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# Genetic suppression\*

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## Abstract

Genetic suppression has provided a very powerful tool for analyzing *C. elegans*. Suppression experiments are facilitated by the ability to handle very large numbers of individuals and to apply powerful selections. Because the animal grows as a self-fertilizing diploid, both dominant and recessive suppressors can be recovered. Many different kinds of suppression have been reported. These are discussed by category, with examples, together with discussion of how suppressors can be used to interpret the underlying biology, and to enable further experimentation. Suppression phenomena can be divided into intragenic and extragenic classes, depending on whether the suppressor lies in the same gene as the starting mutation, or in a different gene. Intragenic types include same-site replacement, compensatory mutation, alteration in splicing, and reversion of dominant mutations by *cis*-knockout. Extragenic suppression can occur by a variety of informational mechanisms, such as alterations in splicing, translation or nonsense-mediated decay. In

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**addition, extragenic suppression can occur by bypass, dosage effects, product interaction, or removal of toxic products. Within signaling pathways, suppression can occur by modulating the strength of signal transmission, or by epistatic interactions that can reveal the underlying regulatory hierarchies. In *C. elegans* biology, the processes of muscle development, vulva formation and sex determination have provided remarkably rich arenas for the investigation and exploitation of suppression.**

## 1. Introduction

Genetic suppressors provide some of the most powerful tools available for exploring gene expression, function and interaction. A suppressor mutation can be defined as a second mutation, at a site distinct from the mutation under study, that suppresses the phenotypic effect of the first mutation. *C. elegans* is especially suited for suppression analysis, for several practical reasons:

1. Very large numbers of individuals can be handled, so that extremely rare mutational events can occur within a single experimental population.
2. Strong selections can be applied, which may permit the detection and recovery of a single individual from a population of one hundred thousand or more.
3. Mutations are easily generated, particularly by the chemical mutagen EMS.
4. The predominant mode of reproduction is by self-fertilization, so that both recessive and dominant suppressors can be recovered readily.

Genetic suppression can arise in a variety of ways, which are described in the sections below. Depending on the nature of the suppressive interaction, different kinds of information can be obtained about the initial gene studied.

## 2. Intragenic suppression by same site replacement

In the simplest and least informative scenario, if a mutant carries a single base change difference from wildtype, then reversion can occur simply by mutating the sequence back to wildtype. However, intracodonic suppression can also occur, if a different base change can result in a functional coding sequence. An example is provided by a Gln-to-Pro mutant of *dpy-31*, encoding a collagen-processing protease, which has been repeatedly reverted either by back mutation, Pro-to-Gln (CCT -> CTT), or by the only other acceptable change in this codon, Pro-to-Ser (CCT -> TCT) (Novelli et al., 2004).

## 3. Intragenic suppression by compensatory second site mutation

In principle, missense mutations that result in a non-functional protein can sometimes be suppressed by introducing another change elsewhere in the protein. Relatively few cases of this effect have been studied so far in *C. elegans*. An example is provided by the reversion of temperature-sensitive missense mutations in *glp-1*, which encodes a cell interaction protein; this experiment yielded a variety of second-site missense changes that are able to correct the original defect (Lissemore et al., 1993).

## 4. Intragenic suppression by altered splicing

Sometimes intragenic reversion can be achieved by a second mutation that leads to omission of a deleterious mutation by means of exon skipping. This is particularly likely to happen with genes that produce multiple splice variants, as illustrated by studies of *unc-52*. This gene encodes multiple isoforms of perlecan, which are proteins required for myofilament anchorage to the basement membrane underlying muscle cells. Nonsense mutations located in some of the alternatively spliced exons can be suppressed by a G-to-A change in the acceptor site adjoining the mutant exon, which leads to its omission; the resulting RNA remains in frame and produces a functional protein (Rogalski et al., 1995).

## 5. Intragenic suppression of dominant mutations by loss-of-function in *cis*.

Most genes in *C. elegans* are haplo-sufficient, so the majority of dominant mutations are associated with gain-of-function effects, which result in a toxic or inappropriately expressed gene product (Park and Horvitz, 1986).

The dominant effect of such a mutation can be suppressed by introducing a second mutation elsewhere in the mutated gene that results in partial or complete loss-of-function in its product.

An early example was provided by studies of the myosin gene *unc-54*. Some dominant mutations of *unc-54* cause slow movement or paralysis in single dose, by poisoning thick filament assembly. These mutations, such as the allele *e1152*, can be reverted by introducing null or partial loss-of-function mutations in *cis*. Heterozygotes carrying just the dominant allele (*e1152/+*) are slow, whereas *e1152 null/+* heterozygotes are wildtype. The *unc-54* gene is essential for movement, so *e1152 null* double homozygotes are paralysed, but the mutations are initially recovered in healthy heterozygotes with normal movement. Selection for improved movement of *e1152/+* heterozygotes therefore provided an efficient means of recovering many mutations in this gene (Anderson and Brenner, 1984).

Similar strategies have been used frequently and effectively to generate multiple loss-of-function mutations in important or essential genes.

## 6. Extragenic suppression — overview

Suppressing the effects of a mutant gene by changes elsewhere in the genome can occur in a number of ways. Extragenic suppression is usually revealed by crossing a revertant line with wildtype, and observing the re-appearance of the original mutant after Mendelian segregation. Numerous cases of extragenic suppression have now been identified and analysed in greater or lesser depth. Many different phenomena are involved in the various cases. About 40 genes in the *sup* class have been defined thus far, and in addition separate gene classes have been established for many of the more specific processes that have been studied. As shown in Table 1, research on 21 suppressor gene classes has been published. At least 20 further classes have been established (see Wormbase) but their properties are as yet unpublished. It should be noted that suppressor mutations frequently turn out to be special alleles of genes that have already been well studied and given more informative names, so the names of suppressor loci are more transient than most.

Table 1. Suppressor gene classes

Gene name	Explanation	Initial references
<i>cps</i>	CED-3 Protease Suppressor	Wang et al., 2002
<i>des</i>	DEgeneration Suppressor	Treinin et al., 1998
<i>eor</i>	Egl-1 suppressor/DiO uptake defect/Raf enhancer	Howard and Sundaram, 2002
<i>ksr</i>	Kinase Suppressor of activated Ras	Sundaram and Han, 1995; Kornfeld et al., 1995
<i>scd</i>	Suppressor of Constitutive Dauer formation	Inoue and Thomas, 2000
<i>sel</i>	Suppressor/Enhancer of Lin-12	Sundaram and Greenwald, 1993
<i>seu</i>	Suppressor of Ectopic Unc-5	Colavita and Culotti, 1998
<i>sgs</i>	Suppressor of G protein S defect	Korswagen et al., 1998
<i>sli</i>	Suppressor of LIneage defect	Jongeward et al., 1995
<i>smg</i>	Suppressor, Morphological effect on Genitalia	Hodgkin et al., 1989
<i>smu</i>	Suppressor of Mec and Unc defects	Lundquist and Herman, 1994
<i>soc</i>	Suppressor Of Clr	Selfors et al., 1998
<i>sog</i>	Suppressor Of Glp	Maine and Kimble, 1993
<i>sol</i>	Suppressor Of Lurcher movement defect	Zheng et al., 2004
<i>sop</i>	Suppressor Of Pal	Zhang and Emmons, 2000
<i>spr</i>	Suppressor of PResenilin defect	Wen et al. 2000

Gene name	Explanation	Initial references
<i>srl</i>	Suppressor of Roller Lethality	Barbazuk et al., 1994
<i>sup</i>	SUPpressor	Riddle and Brenner, 1978; Waterston and Brenner, 1978
<i>sur</i>	SUPpressor of activated Ras	Lackner et al., 1994; Wu and Han, 1994
<i>sus</i>	SUPpressor of Suppressor	Brown and Riddle, 1985
<i>suv</i>	SUPpressor of Vulvaless	Hajnal et al., 1997

Space does not permit a full description of all the classes listed in Table 1, but some of the major types of suppression that have been encountered are discussed below, using particular examples.

## 7. Informational suppression — overview

Alteration of the general machinery for transcription, RNA processing and translation can sometimes ameliorate or suppress certain kinds of mutation in many different genes. The hallmarks of informational suppression, as this phenomenon is known, are allele-specificity and gene-non-specificity. Three kinds of informational suppression have been described so far in *C. elegans*: nonsense suppression; suppression by modified splicing; suppression by loss of nonsense-mediated decay (NMD).

## 8. Nonsense suppression

A large fraction of point mutations identified in *C. elegans* are changes from sense to nonsense codons, either UAG (amber), UAA (ochre) or UGA (opal), resulting in polypeptide chain termination and (usually) complete or almost complete loss of gene function. These nonsense mutants can potentially be suppressed by nonsense suppressor tRNAs, in which a sense codon has been changed to be able to decode the nonsense triplet. In practice, only amber suppressors have been recovered. Early work identified two suppressor loci, *sup-5* and *sup-7*, which could act to suppress certain mutations in a wide variety of genes (Waterston and Brenner, 1978; Waterston, 1981). These were subsequently shown to be amber suppressor mutations of two Trp tRNA genes, carrying alterations in their anticodons from CCA to CTA (Wills, et al., 1983; Bolten et al., 1984).

There are twelve members of the Trp tRNA family in *C. elegans*, but only two can be mutated to yield strong amber suppressors. The same two mutations, *sup-5* and *sup-7*, were isolated repeatedly in independent experiments (Waterston, 1981). Weak amber suppressors were subsequently obtained for six additional members of the set (see Table 2), by selecting for suppressors using amber mutants that are very easily suppressed such as several in the sex determination gene *tra-3* (Hodgkin, 1985; Kondo et al., 1988; Kondo et al., 1990). Probably only a trace of full length TRA-3 protein is sufficient to provide enough activity for normal sexual development, so weak suppressors can be recovered. As indicated in Table 2, the eight identified amber suppressors appear to be expressed at different levels, and they also exhibit some differences between tissues in the strength of suppression (Kondo et al., 1990). In vitro observations on the transcription of these genes that are consistent with these different strengths have been reported (Li et al, 1988). The differences in strength show that flanking sequences can exert a strong influence on expression level of these tRNAs, despite the fact that all eight are identical in the internal promoter sequences believed to be sufficient for Pol III transcription of tRNA genes.

Table 2. Amber nonsense suppressors

Gene name (allele)	Class (other name)	Approximate relative strength	Reference
<i>sup-5(e1464)</i>	Trp ( <i>rtw-1</i> )	7	Waterston and Brenner (1978)
<i>sup-7(st5)</i>	Trp ( <i>rtw-2</i> )	8	Waterston (1981)
<i>sup-21(e1957)</i>	Trp ( <i>rtw-10</i> )	3	Hodgkin (1985)
<i>sup-24(st354)</i>	Trp ( <i>rtw-4</i> )	4	Kondo et al. (1988)

Gene name (allele)	Class (other name)	Approximate relative strength	Reference
<i>sup-28(e2058)</i>	Trp ( <i>rtw-8</i> )	2	Kondo et al. (1988)
<i>sup-29(e1986)</i>	Trp ( <i>rtw-9</i> )	1	Kondo et al. (1988)
<i>sup-33(st389)</i>	Trp ( <i>rtw-11</i> )	4	Kondo et al. (1990)
<i>sup-34(e2227)</i>	Trp ( <i>rtw-12</i> )	3	Kondo et al. (1990)
<i>sup-22(e2057)</i>	Non-Trp	2	Hodgkin (1985)
<i>sup-23(e2059)</i>	?Non-Trp	2	Hodgkin (1985)
<i>sup-32(e1958)</i>	Non-Trp	2	Hodgkin (1985)
<i>sup-?(st402)</i>	Non-Trp	3	Kondo et al. (1990)
<i>sup-?(st414)</i>	Non-Trp	2	Kondo et al. (1990)

Too much suppression is clearly deleterious, because animals carrying two doses of *sup-7* or *sup-5* are inviable or subviable, especially at low temperature (Waterston, 1981). Presumably the deleterious effects result from too much readthrough of natural stop codons. Selection for improved low-temperature viability of *sup-7* animals was used to generate deletions or loss-of-function mutations of *sup-7*, suggesting that the inviability is due to readthrough rather than loss of the normal function of the gene. The same effect may explain why ochre or opal suppressors have not been recovered.

Additional weak amber suppressors have been isolated, which do not correspond to Trp tRNA genes, but they have not yet been identified at the molecular level (Table 2). Some of these are likely to be Gln tRNA genes, because a Gln CTG anticodon can be readily mutated to read amber codons. Suppression by an amber-suppressing Ser tRNA from *Drosophila*, introduced as a transgene, has been reported (Pilgrim and Bell, 1993), demonstrating that endogenous Ser tRNA genes might also yield amber suppressors. As well as providing valuable tools for examining mutations in particular genes and for studying tRNA gene expression, amber suppressors have been useful in the development of methods for microinjection and transformation of *C. elegans* (Kimble et al., 1982; Fire, 1986). The dose-sensitivity of strong amber suppressors also allows selection of single-copy transformants (Fire et al., 1990).

## 9. Extragenic suppression by modified splicing

Two kinds of suppression by modified splicing have been reported. The first kind depends on compensatory mutations in splice-site recognition machinery, and the second is associated with mutations that affect alternative splicing.

The first kind involves allele-specific suppression of mutations that change the conserved and essential GU donor sequence at the 5' end of an intron, by means of compensatory changes in the sequence of a U1 snRNA gene. In the case of *sup-6*, a donor site mutation (AGgu --> AGau) in allele *e309* of *unc-13* is suppressed by a change in one of the U1 snRNA genes (...UCCAUU... --> UCUAAU) (Zahler et al., 2004). In a related case, *sup-39*, a donor site mutation (AGgu --> AGuu) in allele *e936* of *unc-73* is suppressed by a change in a different U1 snRNA gene (...UCCAUU... -->... UCAAUU...) (Run et al., 1996; Roller et al., 2000; Zahler et al., 2004). These effects are consistent with the proposed role of U1 in pairing at the donor site during splicing.

In *C. elegans*, there are 12 genes encoding U1 snRNA, yet only two have yielded suppressors of this type (Zahler et al., 2004). As with the failure to find ochre or opal suppressors discussed above, it is possible that most U1 suppressor mutations will be either too weak, because of redundancy, or too deleterious, because of effects on normal genes. In support of the latter possibility, both *sup-6* and *sup-39* exhibit defects when homozygous: *sup-6* homozygotes arrest in late-larval development, and 50% of *sup-39* homozygotes die as embryos. In addition, *sup-39* animals that escape this embryonic lethality frequently exhibit two rows of oocytes rather than one in adult hermaphrodites, an unusual and unexplained phenotype (Run et al., 1996).

The second kind of extragenic suppression by altered splicing involves the regulation of alternative splicing. As described above, some mutations of the *unc-52* perlecan gene are located in alternatively spliced exons and can

be suppressed by intragenic mutations that cause exon-skipping. A subset of these, those located in exon 17, can also be suppressed by loss-of-function mutations in two genes, *smu-1* and *smu-2*, which encode proteins homologous to mammalian spliceosome-associated factors (Spartz et al., 2004). Loss of these factors leads to enhanced skipping of exon 17, and consequently to suppression of mutations affecting this exon. The *smu-1* and *smu-2* mutations also weakly suppress the pleiotropic effects of mutations in *mec-8*, which encodes another regulator of alternative splicing (Lundquist and Herman, 1994). This suggests that the SMU-1 and SMU-2 factors can affect the splicing of multiple genes, although neither is essential for viability. It seems very likely that further cases of suppression by modified splicing will be encountered in the future.

## 10. Suppression by loss of NMD (Nonsense Mediated Decay)

Mutations resulting in a premature stop codon trigger the NMD system of message degradation. If the NMD system is absent, then mRNAs with such codons have longer lifetimes, increasing the chance of readthrough at the stop codon and the production of a functional protein. Alternatively, if the stop codon is located close to the end of the message, the truncated polypeptide produced after translation of the stabilized message may be long enough to have substantial activity. Nonsense mutations are frequently generated in *C. elegans* forward mutagenesis screens, so a variety of suppressor screens have yielded mutations in the *C. elegans* NMD genes (Hodgkin et al., 1989). Seven genes required for NMD have been identified in this way, which are called *smg* (for Suppressor with Morphogenetic effect on Genitalia). Loss of NMD does not lead to inviability in *C. elegans*, but does result in an abnormal morphology of the hermaphrodite vulva and of the male tail, hence the name. Suppression is generally weaker than with amber suppression.

## 11. Extragenic suppression by non-specific physiological effects

Some cases of suppression probably result from general effects on the physiology of the animal, which can alter the expression of a variety of different genes. An example is provided by the recovery of mutations in eight or more cuticle collagen genes, all of which cause a ‘dumpy left roller’ phenotype, as weak suppressors of two quite different mutant defects: germline proliferation, in the case of *glp-1* (Maine and Kimble, 1989), and embryonic gastrulation, in the case of *emb-5* (Nishiwaki and Miwa, 1998). The mechanism involved is unknown, but indirect effects via alteration of extracellular matrix or of stress response have been suggested as possible explanations.

## 12. Suppression by altered gene dosage

Sometimes the lack of a particular protein can be compensated for by increasing the amount of an alternative, related protein. This is illustrated by the analysis of a gene initially called *sup-3* (Riddle and Brenner, 1978), identified by mutations that acted to suppress *unc-54* or *unc-15* mutants; these lack either the major body wall myosin (UNC-54) or another thick filament component, paramyosin (UNC-15). Molecular analysis of *sup-3* showed that its mutant alleles are duplications or triplications of *myo-3*, which encodes a minor myosin. Normally MYO-3 accounts for only 15% of the body wall myosin, but its level is increased in the duplication alleles, and can thereby compensate for the lack of UNC-54 or UNC-15 (Maruyama et al., 1989).

## 13. Bypass suppression

Suppression by increased dosage can be regarded as a special case of bypass suppression, wherein an alternative protein or pathway is up-regulated or de-regulated to remove the need for a defective component. Mutations that activate alternative pathways are often encountered as a result of suppressor analysis. Screens for suppressors of *pal-1*, the *C. elegans* homolog of the developmental regulator caudal, illustrate this effect (Zhang and Emmons, 2000). In normal development, *pal-1* expression in certain tail cells depends on a positive regulatory element located in one of its introns. A screen was carried out for suppressors of a *pal-1* mutation affecting this intronic element, which led to the identification of three *sop* (Suppressor Of Pal) genes. These encode factors that act as general repressors of an alternative mode of *pal-1* transcription. Two of the *sop* genes encode components of the transcriptional Mediator complex. Loss of these factors results in expression of *pal-1* in the tail cells, and hence to suppression by bypassing the need for the intronic regulatory element.

## 14. Extragenic suppression by interacting components

An abnormal protein can sometimes be restored to greater functionality by changes in an interacting protein. Suppression by such a mechanism is usually allele-specific, and provides *in vivo* evidence for protein interaction. An



example is provided by mutations of the muscle protein gene *unc-22*, which cause a ‘twitcher’ abnormal movement phenotype. Some revertants of certain *unc-22* alleles were found to be unusual missense alleles of the body wall myosin gene *unc-54*, suggesting that the UNC-22 and UNC-54 proteins interact directly (Moerman et al., 1982). Both these proteins are, indeed, located in the thick filaments of the myofilament lattice (Sarcomere assembly in *C. elegans* muscle).

Another example is provided by suppression of certain dominant mutations in *unc-105*, a gene encoding a putative mechanosensory channel in muscle cells, by a specific missense alteration in *let-2*, which encodes a basement membrane collagen (Liu et al., 1996). A plausible interpretation is that UNC-105 and LET-2 interact directly, providing anchorage for the sensory channel and allowing it to act as a stretch sensor.

## 15. Suppression by removal of a toxic protein or protein complex

A situation sometimes encountered in *C. elegans* genetics is the appearance of an abnormal phenotype as the result of a neomorphic change in a gene, resulting in the production of a toxic protein. Such mutations are usually but not always dominant (sometimes they are recessive because in heterozygotes the abnormal protein is outcompeted by normal protein). Suppression studies have been carried out with such mutations in a variety of different genes. Any alteration that removes the toxic protein, or prevents it from acting, will behave as a suppressor.

The simplest situation involves the introduction of a second inactivating mutation in *cis* to the dominant allele. This is exemplified by dominant mutations of the myosin gene *unc-54*, as already described.

A more complex situation was encountered with the gene *unc-93*, which has been subjected to extensive suppression analysis. A rare mutation of *unc-93*, *e1500*, causes a distinctive movement and egg-laying defect referred to as the ‘rubber-band’ phenotype. This phenotype is almost completely recessive, although genetic tests indicate that *e1500* is neomorphic in character. Null mutations introduced in *cis* to *e1500* result in normally moving animals, when homozygous. Therefore, the *unc-93* gene is dispensable, but can be mutated to generate a product that is able to poison muscle contraction (Greenwald and Horvitz, 1980). The *e1500* phenotype can also be suppressed by null mutations in three unlinked genes, *sup-9*, *sup-10* and *sup-18*, which act as recessive suppressors, as well as by specific dominant mutations of another gene, *sup-11* (Greenwald and Horvitz, 1982). Moreover, ‘rubber-band’ alleles of *sup-9* and *sup-10* were subsequently found, which can be suppressed by null alleles of *unc-93* (Greenwald and Horvitz, 1986; Levin and Horvitz, 1992). Molecular analysis indicates that these genes encode components of a muscle-specific potassium channel: the SUP-9 sequence reveals that it belongs to the family of two-pore K<sup>+</sup> channel proteins, and SUP-10 and UNC-93 are membrane proteins that co-localize with SUP-9 in muscle cells (Levin and Horvitz, 1993; de la Cruz et al., 2003). The current interpretation is therefore that this multi-protein channel complex is dispensable for normal muscle function, but it can be mutated so as to interfere severely with contraction, presumably by affecting K<sup>+</sup> flux. The other two interacting genes, *sup-11* and *sup-18*, likely encode additional components, but *sup-11* is known to be an essential gene, in contrast to the other four (because null mutations of *sup-11* are embryonic lethal), so it must be involved in other processes as well.

A comparable situation was found in the case of the *deg-3* gene, which encodes a nicotinic acetylcholine receptor subunit. Rare dominant alleles of *deg-3* cause neuronal degeneration (hence the name), but the degeneration can be suppressed by loss-of-function mutations of *des-2* (DEgeneration Suppressor). The *des-2* gene was found to encode another receptor subunit, and co-expression of wildtype DEG-3 and DES-2 in *Xenopus* oocytes resulted in formation of a functional acetylcholine-gated channel. Co-expression of mutant DEG-3 with wildtype DES-2 resulted in an abnormal channel. Expression of either subunit alone, however, does not allow formation of a channel, thereby explaining the mechanism of suppression (Treinin et al., 1998).

## 16. Suppression by modulators of activity

The efficiency of a signal transduction pathway is usually modulated by interacting components that can increase or decrease the strength of a signal. This is especially true in multicellular organisms, where the same pathway may be used for multiple purposes in different tissues, and can be fine-tuned by these modulators so as to allow optimal function in each context. The presence of modulators is often revealed by suppression analysis. Good illustrations of this type of suppression are provided by extensive work on the LIN-12/NOTCH receptor, which is involved in numerous important signaling events during development (see LIN-12/Notch signaling in *C. elegans*). Suppression experiments have been carried out using both hypomorphic, partial loss-of-function alleles (Sundaram and Greenwald, 1993) and hypermorphic, gain-of-function alleles of *lin-12* (Levitan and Greenwald, 1995; Tax et al., 1997). The first kind of experiment yielded mutations in negative regulators of LIN-12 activity, such as *sel-1*,

encoding an extracellular protein (Grant and Greenwald, 1996), and *sel-10*, encoding an F-box protein predicted to be involved in ubiquitin-mediated turnover of LIN-12 (Hubbard et al., 1997). SEL-10 was independently identified as a factor affecting sex determination, again partly as a result of suppressor analysis (Jager et al., 2004).

The second kind of experiment, suppressing dominant gain-of-function alleles of *lin-12*, generated mutations in positive regulators. These included SUP-17, a protein belonging to the ADAM protease family, which perhaps acts by cleaving and activating the extracellular domain of the LIN-12 receptor (Wen et al., 1997). Also identified were SEL-5, a serine/threonine kinase (Fares and Greenwald, 1999) and SEL-12, which is related to mammalian presenilin proteins and therefore of great medical interest (Levitan and Greenwald, 1995). Not surprisingly, *sel-12* mutations have an egg-laying defect in a *lin-12(+)* background; this in turn has allowed further suppressor screens for mutations correcting this defect, and the identification of *spr* (Suppressor of Presenilin) genes (Wen et al. 2000).

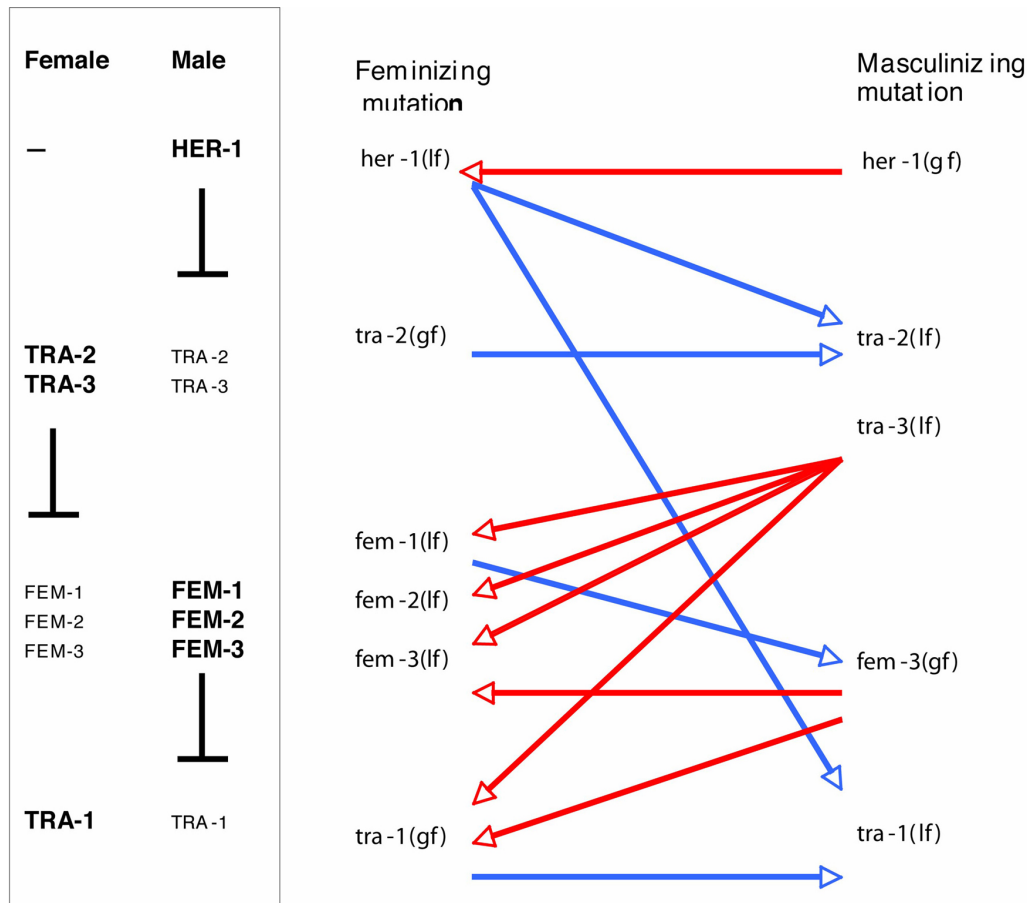
## 17. Suppression by epistasis

Suppression analysis has been invaluable in unraveling regulatory pathways in *C. elegans*. Many important events in development depend on signal transduction events, whereby an inductive signal emanating from one cell leads to a transcriptional response in target cells or tissues. The transduction of the signal involves a chain of interactions, with receptors, second messengers and transcription factors, and the interactions may be either positive or negative at each step. Mutations that decrease or increase signaling at one step can be epistatically suppressed by antagonistic mutations acting at a downstream step, which in turn can be subjected to further analysis. If the pathway involves a linear series of interactions, then the epistatic relationships of mutations in the different genes can be used to deduce the order and nature of the steps involved. More detailed discussion of the rules and interpretation of epistasis can be found elsewhere (Avery and Wasserman 1992).

Two good examples are provided by the vulval induction pathway (see RTKRas/MAP kinase signaling and the sex determination pathway (see Somatic sex determination and Sex-determination in the germ line). In the first case, opposite mutant phenotypes result from either too little or too much activation of the ERK MAP kinase pathway in the vulval precursor cells. These phenotypes are called Vul (vulvaless, failure to induce or complete vulval differentiation) and Muv (multivulva, excess vulval differentiation) respectively. Both kinds of phenotype are very conspicuous in adult hermaphrodites, and both are associated with egg-laying defects and reduced fertility, so suppressors are easily detected. Mutations that suppress a Vul phenotype often lead to a Muv phenotype on their own, and vice versa. For example, screens for suppressors of a loss-of-function Vul mutation in *let-23*, encoding the EGF receptor required for vulval induction, yielded a gain-of-function in *let-60*, encoding the downstream Ras protein in this signal transduction pathway. The *let-60(gf)* mutation has a Muv phenotype on its own, and screens for suppressors of the Muv phenotype led to the isolation of mutations in ten or more genes encoding further downstream components or modifiers of the pathway, most of which have a Vul phenotype on their own. This kind of analysis has led to the identification of thirty or more genes involved in EGF signaling, and has played a major role in the very detailed elucidation of its properties and function (see RTKRas/MAP kinase signaling). For a summary of the suppressor screens that have been carried out, see Sternberg and Han (1998).

In the case of sex determination, the opposite phenotypes caused by mutation are Masculinized and Feminized. It is frequently possible (for example by use of temperature-sensitive mutants) to set up a situation in which a large population of worms develops only along one sexual path. Such a pure male or pure female population will yield no progeny, so there is extremely effective selection for any suppressor mutation that reverts or ameliorates the phenotype, and thereby allows cross- or self-fertilization, and the production of progeny. An extensive series of experiments of this type has been carried out in a number of different laboratories, as summarized in Figure 1. Both loss-of-function and gain-of-function mutations have been isolated at each of four regulatory steps, largely as a result of this approach. For example, selections for suppressors of the masculinizing mutations of *tra-3* yielded feminizing (loss-of-function) alleles of *fem-1*, *fem-2* and *fem-3*, as well as gain-of-function alleles of *tra-1* (Hodgkin, 1986). Amber alleles of *tra-3* were used in some of these selections, which consequently also yielded many nonsense suppressors and NMD (*smg*) suppressors (Hodgkin, 1985; Hodgkin, 1986).





**Figure 1. Suppressor screens and selections for sex determination mutants.** Part of the signal transduction cascade controlling sex in *C. elegans* is shown at left. In hermaphrodites, the feminizing transcription factor TRA-1 promotes female development, and the membrane receptor TRA-2 prevents inhibition of TRA-1 by the FEM proteins. In males, TRA-1 is prevented from acting by the FEM proteins, because TRA-2 action is blocked by binding the extracellular male-specific protein HER-1. At right are shown some of the suppressive interactions that led to this model. Red arrows indicate isolation of feminizing suppressors that counteract masculinization, and blue arrows indicate isolation of masculinizing suppressors that counteract feminization. For more detailed explanation, see text and chapters on sex determination.

Almost every known control pathway in *C. elegans* has been subjected to some kind of suppressor analysis, and the results have frequently been critical in elucidating properties and testing models. Genetic suppression must also happen naturally, and is certain to play an important role in evolution. Whether new kinds of suppression will become apparent, as further genes and processes are subjected to investigation, remains to be seen.

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