A New Kind of Informational Suppression in the Nematode *Caenorhabditis elegans*

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ABSTRACT

Independent reversions of mutations affecting three different *Caenorhabditis elegans* genes have each yielded representatives of the same set of extragenic suppressors. Mutations at any one of six loci act as allele-specific recessive suppressors of certain alleles of *unc-54* (a myosin heavy chain gene), *lin-29* (a heterochronic gene), and *tra-2* (a sex determination gene). The same mutations also suppress certain alleles of another sex determination gene, *tra-1*, and of a morphogenetic gene, *dpy-5*. In addition to their suppression phenotype, the suppressor mutations cause abnormal morphogenesis of the male bursa and the hermaphrodite vulva. We name these genes *smg-1* through *smg-6* (suppressor with *m*orphogenetic effect on *g*enitalia), in order to distinguish them from *mab* (*m*ale *ab*normal) genes that can mutate to produce abnormal genitalia but which do not act as suppressors (*smg-1* and *smg-2* are new names for two previously described genes, *mab-1* and *mab-11*). The patterns of suppression, and the interactions between the different *smg* genes, are described and discussed. In general, suppression is recessive and incomplete, and at least some of the suppressed mutations are hypomorphic in nature. A suppressible allele of *unc-54* contains a deletion in the 3′ noncoding region of the gene; the protein coding region of the gene is apparently unaffected. This suggests that the *smg* suppressors affect a process other than translation, for example mRNA processing, transport, or stability.

A mutant gene can sometimes be restored to approximately normal function by modifying the general machinery with which genetic information is transcribed and translated into active protein (HARTMAN and ROTH 1973). The most familiar example of such "informational suppression" is nonsense suppression: mutations in a gene that result in premature termination of translation can be suppressed by altered tRNAs that recognize a nonsense codon and insert an amino acid (EGGERTSSON and SOLL 1988). Other examples of informational suppression acting at the level of translation are missense suppression (MURGOLA 1985), frameshift suppression (ROTH 1981) and "omnipotent" suppression (SURGACHOV 1988). These phenomena, as studied in bacteria and yeast, have been very useful both in exploring the mechanism of translation, and in defining the nature of particular mutations.

The process leading from gene to functional product involves many steps, especially in higher eukaryotes, and each of these steps might be a target for additional kinds of informational suppression. For example, modification of a component of the "spliceosome" can overcome defects resulting from a mutated splice site (PARKER, SICILIANO and GUTHRIE 1987). Or, an alteration in the mechanisms that sort proteins to different cellular compartments could reroute a misrouted mutant protein to the correct location. As with the study of translational suppression, analysis of such suppressors would probably provide insight both into basic cellular machinery and into the molecular consequences of suppressible mutations.

Informational suppressors are expected to have certain genetic characteristics. They are expected to act on specific mutations of a variety of different genes (that is, to exhibit allele-specific but gene nonspecific suppression). In addition, it is likely that the suppressors themselves will have some deleterious effects, because any significant alteration in the general processes of gene expression will have pleiotropic consequences. For example, most nonsense suppressors in microbial systems lead to decreased viability and sometimes to lethality. Strong nonsense suppressors in the yeast *Saccharomyces cerevisiae* are always associated with slow or impaired growth (SHERMAN 1982). In *Caenorhabditis elegans*, the strong amber suppressor *sup-7* (WATERSTON 1981) leads to sterility or lethality in homozygous animals grown at low temperature. Such pleiotropic effects are likely to restrict severely the kinds of informational suppression that can occur, and may explain why a greater variety of mechanisms of suppression have not been discovered.

Research on suppressor genetics in higher eukar-

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yotes has been limited, partly because of the difficulty of handling large numbers of individuals. This problem can be overcome by working with cells in culture, but these have the disadvantage that usually only dominant suppressors can be selected, and further genetic analysis is difficult. The small nematode *C. elegans* is a favorable organism for studying suppression in a higher eukaryote. As reviewed elsewhere (HODGKIN, KONDO and WATERSTON 1987), large numbers of individuals can be manipulated, powerful selections can be applied, and sophisticated genetic analysis is easy. For example, a set of amber nonsense suppressors have been identified in *C. elegans*, and shown to be anticodon mutations of $tRNA^{Trp}$ genes (WATERSTON and BRENNER 1978; WATERSTON 1981; WILLS *et al.* 1983; KONDO, 1988). Amber suppressors of other types have also been identified (HODGKIN 1985; K. KONDO, unpublished data) but ochre and opal suppressors have not yet been obtained.

Over 30 additional suppressor loci have been identified in *C. elegans*, but in most cases these suppressors are probably not informational suppressors. They often display gene-specific, allele nonspecific suppression, and in certain cases have been shown to result from phenomena such as gene duplication (*e.g.*, *sup-3*: RIDDLE and BRENNER 1978; MILLER and MARUYAMA 1986) or protein-protein interaction [*e.g.*, *unc-22* and *unc-54*: MOERMAN *et al.* (1982); and probably *unc-93* and *sup-10*: GREENWALD and HORVITZ (1986)].

Here we describe a new set of suppressor loci which have all the hallmarks of informational suppression, but which are unlikely to act at the level of translation. Independent work on three genes, affecting sex determination (HODGKIN 1986), developmental timing (AMBROS and HORVITZ 1984) and muscle proteins (WATERSTON 1988), has converged on the same set of six suppressors. Two of the suppressors had previously been identified as causing a characteristic morphological abnormality in the adult male genital structures, for which reason they were initially assigned to the *mab* (male abnormal) class of genes. In this paper we rename them *smg* (for *s*uppressor with *m*orphological effect on *g*enitalia), because they constitute a class of gene with distinctive common features that distinguish them from other *mab* or *sup* (suppressor) loci. We describe the isolation and mapping of suppressor mutations, cross-suppression tests, morphological effects of suppressor mutations, and interactions between the suppressor loci. We also discuss the nature of the suppressor mutations and possible mechanisms for their action.

The existence of these suppressors was first recognized during reversion analysis of the sex determining genes *tra-3* and *tra-2* (HODGKIN 1986). Their identity as general informational suppressors was established subsequently with work on the heterochronic gene *lin-29*, and most recently with the myosin heavy chain gene *unc-54*. We report the reversion experiments in reverse order, because the phenomenology is most complicated with the *tra* genes and least complicated with *unc-54*. Moreover, the molecular basis of suppression is at present most easily investigated using the myosin system.

MATERIALS AND METHODS

General methods of culture, handling and crossing were used (SULSTON and HODGKIN 1988). Nomenclature follows HORVITZ *et al.* (1979). The term "non-amber allele" is used to refer to mutations that show no sign of suppression by the strongest *C. elegans* amber suppressor, *sup-7*.

Strains: The following genes and alleles were used in this study (*smg* alleles are listed separately). Descriptions can be found in HODGKIN *et al.* (1988).

LGI *bli-3(e767)*, *dpy-14(e188)*, *dpy-5(e61, e565, e907)*, *him-1(e879)*, *unc-13(e51, e450)*, *him-2(e1065)*, *unc-54(e190, e1300, r259, r293, r661)*

LGII *eg1-26 (n481, e1952)*, *dpy-10(e128)*, *unc-4(e120)*, *tra-2(e1095, e1209, e1403, e1425)*, *mab-6(e1249)*, *mab-8(e1250)*, *lin-29(n333, n546, n836, n1368, n1440)*, *ro1-1(e91)*, *mnDf87*

LGIII *unc-45(r450)*, *dpy-1(e1)*, *unc-32(e189)*, *sup-5(e1464)*, *mab-4(e1252)*, *tra-1(e1732, e2270)*

LGIV *unc-5(e53)*, *bli-6(sc16)*, *unc-24(e138)*, *dpy-9(e12)*, *unc-17(e245)*, *him-8(e1489)*, *dpy-20(e1282)*, *tra-3(e1107, e1525, e1767, e1903)*, *mab-12(e2186)*.

LGV *dpy-11(e224)*, *unc-42(e270)*, *him-5(e1490)*, *unc-76-(e911)*, *mDf1*, *eDf1*.

LGX *sup-7(st5)*

Alleles of *smg* genes: those with prefix *r* were obtained as suppressors of *unc-54(r293)*, and those with prefix *ma* were obtained as suppressors of *lin-29(n546)*. Two others, *smg-1(e2134)* and *smg-2(e2008)* were obtained as suppressors of *tra-3(e1107)* (FIRE 1986) and *tra-3(e1767)* (HODGKIN 1986) respectively. The remaining *smg* mutations were detected on the basis of the male morphological phenotype (HODGKIN 1983; J. HODGKIN, S. EMMONS and M. M. SHEN, unpublished results). The reference allele for each gene is listed first. Unless otherwise specified, the reference allele for each gene was the allele used in all experiments reported in the text.

smg-1 (formerly *mab-1*): *e1228, e1233, e2134, e2263, ma127, ma129, ma131, r861, r864, r871, r874, r878, r879, r880, r883, r884, r885, r887, r888, r889, r891, r894, r897.*

smg-2 (formerly *mab-11*): *e2008, e1229, e2164, e2261, ma114, ma122, ma123, ma128, r863, r865, r866, r868, r870, r875, r881, r882, r890, r893, r895, r898.*

smg-3: *ma117, ma115, r867, r877.*

smg-4: *ma116.*

smg-5: *r860, r862, r869, r873.*

smg-6: *r896, r886.*

Reversion of *unc-54(r293)*: Worms of genotype *unc-54(r293)* were mutagenized in 50 mM ethylmethane sulfonate (EMS) for 4 hr at room temperature. Approximately 20 L4 larvae or young adults were transferred to 10-cm plates seeded with *Escherichia coli* OP50. F_1 progeny were screened for dominant revertants and F_2 progeny for recessive revertants. Only one revertant was kept from any given plate, to ensure independence. Revertants were identified on the basis of their increased motility or their ability to lay eggs. The 37 strongest revertants were further analysed: each was crossed

to *r293*/+ males to determine if the revertants were dominant or recessive. 36 proved to be recessive and one dominant. Each revertant was crossed to N2 (wild type) males and F_2 progeny screened for Unc animals, to determine if the reversion events were tightly linked to *unc-54*. None of the 36 recessive suppressors was closely linked to the *unc-54* locus. Complementation tests between the 36 suppressors identified five complementation groups.

Mutation frequencies were estimated by Poisson analysis of the distribution of revertants among total independent cultures. The proportion of total cultures not containing a revertant (P_0 class) was used to calculate the frequency. For example, in the first of three experiments, 39/45 plates yielded no revertants, and each plate contained about 5000 F_2 worms, giving a value of 2.9×10^{-5} for the frequency of revertants among total F_2. Two subsequent experiments gave a higher frequency; the mean for all three was 4.5×10^{-5}. This implies a forward mutation frequency for recessive suppressors of approximately 1.8×10^{-4} per mutagenized gamete.

Cross suppression tests for *unc-54*: *unc-54(r293)* hermaphrodites were crossed with wild-type males, and *r293*/+ progeny males crossed with *smg* hermaphrodites. Doubly heterozygous progeny, (*r293*/+; *smg*/+) were selfed and paralysed progeny were picked; if the *smg* mutation acted as a suppressor, then two thirds (or rather less in the case of *smg-1* and *smg-2*, which are linked to *unc-54*) of these paralysed progeny segregated non-Unc progeny. For testing the effects of *smg* mutations on the other *unc-54* alleles *e190*, *e1300*, *r259*, *r661*, and on the heterozygote *r293*/*r259*, the alleles *smg-1(r861)*, *smg-2(r863)*, *smg-3(r867)*, *smg-4(ma116)*, *smg-5(r860)*, and *smg-6(r896)* were used.

Reversion of *lin-29(n546)*: A population of L4 hermaphrodites of genotype *lin-29(n546) rol-1(e91)* was mutagenized with 0.05 M EMS for four hours and distributed over 50 large plates, at about 50 P_0 per plate. The resulting F_1 and F_2 populations (approximately 25,000 F_1 and 250,000–500,000 F_2 hermaphrodites) were screened for revertants. The *lin-29* mutation prevents egg-laying and the expression of the Rol (rolling) phenotype in adults, so revertants were detected by adult rolling and egg-laying competence. Most were picked as F_2 rollers, but in some cases free eggs were picked, and some of these yielded revertant lines. Ten independent lines were obtained (accepting only one revertant line per plate), giving a value of approximately 3×10^{-5} for the frequency of revertants in the F_2 population, or 1.2×10^{-4} for the frequency per mutagenized gamete.

Cross-suppression tests for *lin-29*: Hermaphrodites of genotype *lin-29(n546) rol-1/mnC1* were crossed with wild-type males, and *lin-29 rol-1*/++ males from this cross were mated with *smg* hermaphrodites. The resulting cross progeny of genotype *lin-29 rol-1*/++; *smg*/+ were selfed to yield *lin-29 rol-1*; *smg*/+ hermaphrodites, and these were selfed again to test for suppression of the Lin-29 phenotype.

Cross suppression tests for *tra-2*: *tra-2* alleles were marked using the linked mutation *unc-4*: hermaphrodites of genotype *+tra-2 unc-4/dpy-10+unc-4* were crossed with *smg* males, and non-Unc hermaphrodite progeny of genotype *smg*/+; *tra-2 unc-4*/++ were selfed. F_2 Smg progeny of genotype *smg*; *tra-2 unc-4*/++ were selfed, and Unc progeny examined for suppression of the Tra-2 phenotype. Strains of genotype *smg*; *tra-2(e1209)* were also constructed in a similar manner, without *unc-4*. A few of the strains listed in Table 4 were homozygous for *unc-4*, which does not appear to have any significant effect on fertility.

Mapping: The locations of significant genes and rearrangements used in this study are shown in Figure 1. Map data for *smg-1* and *smg-2* have been reported previously (HODGKIN 1986); the locations of *smg-3* to *smg-6*, and *mab-12*, are based on the data in Tables 1 and 2, together with the following complementation data: *eDf18* and *eDf19* complement *smg-3*; and *mDf1* and *eDf1* fail to complement *smg-4*. All mapping was carried out using the morphological phenotype of adult males.

The mutation *e1952*, originally assigned to *eg1-48* (HODGKIN 1986) has been found to be an allele of *eg1-26* (TRENT, TSUNG and HORVITZ 1983). The reference allele *eg1-26(n481)* has a similar map position to *eg1-48* and fails to complement the egg-laying defect of *e1952*. The *eg1-48* designation is therefore retired.

Suppression of morphological phenotype: Several alleles of *smg-1* and *smg-2* were tested for suppression of their morphological phenotype (both bursal and vulval) by the amber suppressors *sup-5* and *sup-7*. Homozygous *smg*; *sup-5*; *him-5* strains were examined; these were made by first constructing *smg*; *unc-32*; *him-5* strains and crossing these with *unc-32* +/+ *sup-5*; *him-5* males. Resulting non-Unc hermaphrodite progeny (*smg*/+; *unc-32*/*sup-5*; *him-5*) were allowed to self-fertilize and Smg non-Unc progeny were picked. In all cases tested, it was possible to establish *smg*; *sup-5* strains that exhibited the same morphological abnormalities as the parent *smg* strain, indicating that none of the *smg* alleles tested was suppressed. In some cases *smg*; *sup-7* strains were constructed in an analogous manner; these also showed no suppression. We tested the following alleles; *smg-1*: *e1228*, *e1233*, *e2134*, *e2263*, *ma127*, *ma129*, *ma131*; *smg-2*: *e1229*, *e2008*, *e2164*, *e2261*, *ma114*, *ma122*, *ma123*, *ma128*.

Construction of double *smg* mutants: All of the possible double *smg* mutants, with the exception of *smg-1 smg-5*, have been constructed. Double mutants of *smg-3(ma117)* with reference alleles of the five other *smg* genes were constructed by crossing *unc-5 smg-3* hermaphrodites with *smg* males, and picking several Smg non-Unc hermaphrodites from the F_2 progeny of this cross. Single Unc progeny from the next generation, which should be of genotype *smg*; *unc-5 smg-3*, were used to establish double mutant lines. In a similar manner, double mutants of *smg-1* or *smg-5* with other *smg* mutations were constructed using the linked marker *dpy-5(e61)*, and double mutants of *smg-4* were constructed using the linked marker *dpy-11*. The marker *dpy-1* (linked to *smg-6*) was used for the construction of *smg-2*; *smg-6*. Finally, a strain carrying *smg-1(e1228)* and *smg-2(e2008)*, and no other markers, was obtained by first constructing hermaphrodites of genotype *bli-3 +smg-1+/+smg-2+ unc-13*, picking Smg non-Unc non-Bli recombinant progeny, and then removing the *bli* and *unc* markers by segregation in succeeding generations. The genotype of this, and all of the other double *smg* mutants, was confirmed by complementation tests against the relevant parental strains.

RESULTS

Suppression of a myosin mutation: The *unc-54* gene encodes myosin heavy chain B (MHC B), the most abundant myosin heavy chain expressed in the body wall muscle (for review, see WATERSTON 1988). *unc-54(r293)* is a spontaneous mutation that contains a 256-bp deletion in the 3′ noncoding region of the gene [PULAK and ANDERSON (1988); nucleotides 8217–8472 in the sequence of KARN, BRENNER and BARNETT (1983)]. The leftward breakpoint of *r293* is located 38 bases "downstream" from the *unc-54* translational terminator (UAA codon at nucleotides 8176–8178), and its rightward breakpoint is located 123 bases "down-

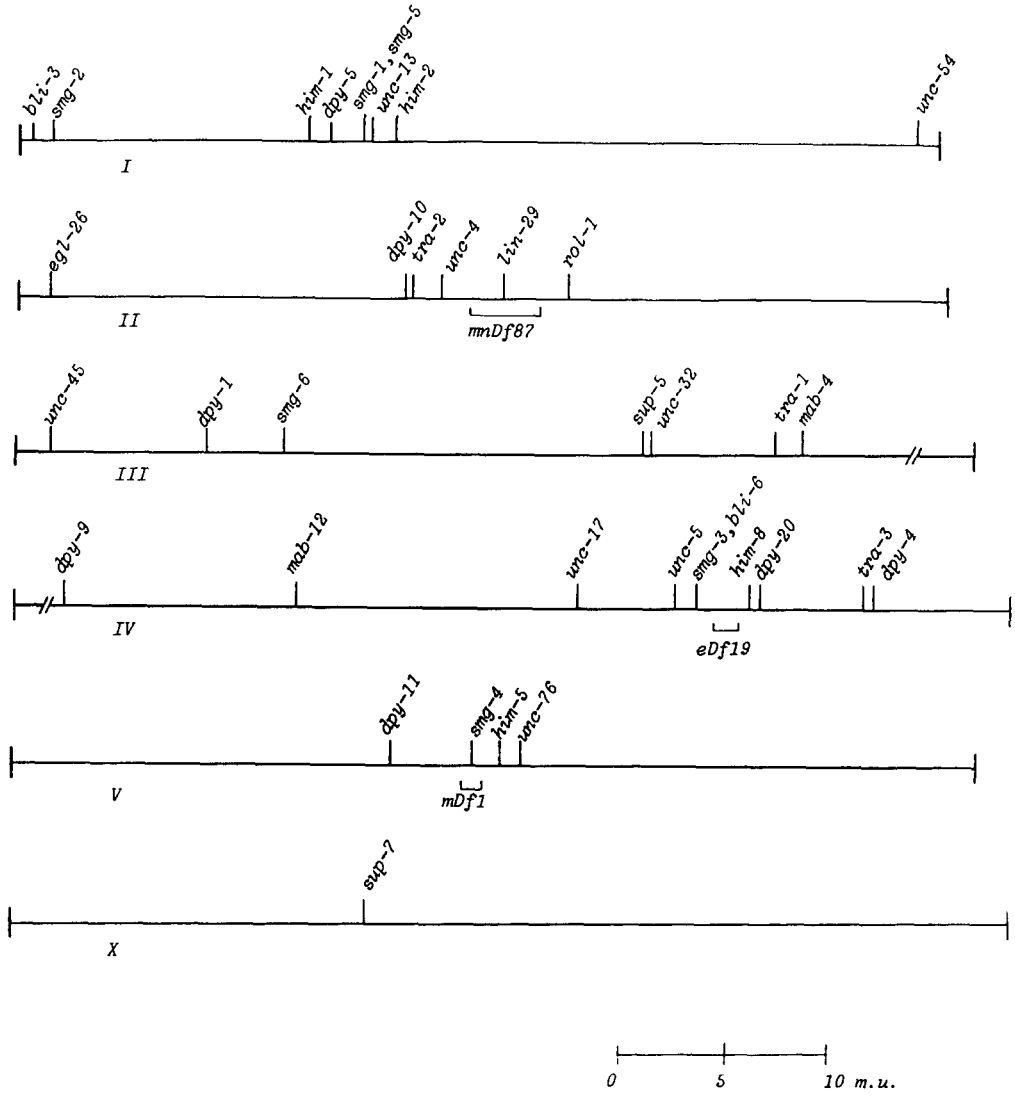

FIGURE 1.—Partial genetic map of *C. elegans*, showing relevant loci in this paper. [Scale in map units (percent recombination).]

TABLE 1

Relevant two-factor data

Cross (hermaphrodite × male)	Number of male progeny		*P*
	Nonrecombinant	Recombinant	
unc-5 smg-3 × *unc-5 smg-3*/++	WT (93) Unc Smg (98)	Unc (1) Smg (1)	1.0
dpy-11 smg-4 × *dpy-11 smg-4*/++	WT (58) Dpy Smg (53)	Dpy (0) Smg (1)	1.0
smg-4 unc-76 × *smg-4 unc-76*/++	WT (52) Smg Unc (53)	Smg (2) Unc (1)	3.0
smg-5 unc-13 × *smg-5 unc-13*/++	WT (137) Smg Unc (107)	Smg (1) Unc (1)	0.8
dpy-1 smg-6 × *dpy-1 smg-6*/++	WT (107) Dpy Smg (115)	Dpy (5) Smg (3)	3.5
smg-6 unc-32 × *smg-6 unc-32*/++	WT (36) Smg Unc (33)	Smg (10) Unc (14)	26.0
dpy-9 mab-12 × *dpy-9 mab-12*/++	WT (142) Dpy Mab (NS)[a]	Dpy (NS) Mab (18)	11.0

[a] NS, not scored.

stream" from the putative polyadenylation signal (AAUAAA at nucleotides 8344–8349). Thus, *r293* does not affect the protein coding region of *unc-54*, but it deletes the polyadenylation signal and the probable sites of pre-mRNA cleavage and polyadenylation (WICKENS and STEPHENSON 1984). Animals homozygous for *r293* are paralysed, though slightly less so than the *unc-54* null mutants *e190* and *e1092* (a small deletion and a nonsense mutant, respectively: DIBB *et al.* 1985). By comparing the phenotype of *r293* to that of a suppressed amber mutant such as *unc-54(1108); sup-5(e1464)* (which contains 5–10% full length protein: MACLEOD *et al.* 1979), we estimate that *r293* accumulates at most 5% of the wild-type level of MHC B.

TABLE 2

Relevant three-factor data

Heterozygote genotype	Recombinants picked	Genotypes
unc-5 bli-6+/++ smg-3	Unc non-Bli	5/5 *smg/+*
dpy-11 smg-4 him-5 unc-76/++++	Unc Him non-Dpy	5/7 *smg/+*
dpy-5+ unc-13/+ smg-5+	Dpy non-Unc	6/6 *smg/+*
	Unc non-Dpy	1/4 *smg/+*
unc-45 dpy-1+/++ smg-6	Unc non-Dpy	5/5 *smg/+*
unc-45 dpy-1 smg-6/+++	Unc non-Dpy	5/5 *smg/+*
	Dpy non-Unc	5/5 *smg/smg*
dpy-9+ unc-17/+ mab-12+	Mab	8/25 *unc/+*
		3/25 *dpy/+*

TABLE 3

Cross-suppression tests

smg gene	Suppression of: *unc-54 (r293)*	*lin-29 (n546)*	*tra-2 (e1209)*	*dpy-5 (e61)*
smg-1(e1228)	S	S	S	S
smg-2(e2008)	S	S	S	S
smg-3(ma117)	S(M)	S	S	S
smg-4(ma116)	S(M)	S	S	S
smg-5(r860)	S	S	S	S
smg-6(r896)	S(M)	S(M)	S	S

S = suppressed. S(M) = suppressed with maternal effect. Maternal effects have not been examined in the suppression of *tra-2(e1209)* and *dpy-5(e61)*.

In a series of reversion experiments (see MATERIALS AND METHODS for details), 37 independent revertants of *r293* were obtained from the F_2 progeny of EMS-treated *r293* hermaphrodites, at a frequency of 10^{-4} per mutagenized gamete. One revertant contained a dominant mutation that is tightly linked to the *unc-54* locus, and the remaining 36 contained recessive suppressors that were either unlinked or loosely linked to *unc-54*. Complementation tests showed that these suppressors represent five complementation groups.

Animals homozygous for any of these suppressors exhibit morphological abnormalities in their genitalia. The abnormalities are more obvious in the male than in the hermaphrodite. This suggested that the suppressors might be alleles of the *mab-1* and *mab-11* suppressors described by HODGKIN (1986). Complementation tests between representative suppressor alleles (using the visible morphological phenotype) demonstrated that the two most frequent classes of suppressor (accounting for 28 of 36 revertants) correspond to *mab-1* and *mab-11*. The three rarer classes represent other suppressor genes that have morphological defects similar to those of *mab-1* and *mab-11*. One of these had been identified as a *lin-29* suppressor (reference allele *smg-3(ma117)*) and the other two are new genes (reference alleles *smg-5(r860)* and *smg-6(r896)*).

The reference alleles for each of four previously defined genes in this class (*smg-1(e1228)*, formerly *mab-1*, *smg-2(e2008)*, formerly *mab-11*, *smg-3(ma117)*, and *smg-4(ma116)*) were tested for suppression of *unc-54(r293)* and found to be as effective as the original set of *r293* suppressors (Table 3). *smg-4(ma116)* was first identified as a *lin-29* suppressor, like *smg-3(ma117)*, but no *smg-4* alleles were recovered in the *unc-54(r293)* reversions.

All six loci have been mapped on the basis of the male morphological phenotype (see MATERIALS AND METHODS) and given the names *smg-1* to *smg-6* (*smg* for *s*uppressor, *m*orphological effect on *g*enitalia). *smg-1* and *smg-2* are new designations for the previously used names *mab-1* and *mab-11*.

The *smg* suppression of *unc-54* is allele specific. We have tested suppression of five additional *unc-54* mutations by each of the *smg* suppressors (see MATERIALS AND METHODS). *unc-54(e190)* and *unc-54(r259)* are both deletions that accumulate no MHC B (EPSTEIN, WATERSTON and BRENNER 1974; MACLEOD, KARN and BRENNER 1981; EIDE and ANDERSON 1985). *unc-54(e1300)* is an amber mutation very near the COOH terminus of MHC B (WILLS *et al.* 1983). *unc-54(r661)* is a leaky mutation whose defect is probably due to aberrant mRNA splicing (EIDE and ANDERSON 1988; B. CARR and P. ANDERSON, unpublished results). None of these mutations is suppressible by *smg-1* through *smg-6*. In fact, some of the *unc-54*; *smg* double mutants are less motile than the parental *unc-54* single mutants. It is not clear if this represents an enhancement of the mutant phenotype or merely an additive effect, because *smg-1* and *smg-2* homozygotes are slightly abnormal in movement themselves.

The phenotypes of *unc-54(r293)*; *smg* double mutants indicate that the amounts of MHC B are likely to be at least 50% of wild-type levels. *unc-54* recessive heterozygotes, which contain approximately 50% of the normal amount of wild-type MHC B, are phenotypically wild type (BEJSOVEC and ANDERSON 1988). Hermaphrodites whose only *unc-54*(+) gene is a hemizygous *X*-linked copy (introduced by transformation: A. FIRE, unpublished results) are visibly slow; these animals are predicted to contain approximately 25% normal levels of *unc-54* mRNA, because hermaphrodites which are homozygous for the transgene contain approximately 50% normal levels of *unc-54* mRNA, and move normally (D. HSU and B. MEYER, personal communication). Thus, MHC B protein in the range of 50% wild-type levels is sufficient to confer a wild-type behavioral phenotype. All *r293*; *smg* double mutants are very nearly wild type in movement. Furthermore, *unc-54(r293)/unc-54(r259)*; *smg* heterozygotes are also wild type. Thus, a single copy of a suppressed *r293* expresses an amount of MHC B sufficient for normal motility, which we believe is in the range of 50% wild type levels.

Suppression by some of the *smg* suppressors is affected by maternal genotype. For the *smg-3*, *smg-4* and *smg-6* classes, animals of genotype *unc-54(r293)*; *smg* that are the offspring of a *smg*/+ heterozygous mother are only partly suppressed. They are less motile than offspring of an *unc-54(r293)*; *smg* homozygous mother. No maternal effects have been seen with the other three classes, *smg-1*, *smg-2* and *smg-5*.

Suppression by each of the *smg* classes is recessive, but there are weak dominant interactions between members of different classes. For example, animals of genotype *smg-2(r865)+unc-54(r293)/+smg-5(r862) unc-54(r293)* exhibit some suppression (less than either *smg* homozygote), and so do animals of genotype *smg-2(r865)+unc-54(r293)/+smg-1(r861) unc-54(r293)*. Not all combinations of heterozygotes have been examined, however.

Suppression of a heterochronic mutation: Mutations of the gene *lin-29* have a "retarded heterochronic" developmental phenotype: in mutant adults, hypodermal seam cells fail to differentiate or produce an adult-specific cuticle; instead, they continue to divide and produce supernumerary larval cuticles, consistent with an inability to switch from larval to adult hypodermal cell fates (AMBROS and HORVITZ 1984). A screen for revertants and suppressors of *lin-29* utilized a double mutant, *lin-29(n546) rol-1(e91)*. Animals of this genotype are egg-laying defective (as a result of aberrant vulval morphogenesis) and fail to express the adult-specific roller phenotype, whereas revertants are able to lay eggs and exhibit the rolling movement characteristic of *rol-1* (COX *et al.* 1980). Such animals are therefore readily detected in large populations. Ten independent revertants were obtained from approximately 250,000–500,000 F_2 progeny of EMS-mutagenized *n546 e91* animals. All were found to carry extragenic recessive suppressors of *lin-29(n546)* and these fell into four complementation groups. The suppressors exhibited a morphological phenotype in the adult genitalia, and complementation tests on the basis of the morphological phenotype led to the assignment of these suppressors to the genes *smg-1* through *smg-4*. Cross-suppression tests using the reference alleles *smg-1(e1228)*, *smg-2(e2008)*, *smg-5(r860)* and *smg-6(r896)* showed that all of these mutations were also able to suppress *lin-29(n546)* (Table 3).

The suppression of *lin-29* is allele-specific, because four other *lin-29* mutations (*n333*, *n836*, *n1368*, *n1440*), which appear to have an identical mutant phenotype to *n546*, are unaffected by *smg* suppressors. This phenotype appears likely to be the null phenotype for *lin-29*, in that similar phenotypes are observed in *n546* homozygotes and in *n546/mnDf87* heterozygotes. Suppression of *n546* by *smg* mutations is complete, as *smg-2(ma123); lin-29(n546)* animals are indistinguishable from *smg-2(ma123)* alone, displaying none of the *lin-29* mutant characteristics (Figure 2); *smg-1* is equally effective. However, *smg-1; lin-29(n546)/mnDf87* animals are not suppressed (although *n546*/+ and *mnDf87*/+ are wild type), indicating that *smg-1* does not restore full wild type function to *n546*. This observation also suggests that *lin-29* has a sharp threshold for gene activity, above which animals are wild type and below which animals are fully mutant.

Maternal effects have been observed in the suppression of *lin-29(n546)* by *smg-6*. However, in contrast to the case of *unc-54(r293)*, *smg-3* and *smg-4* show no maternal effect in the suppression of *lin-29*.

A weak dominant interaction between *smg-1* and *smg-2* has been observed in some animals of genotype *smg-2+/+smg-1; lin-29(n546) rol-1*, which exhibit partial suppression.

Suppression of sex determination mutants: Mutations in the gene *tra-3* lead to masculinization of *XX* animals (normally hermaphrodites). As described elsewhere, extensive reversion experiments on both amber alleles (HODGKIN 1985) and nonamber alleles (HODGKIN 1986) of *tra-3* have been carried out. Among the extragenic suppressors obtained and analysed was the reference allele *smg-2(e2008)*. The reference allele *smg-1(e1228)*, although originally identified because of its male abnormal genital phenotype, was subsequently found to act as a *tra-3* suppressor. The spontaneous mutation *smg-1(e2134)* was obtained as a *tra-3* suppressor by FIRE (1986) during transformation experiments using an amber *tra-3* allele.

Representatives of the other four *smg* suppressors (*smg-3* to *smg-6*) were tested for their ability to suppress *tra-3*, and all gave comparable suppression. In contrast to the situation with *unc-54* and *lin-29*, *smg* suppression of *tra-3* mutations is not allele specific. Three amber alleles of *tra-3* (of which at least two can be separated by recombination) and one non-amber allele, all of which appear to be null for *tra-3* activity, were tested for response to *smg-1(e1228)* and *smg-2(e2008)*. All were suppressed. As discussed below, however, it is probable that suppression does not act on *tra-3* itself, but instead on another activity controlled by *tra-3*, such as *tra-2*.

Several lines of evidence indicate that the wild-type function of *tra-3* is to act as a positive regulator of *tra-2*, another sex determination gene with related properties. First, putative null *tra-2* mutants have a similar (but more extreme) masculinized phenotype than *tra-3* null mutants, but there is no synergism between the two. That is, the phenotype of *tra-2(0) XX* is identical to that of *tra-2(0); tra-3(0) XX* (HODGKIN 1980). Second, the epistatic interactions of *tra-3* and *tra-2* with other sex determining genes suggest that they act at the same step in the sex determination pathway (HODGKIN 1980, 1986). Third, certain gain-of-function *tra-2*

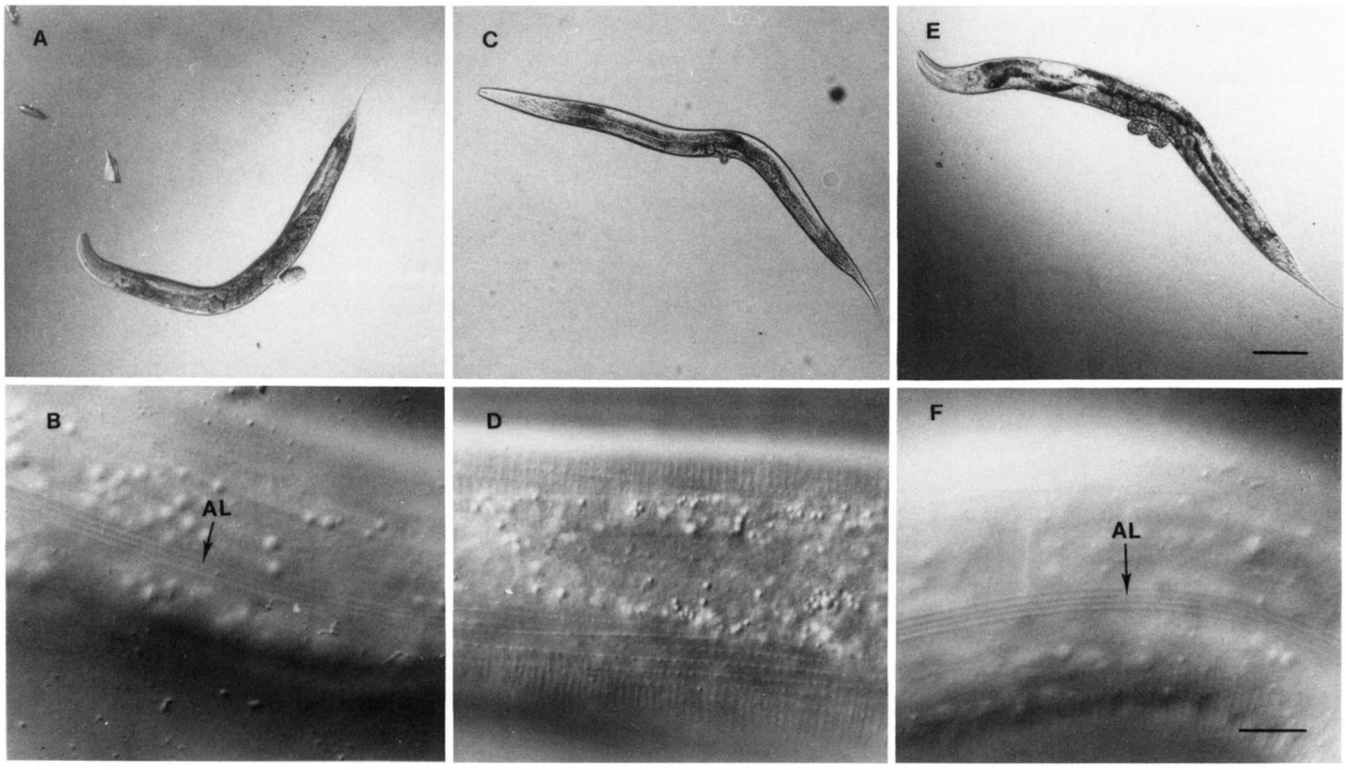

FIGURE 2.—Complete suppression of *lin-29(n546)* by *smg* mutations. A and B, wild-type adult hermaphrodite with normal adult cuticle. C and D, *lin-29(n546)* hermaphrodites with abnormal vulva, no adult lateral alae. E and F, *smg-2(ma123); lin-29(n546)* with normal egg-laying, restored adult cuticle. Lateral views; AL = alae (lateral cuticular ridges). [A, C, E approximately ×70 (scale bar in E, 100 μm). B, D, F approximately ×900 (scale bar in F, 10 μm).]

mutations, which appear to be hypermorphic for *tra-2* function, act as suppressors of *tra-3* (DONIACH 1986; HODGKIN 1986).

For these reasons, four *tra-2* mutations were tested for suppression by *smg-1* and *smg-2*: an amber allele *e1425*, a nonamber allele *e1095* (both of these are either null or close to null for *tra-2*), a weak allele *e1209*, and a very weak allele *e1403*. The allele *e1403* leads to a truncated tail spike in *XX* homozygous hermaphrodites, but does not cause any other obvious masculinization. Neither of the severe mutations, nor the *e1403* allele, showed any suppression, but *e1209* was partly suppressed (Figure 3). The *e1209* allele was chosen because its phenotype most closely resembles that of *tra-3(0) XX* animals: the tail is partly masculinized but the gonad is intersexual, often with some vulval induction. Stronger *tra-2* mutants such as *e1425* have completely male gonads. Occasional *e1209 XX* animals are self-fertile, producing a few self progeny like *tra-3(0) XX* animals (HODGKIN 1985) but the net self fertility is much less than one. In contrast, *tra-2(e1209); smg* animals are usually self fertile (Table 4 and Figure 3) and can be grown as homozygous self-fertilizing populations. Thus, the *smg* mutations act as allele-specific suppressors of *tra-2(e1209)*. The non-specific suppression of all *tra-3* alleles is interpreted as an effect on *tra-2*, rather than on *tra-3*. According to this interpretation, the absence of *tra-3* leads to a reduction in *tra-2* activity, and a similar reduction is caused by the weak *tra-2* allele *e1209*. In both conditions, *smg* mutations can act to increase the residual *tra-2* activity, leading to partial suppression of the Tra phenotype.

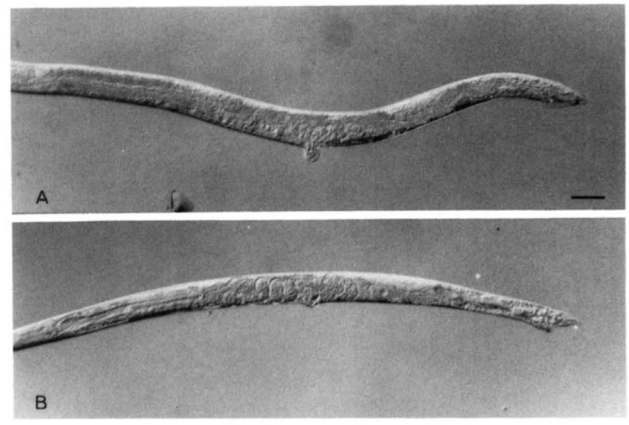

FIGURE 3.—Effect of *smg* mutations on *tra-2(e1209)*. A, *tra-2(e1209) XX*: note partly masculinized tail, sterile gonad with abnormal vulval induction; B, *smg-2(e2008); tra-2(e1209) XX*: note less masculinized tail, eggs in gonad, more normal vulva. Lateral views. (Scale bar 50 μm.)

Suppression of *tra-2(e1209)* is a more convenient assay than suppression of *tra-3*, because the *e1209* phenotype is unaffected by temperature, shows no

TABLE 4

Suppression of *tra-2*: self-progeny broods

Genotype	Broods counted	Range	Mean
tra-2(+)			
WT	14	270–373	327
smg-1	9	147–211	174
smg-2	8	175–269	226
tra-2(e1209)			
e1209	160	0–9	0.1
e1209; smg-1	18	0–36	11
e1209; smg-2	18	0–24	10
e1209; smg-3	9	0–15	6
e1209; smg-4	9	7–27	11
e1209; smg-5	9	0–28	14
e1209; smg-6	9	5–48	18
e1209; smg-4/mDf1	9	5–23	12
eg1–26(e1952) e1209	9	0–15	5
eg1–26(n481) e1209	40	0–8	0.7
e1209; mab-12	25	0	0

maternal effect, and is less variable and slightly more extreme than the *tra-3* masculinized phenotype. Genetic constructions and suppression tests are consequently simpler to carry out. Table 4 summarizes suppression of *e1209* by all the *smg* reference alleles. Certain other mutations were also tested: alleles of *mab-4*, *mab-6*, *mab-8* and *mab-12* (which have morphological effects on phenotype similar to those of *smg* mutations) were tested and found not to suppress. The same *mab* mutations were also tested and found not to suppress *unc-54(r293)*.

In contrast, the *eg1* mutation *e1952*, which was isolated as a *tra-3* suppressor and originally thought to be related to *smg-1* and *smg-2* in its effects (HODGKIN 1986) does suppress *e1209* (Table 4). *e1952* has been shown to be an allele of *eg1-26* (see MATERIALS AND METHODS), and the reference allele, *eg1-26(n481)*, also acts as a weak suppressor of *e1209*. However, *eg1-26(e1952)* has no suppressive effect on *unc-54(r293)* or *lin-29(n546)*, so *eg1-26* cannot be considered a member of the *smg* group.

Suppression of other mutations: In the course of mapping the *smg* mutations and using them in various constructions, mutant alleles of a number of genes were inevitably tested for amelioration of mutant phenotype by one or another of the *smg* mutations. Those that showed no response included *bli-3(e767)*, *unc-13(e51, e450)*, *dpy-14(e188)*, *him-1(e879)* (LGI); *dpy-10(e128)*, *bli-1(e769)*, *unc-4(e120)*, *rol-1(e91)* (LGII); *unc-45(r450)*, *dpy-1(e1)*, *unc-32(e189)* (LGIII); *unc-5(e53)*, *dpy-20(e1282)*, *bli-6(sc16)*, *unc-24(e138)*, *him-8(e1489)* LGIV); *him-5(e1490)*, *unc-76(e911)* (LGV). Of these, many are likely to be hypomorphic with respect to gene function (for example *unc-45*, *dpy-20*, *bli-6*) because more extreme mutations are known for each locus (HODGKIN *et al.* 1988). This provides evidence that *smg* suppressors do not act indiscriminately on all hypomorphic mutations.

TABLE 5

Suppression of *dpy-5*: length measurements

	dpy-5 genotype					
smg genotype	+	*e61*	*e61/+*	*e907*	*e907/+*	*e565*
+	1460	760	1430	760	1330	760
smg-2	1410	950	1090	790	1400	790
smg-1	1390	980				

Mean length measurements, in micrometers, for 20 fully grown adult worms of each genotype [measured as in HODGKIN (1985)]. Coefficients of variation were all less than 6%.

Significant suppression of a mutant phenotype was observed with the *e61* allele of *dpy-5*, as summarized in Table 5. We are grateful to A. FIRE for pointing out the effect of *smg-1* on *e61*. Mutations at each of the six *smg* loci have a similar effect on *e61*, but two other *dpy-5* alleles, *e565* and *e907*, are not affected. The data therefore conform to the general pattern of *smg* effects: suppression is allele-specific but not gene-specific. However, *e61* is no less extreme in its morphological effects than the other two *dpy-5* alleles examined, so this allele is not obviously hypomorphic. A different *dpy* mutation, the hypomorphic allele *dpy-11(e224)*, is also weakly but significantly suppressed by *smg-4* (data not shown).

An additional, paradoxical interaction of *smg* mutations with *dpy-5(e61)* has been observed. The *e61* allele exhibits a very weak dominant phenotype, so that *e61/+* animals are slightly dumpy, but the effect is variable and hard to score reliably. This dominance is greatly increased by *smg* mutations, so that *e61/+; smg* animals are always distinctly dumpy (Table 5). Thus, *smg* mutations suppress two copies of *e61* but enhance one copy. The enhancement effect is specific to *e61*, because it is not seen with a different allele, *e907*, nor with *dpy-11(e224)*.

Interactions of *smg-1* and *smg-2* with certain alleles of *tra-1* have also been observed. The partly suppressed *tra-2(e1209); smg* strains described above (*e.g.*, Table 4) were used to select stronger *tra-2* suppressors (J. HODGKIN and A. SPENCE, unpublished results). In addition to the expected strongly feminizing mutations in genes such as *fem-1* and *fem-3* (which act as epistatic suppressors of *tra-2*), a novel class of *tra-1* mutation was identified. In *smg(+)* backgrounds, these mutations [*e.g.*, *tra-1(e2270)*] do not suppress *tra-2(e1209)* but instead behave as weak recessive *tra-1* alleles, leading to partial masculinization of *XX* homozygotes, which therefore exhibit a sterile intersexual phenotype similar to that of *tra-1(e1732)* (HODGKIN 1987). The *smg* mutations suppress the masculinization, so that *tra-1(e2270); smg XX* animals are self-fertile hermaphrodites. In addition, this genotype leads to weak feminization, evinced by the increased suppres-

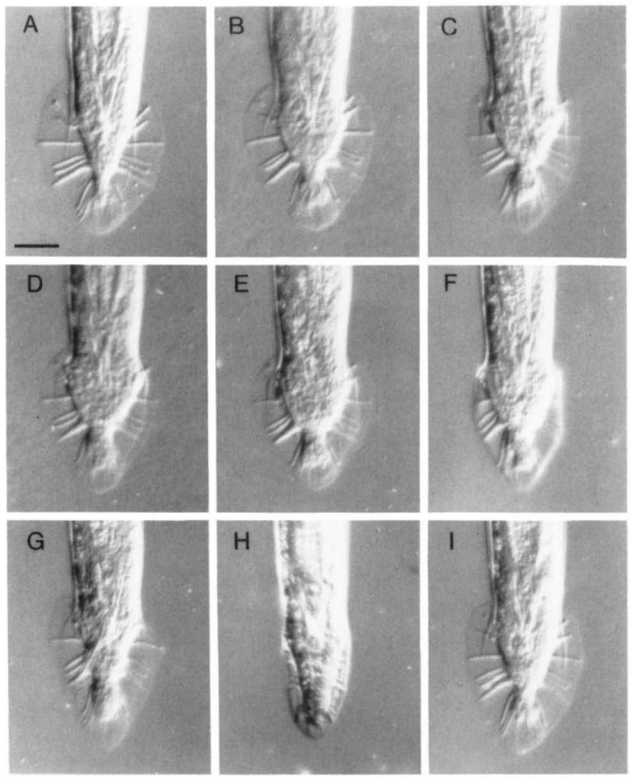

FIGURE 4.—Morphological effect of *smg* and *mab* mutations on adult male tail anatomy. A, wild type. B–G, *smg-1* to *smg-6*. H, *mab-4*, I, *mab-12*. Reference alleles of each gene were used; all animals except A were also homozygous for *him-5* or *him-8*. Tails are viewed ventrally. (Scale bar 20 μm.)

sion of *tra-2(e1209)* (as compared to suppression by *smg* alone) and also by low self-fertility as a result of reduced spermatogenesis in the hermaphrodite. The feminizing effects imply that *e2270* affects the regulation of *tra-1*. These unusual *tra-1* alleles will not be further described here, except to note that they again conform (at least in part) to the general pattern of *smg* effects: interactions are allele-specific and wild-type function is not perfectly restored.

Finally, J. RAND (personal communication) has found that at least some mutations of *smg-1*, *smg-2* and *smg-4* act as allele-specific suppressors of *unc-17(p1156)* (RAND and RUSSELL 1984). The other three *smg* classes have not been tested.

Morphological effects and interactions between *smg* loci: The most conspicuous phenotype caused by *smg* mutations is the abnormal morphology of the adult male tail: the post-anal region (often referred to as the bursa) is swollen and quite different in appearance from the wild type male tail (Figure 4). Despite this abnormality, the anatomical components of the copulatory organs are all present and partly functional, because young mutant males are able to cross-fertilize hermaphrodites at low efficiency. Older mutant males are usually unable to mate successfully, probably because the deformed bursa leads to irreversible extrusion of the copulatory spicules. In contrast, males with lineage defects in the tail such as *mab-3*, *mab-5* or *mab-9* mutants, or males with general sensory defects such as *che-1* mutants (HODGKIN 1983) are never able to mate successfully.

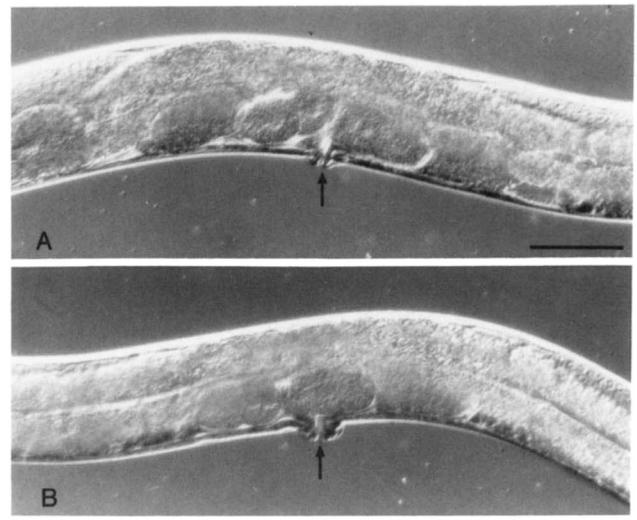

FIGURE 5.—Morphological effect of a *smg* mutation on adult hermaphrodite genital anatomy (vulva arrowed). A, wild type, B, *smg-1(e1228)*. Lateral views. (Scale bar 20 μm.)

Late larval and adult males of *smg-1(e1228)* and *smg-2(e1229)* have been examined in detail using Nomarski light microscopy, by J. E. SULSTON (personal communication). No cell lineage abnormality is apparent in these mutants. Therefore, the defect appears to arise from abnormal morphogenesis, rather than from an alteration in cell lineage or cell fate.

A similar conclusion applies to the vulval phenotype observed in *smg* hermaphrodites. In each of these mutants the adult vulva is functional (permitting both egg-laying and mating) and contains the normal set of nuclei and cells. However, the cells at the lips of the vulva are swollen so that the vulva protrudes from the ventral side of the animal (Figure 5). This phenotype is similar in the reference mutants of all six *smg* loci, though slightly stronger in *smg-1* and *smg-2*.

Comparable bursal and vulval abnormalities are seen in various other *mab* mutants, but these have no obvious suppressive effects. Mutants of *mab-4*, *mab-6*, *mab-8* and *mab-12* all exhibit the combination of swollen bursa and protruding vulva. There are some differences between the phenotypes, for example the deformity of the bursa is more extreme in *mab-4* males than in *smg* males, and slightly less extreme in *mab-12* males (Figure 4), but it would be hard to reliably distinguish *mab-12* from *smg-3* or *smg-4* on the morphological phenotype alone. The reference alleles of *mab-4*, *mab-6*, *mab-8* and *mab-12* have all been tested for the ability to suppress *unc-54(r293)*, *tra-2(e1209)* and *tra-3* mutations, with negative results.

The morphological effects of *smg* mutations are recessive to wild type, but dominant interactions between

TABLE 6

Summary of *smg* isolation methods

Gene	Mab	Suppression of			Total
		unc-54	*lin-29*	*tra-3*	
smg-1	3	16	3	1	23
smg-2	3	12	4	1	20
smg-3		2	2		4
smg-4			1		1
smg-5		4			4
smg-6		2			2

different *smg* loci have been noted. For example, *smg-2+/+smg-1* males sometimes exhibit a weak swollen bursa phenotype [for this reason, one *smg-2* allele, *e1229*, was originally mis-classified as a *smg-1* allele (HODGKIN 1983)]. Dominant interactions of this type have also been observed with respect to *r293* and *n546* suppression, as described above. The full matrix of double heterozygotes has not been examined in detail, but in some cases (*e.g.*, *smg-1/+*; *smg-3/+*) there appear to be no dominant interactions. Also, no interactions have been seen with nonsuppressing *mab* mutations such as *mab-12*.

Most of the possible double homozygote *smg-x*; *smg-y* combinations have been constructed in order to test for synergism between the *smg* genes. All of the combinations tested are viable, though in one case (*smg-1*; *smg-3*) the double mutant grows distinctly more slowly than either parental mutant. When examined, the morphological phenotype of the double mutant is no different from either single mutant. Synergistic effects on the suppression of *dpy-5(e61)* or *dpy-11(e224)* have not been seen.

Nature of *smg* mutations: All *smg* mutations so far obtained are recessive with respect to both morphological and suppression phenotypes. The frequency of isolation of *smg-1* and *smg-2* in both *unc-54(r293)* and *lin-29(n546)* reversions, and in screens for mutants with abnormal male morphology, suggests that these mutations are relatively frequent after EMS mutagenesis, consistent with the belief that they are simple loss-of-function alleles. We obtained many fewer isolates of the other four classes (Table 6), but in the cases of *smg-3*, *smg-4* and *smg-6*, the maternal effects on suppression might have caused revertants to be overlooked among the F_2 populations screened.

Seven *smg-1* and eight *smg-2* alleles have been tested for response to the amber suppressors *sup-5* or *sup-7*, using the male morphological phenotype as a criterion (see MATERIALS AND METHODS). The amber suppressors did not alter this phenotype in any of fifteen cases tested. This indicates that none of these alleles is an amber nonsense mutation, or alternatively that suppression of the morphological phenotype requires very efficient suppression.

Unfortunately, five of the six *smg* loci are located in regions of the genetic map for which deficiencies have not yet been obtained. In one case, *smg-4*, the single known mutation fails to complement both *eDf1* and *mDf1*, and is located in the region spanned by these deficiencies. Since *mDf1* fails to complement mutations flanking *eDf1* (HODGKIN *et al.* 1988), it is safe to assume that *mDf1* is null for the *smg-4* gene. The morphological phenotype of *smg-4(ma116)/mDf1* is the same as that of *smg-4(ma116)* alone, and the suppressive effects of these genotypes are similar (Table 4). Such complementation data would be expected if *ma116* is a loss-of-function mutation, and are consistent with the possibility that it is a null mutation. At this point there is no indication that any of the other five *smg* classes are not also loss-of-function mutations.

DISCUSSION

We have established that *smg* mutations act as allele-specific suppressors of mutations in a wide variety of genes: *unc-54*, *lin-29*, *tra-2*, *dpy-5*. These genes are, respectively, the structural gene for the major body wall myosin heavy chain species; a gene controlling stage-specific differentiation of the hypodermis; a sex-determination gene; and a gene involved in overall body shape. The last, *dpy-5*, may well be a collagen gene, because several other genes of similar phenotypic effect have proved to be collagen genes (KRAMER *et al.* 1988; VON MENDE *et al.* 1988). The four genes have no common feature: they are expressed at different times, affect different tissues, and perform different functions. Therefore, we can assume that the mechanism of suppression is informational, rather than by a fortuitous involvement with each of the separate processes for which the four genes are required.

It is likely that *smg*-suppressible alleles will be found for other genes as well, because they appear to be relatively frequent in occurrence. Suppressible alleles of this type have indeed been identified for two additional genes, *unc-17* (J. RAND, personal communication) and *tra-1*. For three of the genes under discussion (*unc-54*, *lin-29*, *tra-2*), the discovery of suppressible alleles was an incidental product of detailed investigations into particular genes; in the fourth case, *dpy-5*, the discovery of a suppressible allele was accidental, rather than the result of a deliberate search for more suppressible alleles. We have not carried out an exhaustive series of tests on all alleles of any given gene, which might give a better idea of the relative frequency of *smg*-suppressible alleles. As in the case of amber nonsense mutations (HODGKIN 1985), *smg*-suppressible alleles can be expected to be frequent in some genes and rare in others.

The suppressed mutations of each of the four genes do not exhibit any obvious common feature that might shed light on the nature of the mutant lesions or the

mechanism of suppression. Both *unc-54(r293)* and *tra-2(e1209)* are clearly hypomorphic in mutant phenotype, which implies that in each case the gene has residual function. For *unc-54(r293)* the sequence of the mutation (PULAK and ANDERSON 1988) demonstrates that the MHC B protein is unaffected. However, *lin-29(n546)* behaves as a null allele, and *dpy-5(e61)* appears to be among the most severe alleles of this locus. Possibly *lin-29(n546)* is also hypomorphic, and the apparently strong phenotype reflects a threshold effect. That is, at any level of expression less than a certain critical amount, a fully mutant phenotype is expressed. In the case of *dpy-5*, however, the enhancement effect observed with *e61/+* is hard to explain if *e61* is a simple hypomorph. Conceivably *e61* has a latent weak antimorphic effect, which is only apparent in a *smg* background.

Suppression is not complete, for *tra-2(e1209)* and *dpy-5(e61)*. In each case the suppressed phenotype is closer to wild type, but still abnormal. In contrast, amber suppression can be much more complete. For example, *tra-2(e1425am)*; *sup-7 XX* has an almost normal hermaphrodite phenotype (HODGKIN 1985), whereas *smg-1(e1228)*; *tra-2(e1209) XX* is still intersexual. In the case of *lin-29(n546)*, suppression is superficially complete, but this appears to reflect a threshold effect, because the mutant phenotype appears again when the suppressed allele is in *trans* to a *lin-29* deficiency, or to a non-responsive *lin-29* allele. In the case of *unc-54(r293)*, suppression is also superficially complete, but MHC B protein levels have not yet been measured directly.

The molecular nature of the *unc-54(r293)* lesion is known: it is a deletion of material in the 3′ noncoding portion of the gene, spanning the putative poly-A addition site but not affecting the protein coding sequence. It is hard to establish that there is no additional alteration in the *unc-54(r293)* coding sequence, but recent experiments by A. FIRE and S. HARRISON (personal communication) indicate that the *r293* mutant phenotype and its suppression are likely to be associated with the 3′ deletion. FIRE has found that a cloned *unc-54* gene with an experimentally induced deletion in the 3′ non-coding region, similar to the deletion in *r293*, fails to rescue an *unc-54* null mutant when introduced by oocyte injection, but does rescue when injected into a *smg-1(e1228) unc-54* double mutant.

The nature of the mutations in the other genes is unknown, but the *tra-2* gene has been cloned (P. OKKEMA and J. KIMBLE, personal communication), so it should be possible to examine *e1209* for obvious alterations. The gene *tra-1* has been cloned (J. HODGKIN, unpublished results), and one of the *smg*-sensitive alleles, *e2332*, has been shown to carry a deletion of one end of the gene.

Given the nature of *r293* (a 3′ noncoding deletion), it is likely that suppression acts at some step prior to translation, though it is of course conceivable that the 3′ end of *unc-54* mRNA does have some essential role in translation. The deleted *unc-54* gene lacks the putative poly-A addition sequence, so it is probable that *r293* produces a message with an abnormal 3′ terminus, and one likely consequence of this is a decrease in message stability. The *smg* suppressors might then act by stabilizing the message and thereby increasing levels of translated product. Other possibilities can be envisaged: for example, a correct 3′ sequence might be required for packaging into ribonucleoprotein, export from the nucleus, and so on. Alternatively, the *smg* suppressors might act at the level of transcription, by leading to more efficient utilization of a novel 3′ termination site.

If the *smg* suppressors do act by increasing the lifetime of an unstable *unc-54* message, then one would predict that the other sensitive alleles would also be associated with message instabilities. However, instability need not necessarily be associated with an aberrant 3′ terminus, so no prediction can be made about the molecular nature of the mutations in these other cases.

The *smg* suppressors are all recessive, which could be explained by proposing that the wild type functions of these genes are involved with RNA turnover. If there are multiple pathways for RNA degradation, then the absence of any lethal phenotype associated with *smg* mutations could be explained by redundancy. Eliminating any one *smg* function would slow down, but not prevent, RNA turnover. However, we have found no lethal synergism between different classes of *smg* mutation, suggesting that all six affect the same process, and that this process is apparently almost dispensable.

The morphological effects of *smg* mutations on bursal and vulval development are at present mysterious, and not obviously associated with the suppressive effects of these genes. One general scenario that would explain the anatomical effects is that the morphogenetic events required for maturation of the genitalia (which occur rapidly compared to many other processes in nematode development) are associated with a critical transition from one message population to another; if this transition is impeded by *smg* mutations, then the observed defects might ensue. Mutations in several genes other than the *smg* loci have similar morphological effects, suggesting that these processes are particularly sensitive to mutation. Precedents for apparently specific morphological effects caused by defects in basic cellular machinery can be found in Drosophila. For example, *Minute* mutations, which are believed to lie in ribosomal protein genes (KONGSUWAN *et al.* 1985) lead to characteristic eye and bristle phenotypes.

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