + carrier.
† P ≈ 0.001 versus CV-1 cells or CV-1 + carrier.

the total MGF produced by CV-1-ST* (assayed by mast cell proliferation) was higher than that of CV-1-Wt (Table 1). We also compared PGC survival on ST/ST* (BM-ST*) and congenic wild-type (BM-Wt) bone marrow-derived stromal cell lines. BM-ST* supported PGC survival to 40% of BM-Wt after 1 day, but did not support subsequent PGC survival (Fig. 2c). When recombinant MGF (rMGF) was added to PGCs cultured on BM-ST*, PGC survival of over one day improved to 70% of BM-Wt, but subsequent PGC survival was unaffected (Fig. 2c). When rMGF was added to PGCs cultured on NIH-3T3 or STO cells, PGC survival was not enhanced (Fig. 2b and data not shown). Presumably, in the culture conditions used in this study, the amount of MGF produced by NIH-3T3 and STO cells is sufficiently high to support maximal PGC survival in the absence of added rMGF (see Fig. 1b and Table 1). Soluble MGF, such as that produced by ST/ST* cells, is able to support only limited PGC survival and is unable to support long-term survival seen when PGC are cultured on wild-type cells producing both transmembrane and soluble MGF. In a similar study, Godin et al. report that soluble factor is able to enhance initial survival (>48 h) but does not promote long-term survival of PGC cultured in the absence of STO cells.

The reduced ability of soluble MGF to support long-term survival may reflect a requirement for a localized, high concentration of MGF, for a particular conformation of MGF, for a role of MGF in promoting cell adhesion, or for an extended ligand-receptor interaction precluded by internalizable, soluble MGF. That transmembrane MGF is more effective in supporting PGC survival could provide (1) a mechanism both for the haptotactic guidance of PGCs to the gonad anlagen as well as for strict regulation of PGC proliferation and differentiation in the embryo and (2) a possible explanation for the sterility found in ST/ST* mice. The viability, but sterility and lack of pigment, of ST/ST* mice suggests that the haemapoietic lineages can be maintained to some extent by soluble factor, whereas PGCs and melanoblasts cannot. The activity of soluble factor on mast cells and primitive haemapoietic progenitors in vitro14–16 and on mast cells in vivo17,18 is consistent with this idea.

Binding of tyrosine kinase receptors, such as c-kit, by their cognate ligands usually leads to activation of the kinase domain and transduction of signals that lead to cellular proliferation17–19. The MGF/c-kit complex mediates a proliferation response in mast cells14,15,20 and, in combination with other factors, proliferation of primitive haematopoietic progenitors11,15,16,20,21. The data presented here and by Godin et al.16 provide the first evidence of growth factor action on mouse PGCs and suggest a role for MGF in mediating a PGC survival signal rather than a proliferation signal.

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Carboxy-terminal truncation activates glp-1 protein to specify vulval fates in Caenorhabditis elegans

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‡ The glp-1 and lin-12 genes encode homologous transmembrane proteins1–2 that may act as receptors for cell interactions during development3–4. The glp-1 protein is required for induction of germ-line proliferation and for embryogenesis5–6. By contrast, lin-12 mediates somatic cell interactions, including those between the precursor cells that form the vulval hypodermis (VPCs)6. Here we analyse an unusual allele of glp-1, glp-1(q35), which displays a semidominant multivulva phenotype (Mu), as well as the typical recessive, loss-of-function Glp phenotypes (sterility and embryonic lethality)7. We find that the effects of glp-1(q35) on VPC development mimic those of dominant lin-12 mutations, even in the absence of lin-12 activity. The glp-1(q35) gene bears a nonsense mutation predicted to eliminate the 122 C-terminal amino acids, including a ProIQSerThr (PIST) sequence thought to destabilize proteins. We suggest that the carboxy terminus bears a negative regulatory domain which normally inactivates glp-1 in the VPCs. We propose that inappropriate glp-1(q35) activity can substitute for lin-12 to determine vulval fate, perhaps by driving the VPCs to proliferate. During wild-type development, three VPCs (P5p, P6p, P7p) generate 22 descendants that form the vulval hypodermis (Figs 1 and 2a). Several genes are known that, when mutant, interfere with this process (reviewed in ref. 7). One of these is lin-12: animals with elevated lin-12 activity (lin-12(d)) are MuV because all six VPCs adopt the Vh2 fate8. The glp-1 and lin-12 genes

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FIG. 1. Vulval phenotypes. Nomarski differential interference contrast (DIC) photomicrographs of fourth larval stage hermaphrodites. a, c, e. Intact animals that retain their gonad (+GONAD); b, d, f, animals that have had their gonad destroyed in early L1 (−GONAD). a and b, smg-1; c and d, smg-1; glp-1(q35); e and f, smg-1; lin-12(0);glp-1(q35). An arrow indicates the normal, AC-dependent vulval invagination; arrowheads indicate pseudovulval invaginations. Animals in c, d, e and f had additional pseudovulvae not shown in the figure. Scale bar, ~10 μm.

METHODS. Alleles used were the loss-of-function allele smg-1(r867) l (ref. 10), the putative null allele lin-12(n4941) l (ref. 6) and glp-1(q35) III. smg-1(q810) III animals were maintained as a homozygous stock; smg-1(n12(0);glp-1(q35) animals from smg-1(n12(0);glp-1(q35);dpy-19(e1269);ncl-69(e423);ncl-69(e587) mothers were identified by fate transformations typical of lin-12(0), such as an extra AC, or posterior ventral coelomocytes⁴⁶. Nomarski DIC microscopy was performed as described¹⁹. Animals were maintained by standard laboratory techniques¹⁹ at 20 °C (operated animals) and 23 °C (all others). To destroy the gonad, the somatic gonadal precursor cells Z1 and Z2 are killed during the first larval stage of hermaphrodites that had been anesthetized in 20 mM sodium azide/1X M9. Laser-microsurgery was performed as described²⁰ using a Zeiss axiovert microscope, VSL-337 nitrogen laser, and DML-110 dye laser module. Animals were examined in the third larval stage to ensure that the gonad was absent, and that there was no other structural damage.

FIG. 2. a. Model for wild-type vulval development; see ref. 7 for review. Three (P3.p, P6.p, and P7.p) of six vulval precursor cells (VPCs) located in the ventral hypodermis divide three times and form the vulval hypodermis (VH1 (or 1')) and VH2 (or 2') fates¹⁴. The remaining three cells (P3.p, P4.p, and P8.p) divide once and fuse with the hypodermal syncytium (SH (or 3')) fate¹⁴. Each VPC has the potential to adopt the VH1, VH2 or SH fate¹²,¹³,¹⁹. In wild-type, however, the fate of each is invariant¹⁵. VH1 and VH2 fates are induced by the anchor cell (AC), a cell of the somatic gonad¹⁶, whereas SH fate is promoted by a signal from the hypodermis (arrow)¹⁵. Although inductive signals are diagrammed as emanating directly from the AC and hypodermis, these signals may be indirect. The choice between VH1 and VH2 depends on the proximity of a VPC to the AC, and on a signal from the VH1 to the VH2 cell (bar)¹²,¹³. VH1 and VH2 fates are distinguished by the axis of the third division and the adherence of the progeny cells to the ventral cuticle¹⁵. Axes are either transverse (T), or longitudinal (L); no third division is designated as N. Cell divisions that generate adherent daughter cells are underlined; if daughters are nonadherent, the division axis is not underlined. Cells that fuse with the syncytial hypodermis are denoted by an S. The VH1 fate is characterized by four nonadherent divisions (usually TTTT), whereas the VH2 fate is characterized by two adherent divisions and 1-2 non-adherent divisions (usually TTTL or LTTT). b, Vulval fates in glp-1(q35) and lin-12 mutants. The VPC lineages of the following animals were obtained: (1) wild-type (N2 animals with a gonad (smg-1 animals behave like N2 (ref. 10); (2) smg-1 animals in which the gonadal precursors (and hence the AC) were destroyed by laser microsurgery; (3) 4 lin-12(d)/+ and (5) lin-12(d) animals that lack an AC owing to cellular transformation; (6-12) smg-1 glp-1(q35) animals with a gonad; (13-17) smg-1 glp-1(q35) animals in which the gonadal precursors were destroyed by laser microscopy; (18-23) smg-1 lin-12(0);glp-1(q35) animals with a gonad; (24-26) smg-1 lin-12(0);glp-1(q35) animals in which the gonadal precursors were destroyed. Division axes are as described in a in an addition, oblique (Q) divisions were occasionally seen. Cells that divided, but in which the plane of division was not observed, are marked as D. Each line represents an individual VPC lineage. A starched, the AC was centered over P5.p; dagger, P9.p followed a vulval lineage. METHODS. Alleles were the same as those listed in the legend to Fig. 1, as well as the dominant gain-of-function allele lin-12(n4952) III; lin-12(d) and lin-12(d) dpy-19(n69(e423);ncl-69(e587) animals were distinguished by progeny testing. Lineages were performed as described in the legend to Fig. 1.
encode similar proteins. Furthermore, lin-12 and glp-1 are redundant during embryogenesis, and may be able to respond to the same signal from the gonadal anchor cell (AC). These observations raise the possibility that glp-1(q35) is Muv because it is mimicking lin-12(d), so we have compared the effects of glp-1(q35) and lin-12(d) on vulval development.

Several observations indicate that the glp-1(q35) Muv phenotype results from increased or novel glp-1 activity. First, this phenotype is dominant, whereas all glp-1 loss-of-function mutations are recessive (Fig. 3a and b; ref. 3). Second, this class of mutation is rare (one GlpMuv per 10^7 haploid genomes; ref. 3, and our unpublished results). Third, glp-1(q35) homozygotes are more Muv than glp-1(q35)/glp-1(0) or glp-1(q35)/+ heterozygotes (Figure 3a). Fourth, the information suppressor, smg-1(ref. 10), suppresses the glp-1(q35) loss-of-function Glp phenotypes, but enhances its Muv phenotype (Fig. 3b). Certain mutants are rescued by smg-1 mutations, apparently by increasing the abundance of aberrant RNAs (R. Pulak and P. Anderson, personal communication). By analogy, smg-1 is predicted to increase the amount of glp-1(q35) RNA, and consequently protein, thereby suppressing the loss-of-function phenotypes and enhancing the gain-of-function phenotype.

To investigate whether the glp-1(q35) Fate transformations are similar to those of lin-12(d), we examined VPC development in smg-1; glp-1(q35) double mutants (Figs 1c and 2b). The double mutant was used to enhance the Muv phenotype. Whereas VPC fates are not altered in smg-1 mutants, in smg-1; glp-1(q35) homozygotes, P3,p, P4.p and P8.p often follow a VH2-like fate rather than an SH fate (for example, see Fig. 2b, rows 6 and 10). Although other P(3,4,8,p) lineages are harder to characterize, they are never VH1-like. These fate transformations are reminiscent of weak lin-12(d) alleles (Fig. 2b, rows 3–5).

In addition to the ectopic pseudovulvae made by P(3,4,8,p), a normal vulva is always formed in smg-1; glp-1(q35) animals, indicating that the VPCs are still responsive to the anchor cell signal. This is especially apparent when the AC is mispositioned, and the vulva is shifted accordingly (Fig. 2b, row 12). Similarly, when an AC is present in lin-12(d) mutants, the VPCs respond to it.

We next asked whether the glp-1(q35) Muv phenotype, like that of lin-12(d), is independent of the AC. To address this question we destroyed the gonadal precursor cells during the first larval stage, before the AC is born. Although no vulva develops in wild-type and smg-1 animals lacking an AC (Figs 1b and 2b, row 2), pseudovulvae are still made in smg-1; glp-1(q35) animals without an AC (Figs 1d and 2b, rows 13–17). Thus, glp-1(q35) does not act in the gonad to promote pseudovulva formation, nor does glp-1(q35) require a signal from an AC or VH1 cell, as these cell types are not formed in ablated animals. Interestingly, P(5–7,p) are more likely to follow a vulval fate in ablated animals than P(3,4,8,p) (Fig. 2b, rows 13–17). This bias may reflect a difference between the cells themselves (for example, wild-type P(5–7,p) versus P(3,4,8,p), or their environments. Alternatively, glp-1(q35) may affect...
METHODS. The glp-1(q35) mutation was identified using a modification of the mismatch detection method. The glp-1(q35) allele was isolated from a recombinant DNA library constructed from glp-1(q35)/glp-1(q35;QPD3) hermaphrodites. [QPD3 is glp-1(+)]. Genomic DNA was cleaved with XhoI ligated to EMBL3 lambda phage vector cut with SalI. The glp-1(q35) recombinants isolated this way contained ~2 kilobase pairs of 5' flanking sequences. The protocol described in ref. 27 was modified as follows: (1) recombinant DNAs were restricted to produce fragments of 100–800 base pairs in the region to be probed; (2) after hybridization, duplexes were

individual VPCs differently. This same phenomenon is observed in lin-12 (d) mutants (Fig. 2b, rows 3–5; ref. 13, and data not shown).

The glp-1(q35) mutation is temperature-sensitive, which allowed us to determine when glp-1(q35) activity is needed for the Muv phenotype. Temperature-shift experiments show that glp-1(q35) acts before the first P (3–8),p division (Fig. 3c), at the time of VPC determination. At this time most glp-1(+) messenger RNA is found in the somas; lin-12 (d) activity is also required at this stage to determine VH2 fate.

The parallels between the effects of glp-1(q35) and lin-12 (d) on vulval fate are striking. Both promote the VH2 fate among the VPCs, independently of an AC or VH1 cell; both result from a gain of gene function acting at the time of VPC determination. These similarities suggested that glp-1(q35) might affect VPC development by activating lin-12 itself. To test this hypothesis we examined whether functional lin-12 was necessary for the glp-1(q35) Muv phenotype. We found that smg-1,lin-12(0)/glp-1(q35) animals still form pseudovulva, either with a gonad or without one (Fig. 1e,f and 2b, rows 18–26). Thus, lin-12 activity is not required for the glp-1(q35) Muv phenotype. Moreover, this is the first report of a VPC able to adopt a VH2 fate in the absence of functional lin-12. We therefore propose that glp-1(q35) can substitute for lin-12 to direct development of the vulval hypodermis.

Although the effect of glp-1(q35) on the VPCs mimics that of lin-12 (d), other fate transformations typical of lin-12 (d) do not appear to be affected by glp-1(q35) (data not shown). We do not know whether this difference reflects a dissimilarity in gene expression or protein function. For example, glp-1(q35) may not be expressed in the same tissues as lin-12 (d), and therefore could not alter these other cell fate decisions.

The glp-1(q35) mutation is associated with a C to T base transition that changes the triplet encoding arginine at position 1174 to a stop codon, truncating the predicted glp-1 peptide by 122 amino acids (Fig. 4). Both the recessive Glp and dominant Muv phenotypes are likely to stem from this single lesion as both are affected by smg-1 and both are temperature-sensitive. Furthermore, because lin-12 activity is not required, the Muv phenotype associated with glp-1(q35) cannot be explained by a closely linked lin-12(d) mutation.

The region removed by the glp-1(q35) mutation is unlike that of lin-12, except for a short PEST sequence (Fig. 4b). Such sequences are thought to be degradation signals. Supporting this hypothesis, mutations that alter a PEST-like sequence in the f17 gene stabilize the protein and cause dominant, gain-of-function phenotypes. Similarly, the glp-1(q35) Muv phenotype may result from an increase of glp-1 protein. In addition, the carboxy-terminal truncation may either destroy a more specific negative regulatory domain, thereby derepressing normal glp-1 activity, or alter the specificity such that the glp-1(q35) protein can interact with factors that the normal glp-1 product cannot.

The glp-1(q35) mutation generates loss-of-function Glp phenotypes in addition to the gain-of-function Muv phenotype. This reduction in glp-1 activity may reflect a less active protein, or a decreased amount of protein resulting from destabilized mRNA, an effect seen with other nonsense mutations (see for example, ref 16). Consistent with the latter hypothesis is the efficient suppression of the Glp phenotypes by smg-1. Because the glp-1(q35) truncated protein is functional in a smg-1 background we propose that the carboxy-terminal 122 amino acids of the glp-1 protein are not essential. Furthermore, we suggest that the distinct roles of glp-1 and lin-12 in development rely on gene-specific regulation rather than on unique functional domains.

As wild-type glp-1 acts in the germ line to promote mitosis, we speculate that glp-1(q35) may act in the vulval hypodermis by promoting proliferation. Likewise, some cell fate decisions
that require lin-12 are also proliferative. Many mammalian growth factors (for example, colony-stimulating factor-1, ref. 17) promote both proliferation and differentiation. Perhaps these processes are similarly linked in _C. elegans_ and by driving proliferation, 

\[ \text{glp-1(q35)} \] 

influences a VPCs subsequent fate.

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**Human dystrophin expression in mdx mice after intramuscular injection of DNA constructs**

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**DUCHENNE’s muscular dystrophy (MDM), which affects one in 3,500 males, causes progressive myopathy of skeletal and cardiac muscles and premature death.** One approach to treatment would be to introduce the normal dystrophin gene into diseased muscle cells. When pure plasmid DNA is injected into rodent skeletal or cardiac muscle, the cells express reporter genes. We now show that a 12-kilobase full-length human dystrophin complementary DNA gene and a 6.3-kilobase Becker-like gene can be expressed in cultured cells and in vivo. When the human dystrophin expression plasmids are injected intramuscularly into dystrophin-deficient mdx mice, the human dystrophin proteins are present in the cytoplasm and sarcolemma of 1/10 of the myofibres. Myofibres expressing human dystrophin contain an increased proportion of peripheral nuclei. The results indicate that transfer of the dystrophin gene into the myofibres of MDMD patients could be beneficial, but a larger number of genetically modified myofibres will be necessary for clinical efficacy.

A human full-length or a Becker-like cDNA dystrophin sequence was inserted into expression vectors containing either the Rous sarcoma virus (RSV) or cytomegalovirus (CMV) promoters (Fig. 1). In cultured Cos cells transfected with constructs containing either the Becker-like sequence (pCMV-DYB) or the construct containing the full-length dystrophin sequence (pRSV), a dystrophin protein of relative molecular mass (M_r) 200,000 (200K) (Fig. 2a, lane 3) or 427K (Fig. 2a, lane 4), respectively, was detected on immunobots. Some 30% of Cos

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**FIG. 1. Diagrammatic representation of the human dystrophin cDNAs, protein and related antibodies. The full-length dystrophin construct corresponds to a segment of cDNA of ~12 kb (112 bp to 12,101 bp, ref. 25) encoding the principal skeletal muscle isoform of human dystrophin. The 6.3-kb Becker-like dystrophin construct was made in three parts. (1) PCR amplification across the exon 16/49 boundary: plasmid pcP56a and the 2.2-kb EcoRI insert of pcP27 (ref. 26) were amplified by PCR using oligonucleotides M13-forward (BRL) plus 5' -GAAAGAGTACACGAACTCAGATAA-TGAGTT-3' (324-LF) and 5'-CGCCGCTTTTTAAAATGCG-3' (324-JF) plus 5'-AAAGTTCTTGATGTCTTTTCTTTTTT-3' (324-JR), respectively. Oligonucleotides 324-LF and 324-JR are complementary with the dystrophin exon 16/49 boundary at the mid-position in each oligonucleotide. Amplification by PCR comprised of 15 cycles of 94 °C for 1 min, 45 °C for 30 s and 72 °C for 1 min with each oligonucleotide present at 1 μM. One-fifteenth of each PCR was pooled and amplified for 30 cycles using oligonucleotides M13-forward and 324-JF (5' -nucleotide at position 2015).** The DNA product was subsequently digested with XbaI and EcoRI and the 740 bp fragment introduced into double-digested pcP18 (Stratagene). A recombinant plasmid containing the correct PCR product (5' and 3' ends at dystrophin nucleotide positions 2036 and 7879) was determined by restriction enzyme digestion and sequencing of both DNA strands using the Sequenase Kit (USB). (2) 5'-end construction: the plasmid recombinant and pcP27 were digested with EcoRI then with BglII to give complete or partial digestion, respectively, at dystrophin nucleotide position 2083. Fragments of ~980 bp (boundary PCR clone) and ~1971 bp (cP27 from nucleotide position 112 to 2083) were pooled, ligated in equimolar amounts and digested with EcoRI. The 2.7-kb fragment was isolated and ligated into the EcoRI site of pcP18. (3) 3'-end construction: the 4.3-kb EcoRI insert of pcP115 (ref. 26) was partially digested with HindIII (at nucleotide position 11,512), and the 3.6-kb dystrophin fragment ligated into EcoRI plus Smal-digested pcP18. This recombinant plasmid was digested with EcoRI (nucleotide position 7876) plus SalI (3' end in multiple cloning site (MCS) of pcP18) and the dystrophin insert ligated with an equimolar amount of the 2.7-kb insert recovered from the recombinant, which had been digested with complete with SalI (5' end in MCS of pcP18) and partially with EcoRI (nucleotide position 7875). The ligated DNAs were digested with SalI and the 6.3-kb fragment ligated into the SalI site of pcP18. The dystrophin cDNAs were inserted into the RSV promoter construct derived from pRSVLacZ (ref. 24) or the polyclinker site of the pCDNAl plasmid (Invitrogen). pCDNA1 contains a CMV promoter and the SV40 poly(A) addition signal, intron and origin of replication. The regions of dystrophin that were used to produce antibodies are represented by the bars above the protein (110-14). The numbers at the beginning and end of the brackets indicate the corresponding dystrophin cDNA sequences.