Chapter 2

Functional characterization of the adenylyl cyclase gene sgs-1 by analysis of a mutational spectrum in Caenorhabditis elegans
ADENYLYL cyclases convert intracellular adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP), a major second messenger in the cell that regulates many cellular processes. Mammalian membrane-bound adenylyl cyclases consist of a short cytoplasmic N-terminal sequence, a six-transmembrane-spanning region (M1), and a cytoplasmic catalytic domain (C1), followed by a second six-transmembrane-spanning region (M2) and a second cytoplasmic catalytic domain (C2). The C1 and C2 domains together form the catalytic core of the protein. The first 200–250 amino acids of each catalytic domain (the C1a and C2a regions) are similar to each other and to the catalytic domains of other adenylyl and guanylyl cyclases. In mammals, nine different types of adenylyl cyclase genes have been reported, all of which are directly activated by the heterotrimeric G-protein α-subunit Gαs. In addition, certain adenylyl cyclases can be regulated by Go, Gβγ, calcineurin, calmodulin, protein kinase A, protein kinase C, and forskolin (reviewed in Hurley 1999; Simonds 1999; Patel et al. 2001).

We have previously reported that the Caenorhabditis elegans adenylyl cyclase SGS-1 (suppressor of activated Go) is a downstream target of the C. elegans Goα subunit in motoneurons (Korswagen et al. 1998): mutations in SGS-1 suppress the neuronal degeneration induced by a constitutively activating mutation in Goα (Berger et al. 1998; Korswagen et al. 1998). In humans, activating mutations in Goα are implicated in pituitary and thyroid malignancies (Landis et al. 1989; Lyons et al. 1990) and endocrine disorders (Shenker et al. 1993; Iiri et al. 1994). SGS-1 has significant sequence similarity to mammalian membrane-bound adenylyl cyclases and is most similar to the divergent type IX mammalian adenylyl cyclase. Human adenylyl cyclase type IX is predominantly expressed in vital organs, including brain, heart, and pancreas (Paterson et al. 2000). The sgs-1 gene is ubiquitously expressed in the nervous system and muscle cells of C. elegans (Berger et al. 1998; Korswagen et al. 1998). A second adenylyl cyclase gene in C. elegans, acy-2, shows a more restricted expression pattern (Korswagen et al. 1998). This gene is not involved in Goα-induced neuronal degeneration, but probably has an essential function together with Goα in the canal-associated neurons of C. elegans (Korswagen et al. 1998).

Over the last decade, great effort has been undertaken to understand the catalytic mechanism of adenylyl cyclase and its regulation. Most studies have focused on the function of the highly conserved catalytic domains, and several residues that are important for catalytic activity (Tang et al. 1992, 1995; Liu et al. 1997; Yan et al. 1997b; Zimmermann et al. 1998b), Goα binding (Yan et al. 1997a; Zimmermann et al. 1998a), Goα binding (Dessauer et al. 1998), forskolin stimulation (Yan et al. 1998), and ATP binding (Dessauer et al. 1997) have been determined, mainly through in vitro studies. In addition, the determination of the crystal structure of the catalytic domains of adenylyl cyclase (Tesmer et al. 1997, 1999) has provided new insight into the catalytic mechanism and the key residues for catalytic activity and binding of its regulators. The function of the two transmembrane domains of adenylyl cyclase is less well

ABSTRACT
The sgs-1 (suppressor of activated Go) gene encodes one of the four adenylyl cyclases in the nematode C. elegans and is most similar to mammalian adenylyl cyclase type IX. We isolated a complete loss-of-function mutation in sgs-1 and found it to result in animals with retarded development that arrest in variable larval stages. sgs-1 mutant animals exhibit lethargic movement and pharyngeal pumping and (while not reaching adulthood) have a mean life span that is >50% extended compared to wild type. An extensive set of reduction-of-function mutations in sgs-1 was isolated in a screen for suppressors of a neuronal degeneration phenotype induced by the expression of a constitutively active version of the heterotrimeric Gα, subunit of C. elegans. Although most of these mutations change conserved residues within the catalytic domains of sgs-1, mutations in the less-conserved transmembrane domains are also found. The sgs-1 reduction-of-function mutants are viable and have reduced locomotion rates, but do not show defects in pharyngeal pumping or life span.

Functional Characterization of the Adenylyl Cyclase Gene sgs-1 by Analysis of a Mutational Spectrum in Caenorhabditis elegans

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understood. A recent study has now revealed that these domains interact with each other persistently and that this interaction plays a critical role in the targeting and functional assembly of adenyl cyclase (Gu et al. 2001); thus, these domains are very important for the proper functioning of the protein.

In this study, we show that sgs-1 is an essential gene and that sgs-1 is involved in behaviors such as locomotion and pharyngeal pumping. To identify new residues in sgs-1 that are important for normal response to Gα, we performed a genetic screen for suppressors of the activated Gα-induced neuronal degeneration. In total, we identified 14 residues in sgs-1 that can be mutated to suppress the neuronal degeneration. Ten of these residues are located in the catalytic domains of sgs-1, but four mutations in the transmembrane domains were also found. Although none of the mutations are located in the proposed active site of the protein, we show here that catalytic activity of sgs-1 is required for Gα-induced neuronal degeneration.

MATERIALS AND METHODS

Nematode strains and culturing: All strains were maintained as described by Lewis and Fleming (1995). DNA manipulations by microinjection in C. elegans were as described by Mello and Fire (1995). Strains used in this study were Bristol N2, CB1282 (dpy-20(e1282) IV), NL545 (dpy-20(e1362) IV; phks296[hsp::gosa-1QL dpy-20(+)] X) (Korswagen et al. 1997), NL585 (sgs-1(pk310) III, dpy-20(e1362) IV; phks296[hsp::gosa-1QL dpy-20(+)] X) (Korswagen et al. 1998), NL586 (sgs-1(pk310) III, dpy-20(e1362) IV; phks296[hsp::gosa-1QL dpy-20(+)] X) (Korswagen et al. 1998), NL597 (sgs-1(pk384) III, dpy-20(e1362) IV; phks296[hsp::gosa-1QL dpy-20(+)] X) (Korswagen et al. 1998), NL1250 (mut-2(e549) IV; sgs-1(pk450; Tc1) III) (Korswagen et al. 1998), NL3200 (sgs-1(pk1279)/+ III; dpy-20(e1282) IV), and NL3224 (sgs-1(pk1279) dpy-20(e1362) IV; sgs-1(+); dpy-20(e164) III, phks296[hsp::gosa-1QL dpy-20(+) X], sgs-1 alleles phk884, phk887, phk862, phk866, phk871, phk875, phk880, phk884, phk886, phk904, phk907, and phk927 were generated in an N545 background in this study.

Isolation of an sgs-1 knockout animal: We used the outer primers delsgs1-9 GGGGGAAGGTGAAAAATGA and delsgs1-10 TGCAATGCTTCTAACGTC and the nested primers delsgs1-11 CACGGGAAGGGTGAGGACT and delsgs1-12 CGTGTTTTCTGCTGGCAACT, spanning a genomic region of 4.1 kb, to screen for deletion derivatives of phk505; Tc1 (Zwaal et al. 1993), a transposon insertion in the ninth exon of sgs-1 (Korswagen et al. 1998). A 2.9-kb deletion derivative of phk505; Tc1, sgs-1(pk1279), was isolated and could not be maintained as a homozygote. The sgs-1(pk1279) PCR product was isolated from gel and sequenced to determine the deletion end points. The following sequence in capitals was found around the deletion site: (5’T-GAGGTATataacag-gtga-gcactATCtTtTA-3’). The pk1279 allele was backcrossed four times to an N2 background and then crossed with CB164 (dpy-17(e164) III), resulting in strain NL1999 sgs-1(pk1279)/dpy-17(e164). One-fourth of the progeny of NL1999 sg-1(pk1279)/dpy-17(e164) arrested in larval development, and we confirmed by PCR that these are homozygous for sgs-1(pk1279) (data not shown). The homozygous pk1279 phenotype was rescued with a wild-type sgs-1 genomic construct, pRP1522 (Korswagen et al. 1998). This construct was injected at a concentration of 50 μg/ml together with 100 μg/ml PMH86 (Han and Sternberg 1991) in CB1282, and the transgenic array was crossed in NL3200 (sgs-1(pk1279)/+ III; dpy-20(e1282) IV). A rescuing strain, NL3212 (sgs-1(pk1279) III; dpy-20(e1282) IV; pkEx1288[sgs-1(+); dpy-20(+)]), which does not segregate viable Dpy-20 worms, was obtained. Single worm PCR confirmed homozygosity for the pk1279 allele (data not shown).

Isolation of suppressors of the activated Gα, induced neuronal degeneration: The screen that was used to identify suppressors of the activated Gα phenotype was a modified version of an earlier described screen (Korswagen et al. 1998). L4 NL545 animals were treated with 50 nm EMS in M9 buffer for 4 hr, washed, and seeded at 40–60 animals per 9-cm nematode growth medium (NGM) agar plate. The F1 generation was synchronized using NaOCl bleaching, and L1 larvae were heat-shocked for 2 hr at 35° to induce expression of pkh296. The animals that grew out to adults were again synchronized using NaOCl bleaching, and L1 larvae were heat-shocked for 2 hr at 33° to induce expression of pkh296. Animals that did not show neuronal degeneration were collected. sgs-1 alleles were identified by complementation testing.

Activated Gα, induced neuronal degeneration: The activated Gα-induced pattern of neuronal cell death was measured as described in Korswagen et al. (1997). Synchronized L1-L2 larvae were heat-shocked for 2 hr at 35° and after 24 hr the neuronal degeneration pattern was examined in at least 20 animals using a high-powered dissection microscope (Wild M3C). Animals that did not show 100% suppression of the neuronal degeneration were further examined using a Nomarski microscope (Zeiss Axioskop 2): the total number of degenerated neurons was determined in 20 animals. The percentage suppression was calculated by comparing this number with the total number of degenerated neurons in NL545 (dpy-20(e1362) IV; pkh296[hsp::gosa-1QL dpy-20(+)] X) (100% degeneration) and NL586 (sgs-1(pk310) III; dpy-20(e1362) IV; pkh296[hsp::gosa-1QL dpy-20(+)] X) (100% suppression). Statistics were calculated using directed Student’s t-tests.

sgs-1(pk1279) trans experiments: NL1999 sgs-1(pk1279)/ dpy-17(e164) males were crossed with Bristol N2 hermaphrodites and with homozygous sgs-1 mutants. 40 L1 progeny were picked and their growth was followed. After 1-week of growth at 20°, we checked for the presence of the sgs-1(pk1279) allele by PCR.

Construction and expression of a catalytically inactive sgs-1 mutant: SGS-1 R1187 was constructed by PCR using primers R1187-1 TGGTACGCAATAAGCTAGCAGC and R1187-2 GCTG TACATTGCGTACAGCG containing the mutation. The PCR fragment was cloned into a 15.6-kb NheI-BspEI fragment of pRP1322 (Korswagen et al. 1998), giving rise to SGS-1 R1187A. The cloned PCR fragment was sequenced completely and found to be free of errors. SGS-1 R1187A was injected at the same concentration as the wild-type SGS-1 construct pRP1322 (50 μg/ml; Korswagen et al. 1998) together with the markers pRF6 (100 μg/ml; Kramer et al. 1990) and pRP2017 (gkh-2-gkh, 50 μg/ml; Van der Linden et al. 2001) in N2 animals. Three independent SGS-1 R1187 transgenic lines and two independent wild-type SGS-1 transgenic lines were crossed in NL3224 and examined for their rescue of the larval lethality and suppression of the neuronal degeneration.

Behavioral assays: Life span was determined by transferring L1-L2 larvae individually to plates 24 hr after synchronization using NaOCl bleaching. The animals were incubated at 20° and monitored once daily until death. Day of synchronization was used as the first time point. The animals were transferred to fresh plates once in 2 days while producing eggs to keep them separate from their progeny. Animals were scored as dead when they stopped moving and no longer responded to
**sgs-1** is an essential gene

![Phenotypes of *sgs-1(pk1279)* animals.](image)

Figure 1.—Phenotypes of *sgs-1(pk1279)* animals. (A) Genomic representation of the *sgs-1* gene showing the deleted region in *pk1279*. **GENEFINDER** prediction of the *sgs-1* coding sequence was confirmed by sequencing of random and oligo(dT) primed cDNA amplified by reverse transcriptase PCR (Korswagen et al. 1998). (B) The development of the *sgs-1(pk1279)* animal (arrowhead) is compared with similarly aged Bristol N2 animal. (C) Movement levels of N2, *sgs-1(pk1279)* animals and *sgs-1(pk1279)* pkEx1228 [*sgs-1*] animals were assayed 96 hr after synchronization. Each bar represents the average of 10 animals. The error bars represent the standard error of the mean (SEM). Differences between N2 and *sgs-1(pk1279)* were analyzed using Student’s *t*-test. *sgs-1(pk1279)* animals are significantly different from movement when compared with N2 animals (*P < 0.001*). (D) Pharyngeal pumping levels of N2, *sgs-1(pk1279)* animals and *sgs-1(pk1279)* pkEx1288 [*sgs-1*] animals were assayed 96 hr after synchronization. Each bar represents the average of 20 determinations. The error bars represent the SEM. Differences between N2 and *sgs-1(pk1279)* were analyzed using Student’s *t*-test. *sgs-1(pk1279)* animals are significantly different with respect to pharyngeal pumping when compared with N2 animals (*P < 0.001*). (E) In many *sgs-1(pk1279)* animals, a cuticular plug is attached to the mouth opening. (F) The percentage of worms alive on a given day after synchronization: N2, *sgs-1(pk1279)* animals and *sgs-1(pk1279)* pkEx1288 [*sgs-1*] animals. Mean life spans ± SEM, with sample size in parentheses, are 18.0 ± 0.8 (56), 27.9 ± 2.8 (32), and 19.0 ± 1.0 (27), respectively. Significance levels were calculated using Student’s *t*-test. *sgs-1(pk1279)* lived significantly longer than the wild-type strain (*P < 0.001*), whereas there was no significant difference between N2 and *sgs-1(pk1279)* pkEx1288 [*sgs-1*] animals (*P = 0.42*).

RESULTS

**sgs-1** is essential for viability: A transposon-based method (Zwaal et al. 1993) was used to isolate a deletion allele of *sgs-1*, *sgs-1(pk1279)*. As shown in Figure 1A, 2.9 kb of *sgs-1* sequence is deleted in this allele, thereby removing exon 8 up to exon 13. Nearly one-half of the *sgs-1* coding sequence is removed, including the complete second transmembrane and second catalytic domain. Therefore, it is highly likely that *pk1279* is a null allele. Animals homozygous for *pk1279* undergo severely retarded development (Figure 1B). All animals passed the first larval stage, but arrested and died in one of the later larval stages. The length of embryogenesis in *pk1279* homozygotes was comparable to Bristol N2 animals, and *pk1279* homozygotes were morphologically normal at hatching (results not shown). The *pk1279* homozygotes showed reduced body-wall muscle activity (Figure 1C) and pharyngeal muscle activity (Figure 1D). We also observed difficulties with molting (Figure 1E). In spite of these developmental abnormalities, *pk1279* animals had an extended life span; they lived >50% longer than the wild type (Figure 1F).

Rescue of the larval lethality was obtained with an extrachromosomal array containing pRP1522, a 14-kb subclone containing the entire *sgs-1* gene (Korswagen et al. 1998). The rescued *sgs-1(pk1279)* transgenic ani-
mals were used to address whether maternally provided sgs-1 mRNA or protein is needed for development. Ex- trachromosomal arrays are unstable and can be lost during meiosis and mitosis (Stinchcomb et al. 1985; Mel-lo and Fire 1995). This results in mosaic expression of the transgene as it is lost in certain cells and cell lineages. Rescued sgs-1(pk1279) transgenic animals that lost the rescuing transgene in the germ line will have progeny that lack both sgs-1 transgene expression and sgs-1 expression in the form of maternal inheritance of sgs-1 mRNA or protein (Zwaal et al. 1996). Of the rescued pk1279 animals, 1–2% segregated only arrested larvae that were identical to the arrested larvae segregated by heterozygous pk1279 animals. We did not ob- serve any brood consisting of animals with a phenotype more severe than that of the arrested larvae. This result suggests that zygotic sgs-1 performs an essential function during development and that the ability to develop into larva is not the result of the presence of maternal sgs-1 mRNA or protein.

sgs-1 mutants are suppressors of activated Goα-induced neuronal degeneration: sgs-1 mutants were initially se- lected as suppressors of activated Goα-induced neuronal degeneration (Berger et al. 1998; Korswana- gen et al. 1998). The neuronal degeneration phenotype is already visible in the first larval stage, and it is therefore possible to test the sgs-1 knockout animals for suppression of this neuronal degeneration phenotype. Animals homo- zygous for the null allele pk1279 that express the ac- tivated Goα from a heat-shock promoter did not show neuronal degeneration. Thus, sgs-1(pk1279) is a suppressor of the activated Goα-induced neuronal degeneration (Table 1). In screens for suppressors of the activated Goα phenotype, we have now obtained a set of 23 sgs-1 alleles (Table 1), of which 7 were described earlier (Korswana-gen et al. 1998). The sgs-1 alleles were isolated with a frequency of ~1 in 50,000 mutagenized genomes; they are all homozygous viable and recessive for the suppression of the activated Goα phenotype. We con- clude from the viability of these sgs-1 alleles that they are not complete loss-of-function alleles, but partial re- duction-of-function alleles. All 23 sgs-1 alleles were se- quenced and mutations were found in all alleles, except allele pk310, which was not studied further (Table 1). In three independent alleles (pk311, pk474, and pk871), an identical splice donor site mutation in the first cata- lytic domain was found. One allele (pk862) contained a mutation 39 bp upstream of the ATG. In 18 indepen- dent alleles, mutations were found that change 14 dis- tinct (mostly conserved) amino acids. Most mutations that were found to suppress the activated Goα phenotype were located in one of the conserved regions of the catalytic domains of sgs-1, suggesting that catalytic activity of SGS-1 is needed for the activated Goα phenotype. The mutations that did not map in the conserved re- gions of the catalytic domains were located in the first transmembrane domain (pk393, pk884, pk363, pk863, and pk866) or at the border of the second transmem- brane domain and the nonconserved region of the sec- ond catalytic domain (pk907).

SGS-1 mutations are not located in the active site: The availability of the crystal structure of the catalytic domains of adenylyl cyclase (Tesmer et al. 1997, 1999) has made it possible to map the mutations in the cata- lytic domains of sgs-1 to the crystal structure. This analy- sis revealed that none of the 10 residues mutated in the catalytic domains of sgs-1 was located in the proposed active site of the protein (Figure 2). Two of the mutated amino acids (G1068 and R1074) were located at the interaction interface with Goα. It has been shown for mammalian adenylyl cyclases that mutations in the amino acid analogous to R1074 result in decreased bind- ing to Goα, with a consequent decrease in activation (Yan et al. 1997a; Zimmermann et al. 1998a). This suggests that in the mutants G1068R and R1074K suppression of the neuronal degeneration is the result of decreased bind- ing to Goα. The remaining eight mutated amino acids were distributed over the structure and were changed to larger residues (L314F, L347F, A374V, H455Y, and S457F) or to residues that no longer make contact with the backbone or side chain of other amino acids (M411I, S1109N, and R1216H). Biochemical analysis of mamma- lian adenylyl cyclase type II with a mutation analogous to R1216H has shown that mutation of this amino acid does not result in gross structural alterations (Dessauer et al. 1997). This suggests that R1216H has a specific effect on adenylyl cyclase activity or regulation.

Catalytic activity of sgs-1 is necessary for induction of neuronal degeneration: Induction of activated Goα in an sgs-1 null background did not result in neuronal degeneration, showing that sgs-1 is essential for this pro- cess. Indeed, overexpression of the wild-type sgs-1 allele in sgs-1(pk1279) animals both rescued the lethal pheno- type and restored the neuronal degeneration pheno- type upon induction of activated Goα (Figure 3A). Since none of the sgs-1 reduction-of-function mutations di- rectly affected the active site of the SGS-1 protein, we asked whether catalytic activity of SOS-1 is required for the activated Goα-induced neuronal degeneration. We con- structed a point mutation in the active site of SGS-1 (SGS-1 R1187A). R1187 is the residue analogous to R1029 of mammalian type II adenylyl cyclase. R1029 of type II adenylyl cyclase is an important residue for catalysis, and it was shown that changing this residue into an alanine reduces catalysis by 30-fold, but does not cause gross structural alterations (Yan et al. 1997b). To our surprise, overexpression of the SGS-1 R1187 mutant into sgs-1(pk1279) animals rescued the lethality of these animals, most probably due to residual catalytic activity (Yan et al. 1997b). However, we found that this residual activity was not sufficient for the induction of neuronal degeneration, as these animals looked com- pletely wild type upon induction of activated Goα expres- sion (Figure 3B). We conclude from this result that a
Neuronal degeneration induced by activated Go, under heat-shock promoter control as described in MATERIALS AND METHODS. Phenotype was scored in at least 20 L1-L2 larvae for each genotype 24 hr after a 2-hr heat shock at 33°C. Mean total numbers of degenerated neurons ±SEM, with sample size in parentheses, are 23.2 ± 0.3 (60) for NL545 pkIs296[hsp::gsa-1QL]; 0.15 ± 0.1 (20) for sgis-1(pk1279); 21.1 ± 0.4 (20) for sgis-1(pk1279)/+; 1.9 ± 0.4 (20) for sgis-1(pk384); 3.3 ± 0.5 (40) for sgis-1(pk867); and 6.5 ± 0.6 (60) for sgis-1(pk884). The mean total number of degenerated neurons was not determined for the other sgis-1 alleles, but examination using a high-powered dissection microscope showed a level of neuronal degeneration comparable to sgis-1(pk310). Differences between genotypes were calculated using Student’s t-test. There were significant differences between sgis-1(pk310) and sgis-1(pk384) or sgis-1(pk867) or sgis-1(pk884) (for all P < 0.001). There was also a significant difference between NL545 and sgis-1(pk1279)/+ (P < 0.004). The growth in trans to pk1279 was determined as described in MATERIALS AND METHODS. +, none of the animals tested has a growth delay; +/−, ~80% of the animals have a growth delay, but all eventually become adults; −, ~100% of the animals have a growth delay, and ~50% are still in larval stage after 1 week of growth at 20°C; −−, all animals have a growth delay, and none become adults. ND, not determined; C1b, nonconserved part of first catalytic domain.

**sgis-1 alleles form an allelic series:** Most sgis-1 alleles, including the null allele pk1279, showed 100% suppression of the activated Go-induced neuronal degeneration. However, three alleles, pk384 (G1068R), pk867 (M411I), and pk884 (P152S), showed <100% suppression: a few neurons were degenerated in most animals (Table 1). This suggests that the SGS-1 protein is less disturbed in these alleles than in the other alleles. These alleles had mutations in different domains of the SGS-1 protein, and thus there was no correlation between the activity of the mutant proteins and the position of their mutations. Animals heterozygous for pk1279 suppressed the activated Go-induced neuronal degeneration weakly: they showed a slight reduction in the number of degenerated neurons induced by activated Go expression compared to homozygous sgis-1(+) animals (Table 1). A possible explanation for this observation is that in these animals, sgis-1 activity is reduced due to the

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**TABLE 1**

Properties of the sgis-1 mutants

Mutation in the active site suppresses the activated Go-induced neuronal degeneration and that catalytic activity of sgis-1 is required for the neuronal degeneration phenotype. Probably, mutations in the active site are not found in our screen for suppressors of the neuronal degeneration because these mutations are lethal. However, we cannot exclude other explanations for the fact that we have not found these mutations in our screen.

sgis-1 alleles were identified in a previous study (Korswagen et al. 1998).
presence of only one functional copy and that in a small percentage of neurons the sgs-1 activity is reduced to a level that is too low to induce neuronal degeneration.

We were also able to detect variation in SGS-1 activity among the different sgs-1 alleles when we placed all alleles in trans to pk1279 (Table 1). Most heterozygous animals grew normally. However, animals that had allele pk301 (S457F), pk484 (S1109N), or pk907 (E992K) in trans to pk1279 did not show normal growth: their phenotype was an intermediate form between wild-type and homozygous pk1279 animals. These results suggest that the SGS-1 protein was more affected in pk301, pk484, and pk907 than in the other alleles. The growth defect of pk484/ pk1279 and pk907/ pk1279 animals is more severe than the growth defect in pk301/ pk1279 animals, indicating that pk484 and pk907 are the most severe alleles of sgs-1. Both these alleles have mutations in the part of sgs-1 that is deleted in sgs-1(pk1279). We cannot exclude that certain alleles with a mutation in the part of sgs-1 that is not deleted in sgs-1(pk1279) experience intragenic complementation when placed in trans to sgs-1(pk1279) and therefore have a less severe phenotype than pk484 and pk907. When we placed sgs-1(pk484) (S1109N), which has a point mutation in the second catalytic domain, in trans to sgs-1(pk301) (S457F), sgs-1(pk487) (L514F), sgs-1(pk687) (M411L), or sgs-1(pk880) (A374V), which are all mutated in the first catalytic domain, we did not observe an enhancement of the neuronal degeneration phenotype after expression of the activated Gα, from heat shock. This indicates that there is no intragenic complementation between the different sgs-1 reduction-of-function mutants.

sgs-1 reduction-of-function mutants have a normal life span and pharyngeal pumping rates, but reduced locomotion rates: Observation of the sgs-1(pk1279) allele suggests that SGS-1 is involved in locomotion and pharyngeal pumping and life span determination. We measured these three phenotypes in two additional sgs-1 reduction-of-function alleles, one strong sgs-1 allele (pk484) and one weak sgs-1 allele (pk867). We found that there was no difference compared to the background strain for either life span (Figure 4A) or pharyngeal pumping (Figure 4B). This indicates that in these mutants the remaining SGS-1 activity is sufficient for normal function in these processes. Since sgs-1(pk484) is the strongest sgs-1 reduction-of-function allele, it is not likely that any of the other sgs-1 reduction-of-function alleles will show effects on either life span or pharyngeal pumping. The locomotion rate, however, was reduced in the two sgs-1 reduction-of-function mutants tested (Figure 4C), indicating that in these sgs-1 reduction-of-function mutants, SGS-1 activity is not sufficient for wild-type locomotion. The locomotion rate of sgs-1(pk484) was significantly less than the locomotion rate of sgs-1(pk867). This confirms that the allelic series showed that sgs-1(pk484) is a more severe allele than sgs-1(pk867). Since sgs-1(pk867) is one of the weakest alleles we have, probably all sgs-1 reduction-of-function alleles will show reduced locomotion rates. Indeed, sgs-1(pk904) (R1074K) animals also showed significantly reduced locomotion rates compared to the background strain (results not shown). In mammalian adenylyl cyclases, mutation of the amino acid analogous to R1074 was shown to result in de-
creased binding of Goα, but in normal regulation by Goα or the adenyl cyclase activator forskolin (YAN et al. 1997a; ZIMMERMANN et al. 1998a). This suggests that the reduced locomotion of sgs-1(pk904) animals is the result of decreased response to Goα, and thus that Goα functions together with SGS-1 in locomotion. We did not observe any other obvious defects in development or behavior in the sgs-1 reduction-of-function alleles.

**DISCUSSION**

*sgs-1 is an essential gene:* Adenyl cyclases have complex functions and integrate and respond to diverse extracellular and intracellular signals. Genetic and biochemical evidence indicates that both catalytic domains (C1 and C2) are essential for high enzymatic activity (TANG and GILMAN 1995). In this study, we have isolated a deletion allele of an adenyl cyclase of *C. elegans*, sgs-1(pk1279), in which part of the nonconserved first catalytic domain (C1b) and the complete second transmembrane (M2) and second catalytic domain (C2) of the SGS-1 protein are removed. Therefore, pk1279 is likely to be a null allele. Deletion of sgs-1 results in retarded development and eventually in larval lethality. This larval lethal phenotype is different from the larval lethal phenotype of knockout animals for gsa-1 (KORSWAGEN et al. 1997), the heterotrimeric Goα, subunit that acts directly upstream of SGS-1 (KORSWAGEN et al. 1998). This suggests that SGS-1 is not the only downstream target of Goα. Presumably, ACY-2, a second adenyl cyclase protein in *C. elegans* that shows a more limited expression pattern than sgs-1, is one of the other targets of Goα (KORSWAGEN et al. 1998), since acy-2 null mutant animals have a larval lethal phenotype similar to gsa-1 null mutants. Two additional genes encode adenyl cyclases in the *C. elegans* genome, acy-3 (located on chromosome V, our unpublished observation) and acy-4 (located on chromosome III, C44F1.5; *C. ELEGANS SEQUENCING CONSORTIUM* 1998). Little is known about the role played by these molecules in Goα signaling.

*sgs-1 is required for pharyngeal pumping and affects life span:* Pharyngeal pumping is disturbed in homozygous sgs-1(pk1279) animals, indicating that SGS-1 is required for proper functioning of the pharynx. SGS-1 is a downstream target of Goα, but it is not known whether

**Figure 4**—Phenotypes of sgs-1 reduction-of-function animals, sgs-1(pk484) and sgs-1(pk867) were backcrossed two times to NL545 (dpy-20(e1362) IV; phk296[het-gsa-1QL] dpy-20 (+)]X), the background strain in which these alleles were generated. (A) The percentage of worms alive on a given day after synchronization: (●) NL545, (□) sgs-1(pk484) animals, and (X) sgs-1(pk867) animals. Mean life spans ± SEM, with sample size in parentheses, are 17.1 ± 0.7 (29), 17.0 ± 0.6 (32), and 16.0 ± 0.8 (29), respectively. Significance levels were calculated using Student’s t-test. There was no significant difference between NL545 and sgs-1(pk484) (P = 0.91) or sgs-1(pk867) (P = 0.14). (B) Pharyngeal pumping levels of (■) NL545, (□) sgs-1(pk484) animals, and (□) sgs-1(pk867) animals were assayed 96 hr after synchronization. Each bar represents the average of 20 determinations. The error bars represent the SEM. Significance levels were calculated using Student’s t-test. There was no significant difference between NL545 and sgs-1(pk484) (P = 0.076) or sgs-1(pk867) (P = 0.80). (C) Movement levels of (■) NL545, (□) sgs-1(pk484) animals, and (□) sgs-1(pk867) animals were assayed 96 hr after synchronization. Each bar represents the average of 10 animals. The error bars represent the SEM. Differences between the different genotypes for the movement levels were analyzed using Student’s t-test. NL545 animals are significantly different with respect to movement levels when compared to sgs-1(pk484) (P < 0.001) or sgs-1(pk867) (P = 0.048). In addition, sgs-1(pk484) animals are significantly different with respect to movement when compared to sgs-1(pk867) (P < 0.001).
Go, is also involved in pharyngeal pumping. It is therefore possible that in the pharynx, SGS-1 is regulated by a protein other than Go. In several homozygous sgs-1 (pk1279) animals the molting process was not completed, since a cuticular plug remains attached to the mouth opening. The pharynx plays an important role in the molting process (Singh and Sulston 1978), suggesting that the pharyngeal defects seen in sgs-1 deletion animals account for the incomplete molting. sgs-1 reduction-of-function mutants do not have pharyngeal defects, and we do not observe difficulties with molting in these mutants.

We observed that loss of sgs-1 significantly lengthens life span: sgs-1(pk1279) mutants live >50% longer than wild-type animals. Lakowski and co-workers showed that eat mutants, which have defects in pharyngeal function, have significantly increased mean and maximum life span because of food restriction (Lakowski and Hekimi 1998). Furthermore, it has been shown that signals from the reproductive system influence the life span of an animal (Hsin and Kenyon 1999). sgs-1 mutants have both pharyngeal defects and a defective reproductive system, and we therefore suggest that the extended life span is the result of these phenotypes. This is further supported by the fact that sgs-1 reduction-of-function mutants do not show pharyngeal or reproductive system defects and that their life span is comparable with the background strain. However, it is also possible that sgs-1 has a direct effect on life span.

SGS-1 and Go, function together in locomotion: Recent studies have shown that two G-protein α-subunit genes in C. elegans, goa-1 (Goa) and egl-30 (Go), are involved in a complex signaling network that regulates locomotion (Hajdu-Chronin et al. 1999; Lackner et al. 1999; Miller et al. 1999; Nurrish et al. 1999; Chase et al. 2001; Robatzek et al. 2001; Van der Linden et al. 2001). Loss of function of goa-1 results in increased locomotion rates (Mendel et al. 1995; Segalat et al. 1995), whereas reduction of function of egl-30 results in decreased locomotion rates (Brundage et al. 1996). These G-protein pathways regulate diacetylglcerol levels in an antagonistic manner (Lackner et al. 1999; Miller et al. 1999), either stimulating or inhibiting acetylcholine release through the diacylglycerol-binding protein, UNC-13. Our results show that there is yet another G-protein signaling cascade involved in locomotion: Go, signals to the adenylyl cyclase SGS-1 to regulate locomotion. The GOA-1-GL-30 network is shown to act in ventral nerve cord motoneurons (Lackner et al. 1999; Miller et al. 1999). sgs-1 and gsa-1 (Go) are both expressed in ventral nerve cord motor neurons and in body-wall muscle cells. It is therefore possible that the Goα–SGS-1 pathway acts in parallel to the GOA–EGL-30 network in motor neurons, but it is also possible that the Goα–SGS-1 pathway acts downstream of the GOA–EGL-30 network in the body-wall muscles. In the latter hypothesis, acetylcholine release at the neuromuscular junction regulated by EGL-30 and GOA-1 possibly results in regulation of Go in muscle cells.

sgs-1 reduction-of-function mutants reveal important residues in the adenylyl cyclase gene: Several studies have been performed to identify residues that are involved in the catalytic mechanism of adenylyl cyclase and its regulation. However, none of these studies was performed in a complex organism, and in only one study, using the Dictyostelium discoideum adenylyl cyclase ACA, were mutations in the transmembrane domains described (Parent and Devreotes 1995). Here, we have used a genetic selection system in a complex organism, C. elegans, that identified mutations in both the catalytic and transmembrane domains of the adenylyl cyclase gene. sgs-1 is an essential gene, and therefore only mutations that do not completely disrupt sgs-1 function were isolated. Out of the 14 residues that were mutated, 10 residues were located in the catalytic domains. All, except L314, are conserved residues between different adenylyl cyclases. Only 2 of the conserved residues, R1074 and R1216H, were described in other studies, and there it was shown by biochemical analysis that mutation of these residues does not result in gross structural alterations (Dessauer et al. 1997; Yan et al. 1997a; Zimmermann et al. 1998a). Further studies should reveal whether, in the other sgs-1 reduction-of-function alleles, the reduced sgs-1 activity is the result of a defect in a specific function of the protein or the result of improper folding or instability of the protein.

Four mutations revealed residues that are important for a normal response to Go, but that are not located in one of the catalytic domains. Three of these residues (G68, P152, and G181) are located in the first transmembrane domain, and one (E992) is located at the border of the second transmembrane domain and the nonconserved part of the second catalytic domain. Recently, it was shown that the transmembrane domains interact persistently and are involved in two important processes, namely, the proper localization of the protein in the membrane and functional assembly of the two catalytic domains (Gu et al. 2001). It is possible that our transmembrane mutants are disturbed in the localization of the adenylyl cyclase gene, as was shown for the Dictyostelium ACA mutants in the transmembrane domain (Parent and Devreotes 1995), but it is also possible that in these mutants the two catalytic domains are not correctly assembled and therefore result in reduced adenylyl cyclase activity. Further studies should reveal, for each of these transmembrane mutants, which of these two possibilities is correct. In summary, our study shows not only that C. elegans can be used to study gene function, but also that it can be a powerful tool to study protein domain function.

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