G protein hyperactivation of the *Caenorhabditis elegans* adenylyl cyclase SGS-1 induces neuronal degeneration

Hendrik C.Korswagen,
Alexander M.van der Linden and
Ronald H.A.Plasterk¹

Division of Molecular Biology, The Netherlands Cancer Institute,
Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

¹Corresponding author
e-mail: rplas@nki.nl

Expression of a constitutively activated version of the heterotrimeric G protein α -subunit $G\alpha_s$ results in the swelling and vacuolization of a specific subset of ventral nerve cord motoneurons of *Caenorhabditis elegans*. A second site modifier (*sgs-1*) that completely suppresses this neuronal degeneration has been isolated. *sgs-1* was cloned and was shown to encode an adenylyl cyclase which is most similar to mammalian adenylyl cyclase type IX. Mutations in *sgs-1* change residues that are conserved among different adenylyl cyclases. These mutations are located in the two catalytic domains and in the first multiple transmembrane spanning region of the predicted protein. An *sgs-1* reporter construct shows a general neuronal expression pattern, demonstrating that *sgs-1* is expressed in the neurons that are susceptible to activated $G\alpha_s$ -induced cell death. A second *C.elegans* adenylyl cyclase gene (*acy-2*) was analyzed as well. In contrast to *sgs-1*, *acy-2* shows a restricted expression pattern and loss of *acy-2* function results in early larval lethality. These results suggest that SGS-1 is a target of $G\alpha_s$ signaling in motoneurons, whereas an interaction of $G\alpha_s$ with ACY-2, probably in the canal-associated neurons, is required for viability. **Keywords: adenylyl cyclase/*Caenorhabditis elegans*/ $G\alpha_s$ /neuronal degeneration/suppressor**

Introduction

Heterotrimeric G proteins connect serpentine transmembrane receptors to a variety of intracellular effectors. G proteins consist of a guanine nucleotide binding α -subunit and a $\beta\gamma$ -subunit, both of which have signaling capabilities (Kazirot *et al.*, 1991; Simon *et al.*, 1991). The nematode *Caenorhabditis elegans* expresses homologs of a number of mammalian G protein subunits, including $G\alpha_s$ (Park *et al.*, 1997), $G\alpha_o$ (Mendel *et al.*, 1995; Ségalat *et al.*, 1995), $G\alpha_q$ (Brundage *et al.*, 1996) and $G\beta$ (Zwaal *et al.*, 1996), which allows detailed genetic analysis of conserved G protein-coupled signal transduction pathways in a relatively simple organism.

We previously reported that *gsa-1* encodes a conserved nematode $G\alpha_s$ subunit which is essential and is ubiquitously expressed in the nervous system and muscle cells of *C.elegans* (Korswagen *et al.*, 1997). A constitutively

activating mutation in $G\alpha_s$ that inhibits the GTPase activity of the protein and locks it in the GTP-bound active conformation, induces body-wall muscle hypercontraction and swelling, and vacuolization of a specific subset of neurons. Neurons which are susceptible to activated $G\alpha_s$ -induced degeneration are mainly ventral nerve cord motoneurons and some neurons located in the head and tail ganglia. This neuronal cell death is distinct from programmed cell death and is similar in morphology to the neuronal degeneration observed in degenerin mutants of *C.elegans* (Chalfie and Wolinsky, 1990; Driscoll and Chalfie, 1991; Hall *et al.*, 1997). The degenerins *deg-1* and *mec-4* encode proteins which are similar to subunits of mammalian amiloride-sensitive Na^+ channels and form mechanosensory channels in *C.elegans* (Hong and Driscoll, 1994; Huang and Chalfie, 1994), whereas *deg-3* encodes an acetylcholine receptor channel-subunit (Treinin and Chalfie, 1995). The degeneration-inducing mutations in *deg-1*, *deg-3* and *mec-4* presumably hyperactivate these channels, resulting in an increased or altered ion-influx that is followed by cell death. The morphological similarity of activated $G\alpha_s$ -induced neuronal degeneration to degenerin-induced cell death suggests that $G\alpha_s$ directly or indirectly hyperactivates ion-channels. This might be a direct interaction of $G\alpha_s$ with ion-channels (reviewed in Wickman and Clapham, 1995), or could be indirect through, for example, the activation of an adenylyl cyclase, resulting in generation of the second messenger cyclic-3',5'-adenosine monophosphate (cAMP) (reviewed in Sunahara *et al.*, 1996). Increased levels of cAMP can directly modulate cAMP-gated ion-channels or modulate ion channels through activation of cAMP-dependent protein kinase A (PKA) (reviewed in Walsh and Van Patten, 1994; Wickman and Clapham, 1995).

To study the mechanism of activated $G\alpha_s$ -induced neuronal degeneration, we characterized extragenic suppressors which inhibit neuronal cell death (Korswagen *et al.*, 1997). Here we show that one of these suppressors, *sgs-1*, encodes an adenylyl cyclase that is essential for activated $G\alpha_s$ -induced neurotoxicity. Furthermore, we studied a second adenylyl cyclase gene (*acy-2*) of *C.elegans*. We show that *acy-2* is expressed in the canal-associated neurons (CAN) and that loss of *acy-2* results in a larval lethal phenotype which is similar to the lethal phenotype of *gsa-1* null mutant animals.

Results

sgs-1 is a suppressor of activated $G\alpha_s$ -induced neuronal degeneration

In a screen for extragenic suppressors of activated $G\alpha_s$ -induced neuronal degeneration, a locus was identified that suppresses neuronal cell death. This locus was named *sgs-1* (suppressor of activated G_s ; Korswagen *et al.*, 1997).

Table I. Alleles of *sgs-1* suppress activated $G\alpha_s$ -induced neuronal degeneration

Genotype	Mutation	Neuronal degeneration ^a (%)	Body-wall muscle hypercontraction ^b
Wild-type		0	–
Activated $G\alpha_s$ <i>sgs-1</i> alleles ^c :		100	++++
<i>pk301</i>	C1a	0	+
<i>pk310</i>		0	++
<i>pk311</i>	C1a	0	++
<i>pk363</i>	transmembrane	0	++
<i>pk384</i>	C2	0	++
<i>pk474</i>	C1a	0	+
<i>pk393</i>	transmembrane	0	+++
<i>pk450::Tc1</i>	Tc1 insertion	0	++

^aActivated $G\alpha_s$ -induced pattern of neuronal cell deaths as described in Korswagen *et al.* (1997). Phenotypes were scored in synchronized L1-2 larvae, 24 h after a 2 h heat-shock at 33°C.

^bAnimals showing a Dpy appearance and strong folding of the intestine were scored as positive for body-wall muscle hypercontraction (–, 0–10%; +, 5–25%; ++, 25–50%; +++, 50–75%; +++++, >75% of animals that show body-wall muscle hypercontraction).

^c*sgs-1* alleles were analyzed in an activated $G\alpha_s$ (*pkIs296*) background

As is shown in Table I, each of eight alleles of *sgs-1* completely suppress neuronal degeneration. In contrast to the complete suppression of neuronal cell death, suppression of the activated $G\alpha_s$ -induced body-wall muscle hypercontraction is variable.

Animals mutant in *sgs-1* do not show obvious defects in development or behavior. A deletion that spans the *sgs-1* locus was used to determine whether mutant phenotypes associated with *sgs-1* could be uncovered by lowering the level of *sgs-1* by half. When *sgs-1(pk310)* and *sgs-1(pk450::Tc1)* were placed in *trans* to the genetic deficiency *sDf121*, which deletes the *sgs-1* locus (D.Baillie, personal communication), the heterozygous animals did not show any changes in development or behavior. These results suggest that *sgs-1* is not an essential gene.

***sgs-1* encodes an adenylyl cyclase**

sgs-1 was placed on linkage group III, to the left of the deficiency *nDf17*, between markers *unc-93* and *unc-32*, and was found to be closely linked to *dpy-17* (Figure 1A). The area of the physical map of chromosome III that corresponds to this region is completely sequenced (Wilson *et al.*, 1994) and cosmids containing candidate genes were tested for rescue of the Sgs phenotype. Cosmid F17C8 rescued *sgs-1*, resulting in the reappearance of swollen and vacuolized neurons after expression of activated $G\alpha_s$. Unrelated cosmids did not rescue *sgs-1*. Rescue of *sgs-1* was also obtained with a subclone of F17C8 (Figure 1B). This 14 kb subclone contains a single predicted gene that encodes a 1254 amino acid protein (Figure 2A) with significant sequence similarity to mammalian adenylyl cyclases. As is shown in Figure 3, SGS-1 is most similar (39% overall identity, 48% similarity of amino acid positions) to mouse adenylyl cyclase type IX. Sequence similarity is highest in the conserved C1a and C2a regions of the two catalytic domains of adenylyl cyclase, with 67 and 60% identity of amino acid positions, respectively, between SGS-1 and mouse adenylyl cyclase type IX. The

predicted protein shows the characteristic topology of a membrane localized adenylyl cyclase, which consists of a short intracellular N-terminal sequence, two multiple transmembrane spanning regions and two large intracellular catalytic domains (Figure 2B).

Mutations in sgs-1 change conserved residues in the adenylyl cyclase coding sequence

The coding sequence of *sgs-1* was scanned for mutations in different alleles that suppress neuronal cell death. All mutations found were G–A or C–T substitutions, which correspond to the canonical mutation spectrum of ethyl methanesulfonate (EMS) (Anderson, 1995), the mutagen used in the genetic screens to isolate alleles of *sgs-1* (Korswagen *et al.*, 1997). With the exception of mutations in *pk310* and *pk393*, only mutations were found that change amino acid positions which are conserved between different adenylyl cyclases (Figure 2A); in *pk310* no mutational change could be detected in the *sgs-1* coding sequence, in *pk393*, a non-conserved Gly at position 68 is changed into an Arg. In *pk363*, a conserved Gly at position 199 in the first region of transmembrane domains is changed into a Glu. Two mutations are in the catalytic domains. In *pk301* a Ser at position 486 in the first catalytic domain is changed into a Tyr, in *pk384* a Gly at position 1068 in the second catalytic domain is changed into an Arg. Two independent alleles of *sgs-1* (*pk311* and *pk474*) contain an identical splice donor site mutation located in the first catalytic domain that changes the canonical GT splice donor sequence to AT. Sequencing of *pk311 sgs-1* cDNA revealed that cryptic splice donor sites surrounding the mutated splice donor are used, some of which may retain the correct reading frame (data not shown).

The isolation of an identical mutational change in the independent alleles *pk311* and *pk474* implies that only a limited spectrum of mutations are tolerated in *sgs-1*. To address whether only specific mutational changes in *sgs-1* can result in suppression of neuronal cell death, or whether any loss-of-function mutation in *sgs-1* can produce this phenotype, a Tc1 transposon insertion located in the *sgs-1* coding sequence (Figure 2A) was isolated from a library of random Tc1 insertion mutants (Zwaal *et al.*, 1993). Insertion of Tc1 can disrupt a gene by truncation of the coding sequence or can affect the protein product by cryptic splicing of the transcript (Rushforth *et al.*, 1993). As is shown in Table I, the *sgs-1* transposon allele *pk450::Tc1* completely suppresses activated $G\alpha_s$ -induced neuronal degeneration, demonstrating that a randomly isolated presumed loss-of-function allele of *sgs-1* suppresses neuronal cell death as well. Furthermore, these results suggest that loss of *sgs-1* function does not result in severe developmental or behavioral phenotypes.

***sgs-1* is expressed in the nervous system**

Presumptive upstream control sequence of *sgs-1* was used to direct expression of a Green Fluorescent Protein (GFP) (Chalfie *et al.*, 1994) encoding reporter gene. The *gfp* reporter gene was inserted close to the 3' end of *sgs-1* and the fusion construct includes all intron sequences of *sgs-1* (Figure 2A). Since this fusion contains almost the complete *sgs-1* gene, it is likely that this fusion closely

Fig. 1. Positional cloning of *sgs-1*. (A) Genetic map of linkage group III, showing the position of *sgs-1* and markers used in this study. (B) Cosmid clone F17C8 and the subclone pRP1522. pRP1522 contains a single predicted gene encoding an adenylyl cyclase. Both F17C8 and pRP1522 rescue *sgs-1*. (C) Genomic representation of the *acy-2* gene, showing the deleted region in *pk465* and the site of *gfp* fusion in pRP1525.

resembles the endogenous *sgs-1* expression pattern. *sgs-1::gfp* shows a general neuronal expression pattern, with expression in neurons of the head ganglia (Figure 4A), the ventral nerve cord (Figure 4B and C) and the tail ganglia (Figure 4C). Not all neurons express the *sgs-1* reporter; expression is faint or absent in the CAN cells and is less extensive in the anterior head ganglion. *sgs-1* is also expressed in the muscle cells of the vulva (Figure 4B) and in adult animals at low levels in body-wall muscle cells. Based on this reporter construct, *sgs-1* is not expressed in the muscle cells of the pharynx, nor is *sgs-1* expressed in hypodermal or intestinal cells.

***acy-2* encodes a second *C.elegans* adenylyl cyclase**

The identification of *sgs-1* as an adenylyl cyclase, prompted us to search the *C.elegans* genome database for additional adenylyl cyclase genes. A gene encoding a second predicted adenylyl cyclase was identified by the *C.elegans* genome project (*C.elegans* genome consortium, unpublished observation). C10F3.3 encodes a predicted protein (ACY-2) of 1080 amino acids which shows significant sequence similarity to mammalian adenylyl cyclases (35% overall identity, 45% similarity to adenylyl cyclase type II; Figure 3). Like SGS-1, ACY-2 contains the highly conserved C1a and C2a sequences (64% and 80% identity to adenylyl cyclase type II, respectively), and shows the expected structural organization of two multiple transmembrane regions and two large intracellular catalytic domains (Figure 2B). *acy-2* shows a more limited expression pattern than *sgs-1*. A translational fusion of *acy-2* to *gfp* is expressed at high levels in the CAN cells (Figure 4E) and some neurons in the head ganglia (Figure 4D), and at low levels in a small number of ventral nerve cord neurons. We isolated a deletion of *acy-2* genomic sequence from a library of chemically-induced deletion mutants (Jansen *et al.*, 1997). In *acy-2* (*pk465*), a 2.7 kb region of the *acy-2* sequence is deleted, removing the first catalytic

domain and the two multiple transmembrane regions (Figure 1C). Loss of *acy-2* results in early larval lethality; animals homozygous for *pk465* arrest at the first stage of larval development (Figure 4F), a phenotype that is similar to the larval lethal null phenotype of *gsa-1* (Korswagen *et al.*, 1997). This lethal phenotype was rescued by introduction of the *acy-2*(+) containing cosmid C10F3 in *pk465* animals. Interestingly, rescued *pk465* lines segregated animals that appeared fluid filled, a phenotype that is reminiscent of *clr-1* mutants and suggests a defect in the CAN cells. The CAN cells have a function in osmoregulation (Forrester and Garriga, 1997). Taken together, these results suggest that $G\alpha_s$ and ACY-2 perform an essential function in the CAN cells.

Discussion

Adenylyl cyclases are a family of signaling molecules that generate the second messenger cAMP. Mammals express at least nine different adenylyl cyclase isoforms which are all stimulated by $G\alpha_s$ and the diterpene forskolin, but differ otherwise in stimulation or inhibition by other $G\alpha$ subunits, $G\beta\gamma$, Ca^{2+} -calmodulin and protein kinase C (PKC) (reviewed in Sunahara *et al.*, 1996). Since adenylyl cyclases are modulated by components of different signal transduction pathways, they constitute an important level of integration of diverse signals. Some isoforms of adenylyl cyclase require, for instance, stimulatory input from both $G\alpha_s$ and Ca^{2+} -calmodulin and function as coincidence detectors. An example of an adenylyl cyclase acting as such a coincidence detector is provided by the *Drosophila Rutabaga* gene (Levin *et al.*, 1992), which plays a role in associative learning and memory storage (reviewed in Kandel and Abel, 1995). Adenylyl cyclases are transmembrane proteins that consist of a short, non-conserved intracellular N-terminal sequence, a region of six transmembrane regions followed by a large intracellular

Fig. 2. Amino acid sequence and hydrophobicity plot of SGS-1. (A) Amino acid sequence of SGS-1 showing the C1a and C2a regions between brackets and conserved residues in these regions in bold. Mutations in *sgs-1* and the site of *gfp* fusion (*gfp*) are indicated. A region in C2a which is important for $G\alpha_s$ -binding is depicted by a hatched bar. (B) Hydrophobicity plot (Kyte and Doolittle, 1982) showing the multiple transmembrane regions in SGS-1 and ACY-2.

catalytic domain (C1) and a second region of six transmembrane domains followed by a second intracellular catalytic domain (C2). The two catalytic domains each contain a

Fig. 3. Alignment dendrogram generated using the PILEUP algorithm using default program parameters. SGS-1 is most closely related to mouse adenylyl cyclase type IX.

highly conserved region (C1a and C2a, respectively) which is similar between the two catalytic domains. In stimulated adenylyl cyclase, the two catalytic domains come together to form a catalytically active heterodimer (Zhang *et al.*, 1997).

As in mammals, multiple isoforms of adenylyl cyclases are present in *C.elegans*; in addition to *sgs-1* and *acy-2*, the *C.elegans* genome contains at least a third gene that encodes a predicted adenylyl cyclase (*C.elegans* genome consortium, unpublished observation). Study of such conserved signaling molecules in *C.elegans* offers the advantage of a well defined system and allows detailed genetic analysis of adenylyl cyclase function in the nematode. Here we describe two *C.elegans* adenylyl cyclases, SGS-1 and ACY-2. SGS-1 was identified as a suppressor of activated $G\alpha_s$ -induced neuronal cell death, ACY-2 was identified by the *C.elegans* genome project. Both SGS-1 and ACY-2 show significant sequence similarity to mammalian adenylyl cyclases, with highest sequence homology within the C1a and C2a domains. In addition, both predicted proteins show the expected multiple transmembrane regions and large cytoplasmatic domains characteristic of adenylyl cyclases. With the exception of SGS-1, which has a Ser at the position of a conserved Lys in C1a, both proteins contain the Lys and Asp residues in the C1a and C2a regions that confer specificity to adenosine triphosphate as opposed to guanosine triphosphate in the related family of guanylyl cyclases (Liu *et al.*, 1997). Of mammalian adenylyl cyclases, SGS-1 is most similar to mouse adenylyl cyclase type IX, a divergent class of adenylyl cyclases (Premont *et al.*, 1996). Type IX adenylyl cyclase is abundantly expressed, with highest levels in skeletal muscle and brain. Interestingly, type IX adenylyl cyclase is relatively insensitive to forskolin and does not seem to be modulated by $G\beta\gamma$ or Ca^{2+} . It is not known however, whether this is also the case for SGS-1.

Different loss-of-function alleles of *sgs-1* show a range of mutations in the *sgs-1* coding sequence. All single amino

Fig. 4. Localization of *sgs-1::gfp* and *acy-2::gfp*. (A) Expression of *sgs-1::gfp* in the head ganglia. The neurons of the head ganglia shown are located between the two bulbs of the pharynx (ph). *sgs-1* is not expressed in the pharyngeal muscle (ph) cells. Yellow dots result from gut autofluorescence. (B) Expression of *sgs-1::gfp* in the vulva muscle cells (vm) and ventral nerve cord neurons (arrows). (C) Expression of *sgs-1::gfp* in ventral nerve cord neurons (arrows indicate single neurons or groups of neurons) and the tail ganglia (tg). (D) Expression of *acy-2::gfp* in a limited subset of neurons located in the head ganglia (arrows). (E) Expression of *acy-2::gfp* in the canal-associated neurons (CANL is shown). (F) Terminal phenotype of *acy-2* (*pk465*). Note the fluid filled cavity (arrow) between the hypodermis and intestine.

acid substitutions change small non-charged residues such as Gly and Ser into large, highly charged residues such as Arg and Glu or large aromatic residues such as Tyr—mutations which may affect the catalytic action of the protein or may result in a conformational change. Two mutations are located within the C1a domain. In *pk301*, a highly conserved Ser is changed into a Tyr, whereas in *pk311* and *pk474* a splice donor site is disrupted, a change that will affect the C1a sequence as well. Given the importance of the C1a region for the catalytic activity of

adenylyl cyclases, it is likely that these mutations directly influence SGS-1 activity. One mutation (*pk384*) is at the border of the C2a domain, three positions upstream of a sequence which is part of a negatively charged hydrophobic groove that is an important binding region for $G\alpha_s$ (Tesmer *et al.*, 1997; Yan *et al.*, 1997). Introduction of a positively charged Arg close to this domain may interfere with $G\alpha_s$ binding and uncouple SGS-1 from activation by $G\alpha_s$. Two further mutations are within the first multiple transmembrane region of SGS-1. These mutations may

not affect SGS-1 activity directly, but may interfere with sorting and localization of SGS-1, as has been shown for mutations in the first multiple transmembrane region of the *Dictyostelium* adenylyl cyclase ACA (Parent and Devreotes, 1995).

No mutations were found that result in early termination or frame-shift of the SGS-1 protein. Therefore, it is not clear whether any of the mutations described represents a complete loss-of-function allele of *sgs-1*. A putative null allele of *sgs-1* was however isolated from a library of Tc1 insertion mutants. Insertion of Tc1 may lead to truncation of the SGS-1 protein sequence or will affect the *sgs-1* protein product through cryptic splicing (Rushforth *et al.*, 1993). Truncation at this insertion site will remove the complete C2 region, which is essential for adenylyl cyclase activity.

Mutation of *sgs-1* does not result in severe developmental or behavioral phenotypes. This is in contrast to *acy-2*, mutation which results in larval lethality. This lethal phenotype is similar to the larval lethal phenotype of *gsa-1* null mutant animals (Korswagen *et al.*, 1997). The *clr-1*-like appearance of incompletely rescued *acy-2* (*pk465*) animals suggests a defect in the CAN cells (Forrester and Garriga, 1997), a phenotype that is in agreement with the expression of *acy-2* in these cells. Therefore, it is likely that $G\alpha_s$ and ACY-2 perform an essential function in the CAN cells, whereas SGS-1 has a more subtle, but non-redundant function. Reporter fusions of *sgs-1* show a general expression in the nervous system, including neurons in the head ganglia, the ventral nerve cord and the tail ganglia. Consequently, *sgs-1* is expressed in the neurons that are susceptible to activated $G\alpha_s$ -induced cell death. Mutation of *sgs-1* also influences activated $G\alpha_s$ -induced body-wall muscle hypercontraction. It is not known whether this is a direct result of *sgs-1* function in muscle cells, or is the result of a function of *sgs-1* in motoneurons.

At least one other locus (*sgs-2*; H.Korswagen, unpublished observation) was identified that can be mutated to suppress activated $G\alpha_s$ -induced neuronal degeneration. Cloning of additional loci will uncover more components of $G\alpha_s$ -coupled signal transduction in *C.elegans* and may elucidate the mechanism with which overactivity of $G\alpha_s$ and SGS-1 induces neurotoxicity.

Materials and methods

Nematode strains and culturing

General methods for culture, manipulation and genetics of *C.elegans* were as described (Lewis and Fleming, 1995). Strains used in this study were Bristol N2, NL545 (*dpy-20(e1362)IV*; *pkIs296[gsa-1QLdpy-20(+)]X*), MT690 (*unc-93(e1500)* *dpy-17(e164)III*), SP471 (*dpy-17(e164)* *unc-32(e189)III*), MT1169 (*nDf17/dpy-17(e164)tra-1(e1099)III*) and BC4697 (*sDp3(III:f)*; *sDf121(s2098)* *unc-32(e189)III*). All *sgs-1* mutations were generated in an NL545 background as described (Korswagen *et al.*, 1997). *sgs-1* alleles *pk363*, *pk384*, *pk393* and *pk474* were isolated in this study. A Tc1 insertion in *sgs-1* (*pk450::Tc1*) was isolated from a library of Tc1 insertion mutants using primers seq13 GATAGTGGTCACTGGTTG and seq15 GTTGTTCGAATGACACCA-GC, as described (Zwaal *et al.*, 1993). A deletion mutant of *acy-2* (*pk465*) was isolated from a chemical deletion library using primers del5, AATGTCTCATCCCGCTC; del8, CAGCAACACTCGAAGGT-AAG and nested primers del6, GCAAACTTACCGATGTGCTCC and del7, TAGTTTCCACGTACGTTCTC as described (Jansen *et al.*, 1997).

Positional cloning of *sgs-1*

sgs-1 was placed on linkage group III (Figure 1A) (Korswagen *et al.*, 1997). Using further three-factor crosses with a *dpy-17unc-32* marked chromosome, *sgs-1* was placed to the left or close to *dpy-17* (16/16 were SgsUnc, 0/11 were SgsDpy). Crosses with an *unc-93dpy-17* marked strain established that *sgs-1* maps to the right of *unc-93* and close to *dpy-17* (10/10 were SgsUnc, 0/3 were SgsDpy). Furthermore, the deficiency *nDf17* does not uncover *sgs-1* (in 15/15 crosses, *sgs-1(pk310)/nDf17*; *pkIs296/+* animals showed neuronal degeneration after expression of activated $G\alpha_s$). A cosmid containing a putative adenylyl cyclase gene from this region was tested for rescuing activity. Cosmid F17C8 was injected at a concentration of 10 µg/ml with the *rol-6(su1006)* marker plasmid pRF4 (Kramer *et al.*, 1990) in NL586 (*sgs-1(pk310)*; *dpy-20(e1362)*; *pkIs296[gsa-1QLdpy-20(+)]*) (Mello and Fire, 1995). Animals were analyzed for neuronal degeneration after expression of activated $G\alpha_s$ as described (Korswagen *et al.*, 1997). Cosmids T20G5 and F54G8 did not rescue *sgs-1*. pRP1522, a 14 kb *SpeI/XhoI* fragment containing the adenylyl cyclase gene, was subcloned from F17C8 (Figure 1B). The GENEFINDER prediction of the *sgs-1* (and *acy-2*) coding sequence, as annotated in the *C.elegans* database ACeDB (Eeckman and Durbin, 1995), was confirmed by sequencing of random and oligo-dT primed cDNA amplified by reverse transcriptase PCR. These sequence data have been submitted by the *C.elegans* genome consortium to the DDBJ/EMBL/GenBank databases under accession No. Z35719 for SGS-1 and AF022968 for ACY-2. The open reading frame of *sgs-1* was scanned for mutations in different *sgs-1* alleles by sequence analysis of genomic DNA.

Sequence analysis of SGS-1 and ACY-2

Multiple alignments of SGS-1 and mammalian and *Drosophila* adenylyl cyclase sequences (Swiss-Prot accession Nos P51830, P26769, P26770, P51829, Q04400, Q01341, P19754, P97490 and P21932) were made using the Genetics Computer Group (version 9.1) PILEUP program (Genetics Computer Group Inc., Wisconsin). A Kyte–Doolittle hydrophobicity plot (Kyte and Doolittle, 1982) was made using the Genetics Computer Group PEPLOT program.

GFP reporter constructs

A fusion of *gfp* (Chalfie *et al.*, 1994) close to the predicted 3' end of *sgs-1* (pRP1523) was constructed by cloning an *XbaI/ApaI* fragment of pPD95.77 (A.Fire, personal communication) in unique *NheI* and *ApaI* sites of pRP1522. pRP1523 was injected at 50 µg/ml (plus 50 µg/ml pMH86 and 100 µg/ml pBluescript) in CB1362 [*dpy-20(e1362)IV*] (Han and Sternberg, 1991). The transgenic array was integrated by irradiating animals with 40 Gy of gamma radiation from a ¹³⁷Cs source (Way *et al.*, 1991). A fusion of *gfp* to *acy-2* was constructed by cloning an 8.7 kb *PstI/SpeI* fragment of C10F3 in the *PstI* and *XbaI* sites of pPD95.77 (Figure 1C). pRP1525 was injected at a concentration of 100 µg/ml with 100 µg/ml pRF4 in Bristol N2 animals. Cells were identified in reference to Sulston and Horvitz (1977) and White *et al.* (1986).

Acknowledgements

We thank Stephen Wicks and Gert Jansen for critically reading the manuscript and David Baillie for *sDf121*. The *Caenorhabditis* Genetics Center provided some of the strains used in this study. This work was supported by a Human Frontier Science Program grant to R.H.A.P. The *sgs-1::Tc1* insertion allele used in this study was isolated from a mutant library supported by a National Institute of Health–National Center for Research Resources grant 5 RO1 RR10082–02 to R.H.A.P.

References

- Anderson, P. (1995) Mutagenesis. *Methods Cell Biol.*, **45**, 31–58.
- Brundage, L., Avery, L., Katz, A., Kim, U.-J., Mendel, J.E., Sternberg, P.W. and Simon, M.I. (1996) Mutations in a *C.elegans* $G_{\alpha q}$ gene disrupt movement, egg-laying and viability. *Neuron*, **16**, 999–1009.
- Chalfie, M. and Wolinsky, E. (1990) The identification and suppression of inherited neurodegeneration in *Caenorhabditis elegans*. *Nature*, **345**, 410–416.
- Chalfie, M., Tu, Y., Euskirchen, G., Ward, W.W. and Prasher, D.C. (1994) Green fluorescent protein as a marker for gene expression. *Science*, **263**, 802–805.
- Driscoll, M. and Chalfie, M. (1991) The *mec-4* gene is a member of a family of *Caenorhabditis elegans* genes that can mutate to induce neuronal degeneration. *Nature*, **349**, 588–593.

- Eeckman, F.H. and Durbin, R. (1995) ACeDB and Macace. *Methods Cell Biol.*, **48**, 583–605.
- Forrester, W.C. and Garriga, G. (1997) Genes necessary for *C.elegans* cell and growth cone migrations. *Development*, **124**, 1831–1843.
- Hall, H.H., Gu, G., Garcia-Anoveros, J., Gong, L., Chalfie, M. and Driscoll, M. (1997) Neuropathology of degenerative cell death in *Caenorhabditis elegans*. *J. Neurosci.*, **17**, 1033–1045.
- Han, M. and Sternberg, P.W. (1991) Analysis of dominant-negative mutations of the *Caenorhabditis elegans* *let-60* ras gene. *Genes Dev.*, **5**, 2188–2198.
- Hong, K. and Driscoll, M. (1994) A transmembrane domain of the putative channel subunit MEC-4 influences mechanotransduction and neurodegeneration in *C.elegans*. *Nature*, **367**, 470–473.
- Huang, M. and Chalfie, M. (1994) Gene interactions affecting mechanosensory transduction in *Caenorhabditis elegans*. *Nature*, **367**, 467–470.
- Jansen, G., Hazendonk, E., Thijssen, K.L. and Plasterk, R.H.A. (1997) Reverse genetics by chemical mutagenesis in *Caenorhabditis elegans*. *Nature Genet.*, **17**, 119–121.
- Kandel, E. and Abel, T. (1995) Neuropeptides, adenylyl cyclase and memory storage. *Science*, **268**, 825–826.
- Kaziro, Y., Itoh, H., Kozasa, T., Nakafuku, M. and Satoh, T. (1991) Structure and function of signal transducing GTP binding proteins. *Annu. Rev. Biochem.*, **60**, 349–400.
- Korswagen, H.C., Park, J.H., Ohshima, Y. and Plasterk, R.H.A. (1997) An activating mutation in a *Caenorhabditis elegans* G_s protein induces neuronal degeneration. *Genes Dev.*, **11**, 1493–1503.
- Kramer, J.M., French, R.P., Park, E.-C. and Johnson, J.J. (1990) The *Caenorhabditis elegans* *rol-6* gene, which interacts with the *sqt-1* collagen gene to determine organismal morphology, encodes a collagen. *Mol. Cell. Biol.*, **10**, 2081–2089.
- Kyte, J. and Doolittle, R.F. (1982) A simple method for displaying the hydrophobic character of a protein. *J. Mol. Biol.*, **157**, 105–132.
- Levin, L.R., Han, P.L., Hwang, P.M., Feinstein, P.G., Davis, R.L. and Reed, R.R. (1992) The *Drosophila* learning and memory gene *rutabaga* encodes a Ca^{2+} /Calmodulin-responsive adenylyl cyclase. *Cell*, **68**, 479–489.
- Lewis, J.A. and Fleming, J.T. (1995) Basic culture methods. *Methods Cell Biol.*, **45**, 4–30.
- Liu, Y., Ruoho, A.G., Rao, V.D. and Hurley, J.H. (1997) Catalytic mechanism of the adenylyl and guanylyl cyclases: modeling and mutational analysis. *Proc. Natl Acad. Sci. USA*, **94**, 13414–13419.
- Mello, C.C. and Fire, A. (1995) DNA transformation. *Methods Cell Biol.*, **48**, 451–482.
- Mendel, J.E., Korswagen, H.C., Liu, K.S., Hajdu-Cronin, Y.M., Simon, M.I., Plasterk, R.H.A. and Sternberg, P.W. (1995) Participation of the protein G_o in multiple aspects of behavior in *C.elegans*. *Science*, **267**, 1652–1655.
- Parent, C.A. and Devreotes, P.N. (1995) Isolation of inactive and G protein-resistant adenylyl cyclase mutants using random mutagenesis. *J. Biol. Chem.*, **270**, 22693–22696.
- Park, J.-H., Ohshima, S., Tani, T. and Ohshima, Y. (1997) Structure and expression of the *gsa-1* gene encoding a G protein α (s) subunit in *C. elegans*. *Gene*, **194**, 183–190.
- Premont, R.T., Matsuoka, I., Mattei, M.G., Pouille, Y., Defer, N. and Hanoune, J. (1996) Identification and characterization of a widely expressed form of adenylyl cyclase. *J. Biol. Chem.*, **271**, 13900–13907.
- Rushforth, A.M., Saari, B. and Anderson, P. (1993) Site-selected insertion of the transposon Tc1 into a *Caenorhabditis elegans* myosin light chain gene. *Mol. Cell. Biol.*, **13**, 902–910.
- Ségalat, L., Elkes, D.A. and Kaplan, J.M. (1995) Modulation of serotonin controlled behaviors by G_o in *Caenorhabditis elegans*. *Science*, **267**, 1648–1651.
- Simon, M.I., Strathmann, M.P. and Gautam, N. (1991) Diversity of G proteins in signal transduction. *Science*, **252**, 802–808.
- Sulston, J.E. and Horvitz, H.R. (1977) Post-embryonic cell lineages of the nematode *Caenorhabditis elegans*. *Dev. Biol.*, **56**, 110–156.
- Sunahara, R.K., Dessauer, C.W. and Gilman, A.G. (1996) Complexity and diversity of mammalian adenylyl cyclases. *Annu. Rev. Pharmacol. Toxicol.*, **36**, 461–480.
- Tesmer, J.J.G., Sunahara, R.K., Gilman, A.G., Sprang, S.R. (1997) Crystal structure of the catalytic domain of adenylyl cyclase in a complex with G_{α} . *GTP γ S*. *Science*, **278**, 1907–1916.
- Treinin, M. and Chalfie, M. (1995) A mutated acetylcholine receptor subunit causes neuronal degeneration in *C. elegans*. *Neuron*, **14**, 871–877.
- Walsh, D.A. and Van Patten, S.M. (1994) Multiple pathway signal transduction by the cAMP-dependent protein kinase. *FASEB J.*, **8**, 1227–1236.
- Way, J.C., Wang, L., Run, J.Q. and Wang, A. (1991) The *mec-3* gene contains cis-acting elements mediating positive and negative regulation in cells produced by asymmetric cell division in *Caenorhabditis elegans*. *Genes Dev.*, **5**, 2199–2211.
- White, J.G., Southgate, E., Thomson, J.N. and Brenner, S. (1986) The structure of the nervous system of the nematode *Caenorhabditis elegans*. *Phil. Trans. R. Soc. Lond.*, **314**, 1–340.
- Wickman, K. and Clapham, D.E. (1995) Ion channel regulation by G proteins. *Physiol. Rev.*, **75**, 865–885.
- Wilson, R. et al. (1994) 2.2 Mb of contiguous nucleotide sequence from chromosome III of *C.elegans*. *Nature*, **368**, 32–38.
- Yan, S.Z., Huang, Z.H., Rao, V.D., Hurley, J.H. and Tang, W.J. (1997) Three discrete regions of mammalian adenylyl cyclase form a site for G_{α} activation. *J. Biol. Chem.*, **272**, 18849–18854.
- Zhang, G., Liu, Y., Ruoho, A.G. and Hurley, J.H. (1997) Structure of the adenylyl cyclase catalytic core. *Nature*, **386**, 247–253.
- Zwaal, R.R., Broeks, A., van Meurs, J., Groenen, J.T.M. and Plasterk, R.H.A. (1993) Target selected gene inactivation in *Caenorhabditis elegans* by using a frozen transposon insertion mutant bank. *Proc. Natl Acad. Sci. USA*, **90**, 7431–7435.
- Zwaal, R.R., Ahringer, J., van Luenen, H.G.A.M., Rushfort, A., Anderson, P. and Plasterk, R.H.A. (1996) G proteins are required for spatial orientation of early cell cleavage in *C.elegans* embryos. *Cell*, **86**, 1–20.

Received April 6, 1998; revised and accepted July 6, 1998

Note added in proof

Similar results on the suppression of G_{α_s} -induced neuronal degeneration by mutation of an adenylyl cyclase have recently been reported [Berger, A.J., Hart, A.C. and Kaplan, J.M. (1998) G_{α_s} -induced neurodegeneration in *Caenorhabditis elegans*. *J. Neurosci.*, **18**, 2671–2880].