Deletion of smn-1, the Caenorhabditis elegans ortholog of the spinal muscular atrophy gene, results in locomotor dysfunction and reduced lifespan

Michael Briese1,†, Behrooz Esmaeili1,†, Sandrine Fraboulet1,2, Emma C. Burt1, Stefanos Christodoulou1, Paula R. Towers1, Kay E. Davies1 and David B. Sattelle1,*

1MRC Functional Genomics Unit, Department of Physiology Anatomy and Genetics, University of Oxford, South Parks Road, Oxford OX1 3QX, UK and 2Institut des Neurosciences Grenoble, Centre de recherche Inserm U836-UJF-CEA-CHU, Université Joseph Fourier, Bâtiment Edmond J. Safra, Domaine de la Merci, 38706 La Tronche Cedex, France

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Spinal muscular atrophy is the most common genetic cause of infant mortality and is characterized by degeneration of lower motor neurons leading to muscle wasting. The causative gene has been identified as survival motor neuron (SMN). The invertebrate model organism Caenorhabditis elegans contains smn-1, the ortholog of human SMN. Caenorhabditis elegans smn-1 is expressed in various tissues including the nervous system and body wall muscle, and knockdown of smn-1 by RNA interference is embryonic lethal. Here we show that the smn-1(ok355) deletion, which removes most of smn-1 including the translation start site, produces a pleiotropic phenotype including late larval arrest, reduced lifespan, sterility as well as impaired locomotion and pharyngeal activity. Mutant nematodes develop to late larval stages due to maternal contribution of the smn-1 gene product that allows to study SMN-1 functions beyond embryogenesis. Neuronal, but not muscle-directed, expression of smn-1 partially rescues the smn-1(ok355) phenotype. Thus, the deletion mutant smn-1(ok355) provides a useful platform for functional analysis of an invertebrate ortholog of the human SMN protein.

INTRODUCTION

Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder, mostly of childhood-onset, characterized by degeneration of lower motor neurons in the anterior horn of the spinal cord leading to proximal muscle wasting. It is the most common genetic cause of infant mortality with an incidence of around 1 in 10,000 live births (1,2). Childhood-onset SMA cases show deletions in the survival motor neuron (SMN) gene located as a telomeric copy (SMN1) and a centromeric copy (SMN2) on chromosome 5 in an inverted duplicated region (3). Owing to a single C-to-T transition in exon 7, the majority of transcripts originating from SMN2 are alternatively spliced lacking exon 7, and give rise to a truncated, unstable protein product (4,5). Thus, SMN1, the SMA-determining gene, produces full-length SMN protein almost exclusively, whereas SMN2 acts as a disease modifier gene due to the small amount of functional SMN it produces (6,7). Even though the genetic defect underlying SMA has been shown to reside in the SMN gene, important questions concerning the disease etiology remain unresolved. SMN is expressed widely both within and outside the nervous system (3). Thus, the specific motor neuron defect seen in SMA cannot be explained purely in terms of the expression pattern of SMN but instead may relate to its function (8). Animal models of SMA have been indispensable for the study of physiologic roles of SMN in muscle cells and neurons as well as for the dissection of pathological events
leading to SMA. However, since all model organisms deployed so far possess only one ortholog of the SMN1 gene, progress has been limited by the lethality resulting from complete loss of SMN. To circumvent this difficulty, various strategies have been developed. Expression of human SMN2 in Smn−/− null mice rescues their embryonic lethality and generates animals with a movement defect and motor neuron loss resembling severe SMA (9). In zebrafish, the reduction of Smn levels using antisense morpholinos produces motor axon defects such as truncations and aberrant branching (10). A Drosophila mutant containing a mis-sense mutation in the fly smn gene survives until late larval stages due to maternally contributed smn mRNA (11). This mutant exhibits reduced excitatory postsynaptic currents and structural defects at neuromuscular junctions in the absence of motor neuron loss.

A C. elegans ortholog of the human SMN1 gene has been identified and termed smn-1 (12). A green fluorescent protein (GFP) reporter construct contains full-length smn-1, and its upstream sequence produces fluorescence in various tissues including the nervous system and body wall muscles, demonstrating widespread expression of smn-1. Additionally, the SMN-1 protein was detected by immunofluorescence from the zygotic stage onwards, indicating a maternal contribution of the protein or its mRNA. So far, the functions of C. elegans smn-1 have been studied in vivo by RNA interference (RNAi) induced by injection of double-stranded RNA that produces knockdown effects in all tissues, including maternal transcripts, although silencing of neuronal genes is less efficient (13). Targeting smn-1 with RNAi gives rise to severe developmental defects and embryonic lethality, thus limiting further investigations on postembryonic roles of SMN-1 (12). Here we have characterized the smn-1(ok355) deletion allele, which removes most of smn-1 including the translation start codon, resulting in late larval arrest, a dramatic decrease in lifespan as well as impaired locomotion and pharyngeal pumping. Early larval development of smn-1(ok355) mutant animals appears normal due to maternally contributed SMN-1. As it models aspects of SMA, this smn-1 mutant lends itself to the functional analysis of an SMN ortholog and has potential future utilities in screens searching for phenotypic modifiers as a first step toward potential SMA therapies.

RESULTS

The smn-1(ok355) deletion

Like all model organisms studied to date, C. elegans has only a single copy of the gene encoding the survival motor neuron protein. The smn-1(ok355) allele described here removes 975 bp comprising 246 bp of the upstream intergenic region and most of the smn-1 gene leaving only 87 bp (including the stop codon) at the 3′ end (part of exon 5; Fig. 1). The coding sequences of the adjacent genes klp-16 and F30A10.10 are unaffected by the smn-1(ok355) deletion. Absence of the smn-1 ATG start codon in the remaining sequence suggests that smn-1(ok355) is a null allele and hence the only SMN-1 present is maternal, originating from balanced smn-1(ok355)/hT2[qIs48] heterozygous mothers (strain LM99).

smn-1(ok355) mutants show defects in motility and pharyngeal pumping

Following larval arrest, smn-1(ok355) nematodes display a progressive decline in motility. To measure the locomotory activity we quantified the thrashing rate of animals in M9 buffer. During early development, i.e. before arrest, the thrashing rate of smn-1(ok355) homozygotes was similar to balanced smn-1(ok355)/hT2[qIs48] heterozygotes and N2 wild-types (Fig. 5). Thrashing of smn-1(ok355) animals then progressively declined and almost completely ceased after 5 days post-L1. In contrast, locomotion of smn-1(ok355)/hT2[qIs48] heterozygous animals was similar to N2 wild-types at all time-points throughout the assay.

Likewise, smn-1(ok355) mutants are unable to feed due to loss of pharyngeal function. The rate of pharyngeal pumping was measured in smn-1(ok355) homozygous mutants over a period of 4 days and compared with that of N2 wild-type animals and smn-1(ok355)/hT2[qIs48] heterozygotes (Fig. 6). On the first day of the assay (1 day after starved L1 larvae were placed on seeded NGM plates), the pharyngeal pumping rate of the mutant was indistinguishable from that of wild-type nematodes. Similar to body wall muscle activity, pharyngeal pumping rates showed a rapid and progressive decline. Taken together, these data indicate a general defect in behaviors requiring rhythmic contractions of muscle tissue in smn-1(ok355) animals.

Neuronal expression of smn-1 partially rescues the smn-1(ok355) phenotype

It has recently been shown that the phenotype of severe SMA mice can be corrected by expressing full-length SMN solely in the nervous system (14). To investigate the sensitivity of different aspects of the C. elegans neuromuscular system to smn-1 loss, we sought to provide wild-type smn-1 to either muscles or neurons of smn-1(ok355) mutants. To this end, rescuing constructs were used expressing full-length smn-1 in body wall and vulval muscles through a myo-3 promoter (Pmyo-3::smn-1) and in all neurons through an unc-119 promoter (Punc-119::smn-1). Promoters of unc-119 and myo-3 have been used previously for neuronal- and muscle-directed
rescue (15,16) and are considered strong transcriptional inducers (17). The rescuing constructs were microinjected into the gonads of smn-1(ok355)/hT2[qIs48] heterozygotes to generate heritable lines. Transgenic heterozygous mothers carry the plasmid DNA as multi-copy, extrachromosomal arrays and transmit the transgene at a certain frequency to their progeny including smn-1(ok355) homozygotes. Extrachromosomal transgenes are frequently lost during cell division resulting in mosaic expression of the transgene (18).

Despite having injected a large number of animals, only one heritable transgenic line each was obtained carrying extrachromosomal Ex[Pmyo-3::smn-1] or Ex[Punc-119::smn-1] for as yet unknown reasons. As an indicator for transgene expression in vivo, the GFP fusions Pmyo-3::GFP and Punc-119::GFP co-injected with the rescuing constructs showed expression in the expected tissues (body wall and vulval muscles for Pmyo-3 and nervous system for Punc-119, data not shown).

Whereas mutant smn-1(ok355) animals expressing muscle-directed smn-1 showed only weak phenotypic rescue in a subset of animals, pan-neuronal expression of smn-1 produced stronger rescue effects (Fig. 7A). Albeit remaining sterile, rescued smn-1(ok355); Ex[Punc-119::smn-1] animals had a larger body size compared with smn-1(ok355) and smn-1 (ok355); Ex[Pmyo-3::smn-1] animals (Fig. 7B) and often retained their intestinal pigmentation, thus appearing less starved. However, they did not reach the size of fully-grown adult smn-1(ok355)/hT2[qIs48] heterozygotes, indicating partial as opposed to full rescue. We noted that the extent of the rescuing effects was variable which, most likely, reflects the mitotic instability of the extrachromosomal transgene. In agreement with the body length measurements, neuronal-expressing smn-1(ok355); Ex[Punc-119::smn-1] animals survived longer than smn-1(ok355) and muscle-expressing smn-1(ok355); Ex[Pmyo-3::smn-1] animals (Fig. 7C) and showed a partial correction of the pharyngeal pumping defect (Fig. 6). Taken together, these data suggest that restoration of neuronal smn-1 activity can, at least partially, correct aspects of the smn-1(ok355) phenotype.

The nervous system of smn-1(ok355) animals

Having shown that neuronal-directed expression of smn-1 is much more effective than muscle-directed expression in rescuing the larval arrest phenotype of smn-1(ok355), we sought to test whether smn-1(ok355) exhibits nervous system abnormalities in general and loss of cholinergic motor neurons in particular. To this end we used the pan-neuronal marker F25B3.3::GFP (19) (strain LM111) and the marker for cholinergic neurons unc-17::GFP (strain LM112) to facilitate neuronal identification. On the basis of their connectivity, motor neurons can be subdivided into classes that innervate dorsal...
muscles by sending their axons dorsally as commissures to form the dorsal nerve cord and those classes that project ventrally. Motor neurons (classes and individual cells) were identified by studying their axonal trajectories and cell body position in the dorsal/ventral nerve cord in LM111 animals (20). We could not detect any gross abnormalities in nervous system morphology of smn-1(ok355); F25B3.3::GFP animals (Fig. 8A). In an attempt to test whether there is loss of neurons over time, LM112 animals were staged and the number of cholinergic neurons in the ventral nerve cord was counted using the marker unc-17::GFP that labels embryonic and postembryonic motor neurons (21,22). No differences were detected in the number of GFP-positive neurons in the ventral cord region between smn-1(ok355) and smn-1(ok355)/hT2[qls48] up to 5 days post-L1 (Fig. 8B).

**DISCUSSION**

In all animal models of SMA studied to date, the ortholog of human SMN is ubiquitously expressed and exists as a single-copy gene. We have shown that, in the *C. elegans* smn-1(ok355) deletion mutant, the loss of SMN-1 protein leads to a progressive decline of motor function. Even though the coding regions of the neighboring genes remain intact, it remains possible that the upstream regulatory sequence of klp-16 may be affected by the smn-1(ok355) deletion thus disrupting klp-16 expression as well. However, the klp-16(ok1505) mutant containing a 1.1 kb deletion within the klp-16 coding sequence has no obvious defects, with normal movement, body size and brood size (data not shown). Thus, taken together with the finding that the smn-1(ok355) mutant phenocopies the less affected progeny originating from smn-1 RNAi (12), it remains unlikely that the smn-1(ok355) deletion phenotype described here is caused by the combined loss of smn-1 and klp-16 function.

The smn-1(ok355) mutation is essentially a null allele, albeit retaining progressively decreasing levels of maternal SMN-1. Immunofluorescence analysis of embryos has previously revealed the presence of SMN-1 at the one-cell stage (P0) prior to initiation of de novo zygotic transcription (12). Considering that RNAi-mediated knockdown of smn-1, which reduces the levels of both maternal and zygotic transcripts, leads to embryonic lethality, we postulate that the maternal contribution of SMN-1 allows progression of smn-1(ok355) animals through early development. Consistent with this notion, the thrashing rate of smn-1(ok355) is unaffected prior to arrest, indicating normal functioning of the neuromuscular system. Presumably, the progressive reduction of maternal SMN-1 results in developmental arrest of homozygous animals at a late larval stage reflected by a defect in gonadogenesis which is followed by paralysis and early lethality.

Closely inspection of the ventral nerve cord of neuronal GFP-expressing smn-1(ok355) animals failed to reveal any loss of cholinergic motor neurons. This finding indicates that embryonic and postembryonic motor neuron development proceeds normally due to sufficient levels of maternally contributed SMN-1 prior to their larval arrest. The absence of a motility defect in the early larval stages underlines the notion that, in smn-1(ok355) animals, motor neurons initially establish normal functional connections with their target muscles. When the movement defects first appeared, we could not detect any loss of cholinergic motor neurons in the ventral nerve cord. Even when paralysis ensues, motor neuron numbers remain unaffected. Thus, in smn-1(ok355) mutants, the maternally contributed SMN-1 can sustain development to late larval stages when the animals arrest in response to SMN protein depletion in the absence of any obvious loss of neuronal cell bodies.

The absence of motor neuron loss in smn-1(ok355) animals is in contrast with mouse models of SMA exhibiting motor defects accompanied by motor neuron degeneration (9), and a zebrafish SMA model showing axon path-finding defects in response to morpholino-induced smn knockdown (10). Rather, the

**Figure 3.** Gonad migration defects in *C. elegans* smn-1(ok355) mutants. (A) In N2 wild-type hermaphrodites the gonad folds back during the L4 stage and develops in a U-shaped manner. (B) In arrested smn-1(ok355) the gonad fails to develop properly.

**Figure 4.** Survival of smn-1(ok355) mutants (*n* = 58) in comparison to smn-1(ok355)/hT2[qls48] heterozygotes (*n* = 56) and N2 wild-type *C. elegans* (*n* = 54). Each data point represents the fraction of synchronized animals alive on a given day.
sma-1(ok355) phenotype described here resembles that of the Drosophila SMA model bearing a miss-sense mutation in smn similar to some of the mutations found in SMA patients (11). The mutant smn flies also survive past embryogenesis due to maternally contributed mRNA and die as late larvae following depletion of the maternal component. However, whereas homozygous smn mutant fly larvae produce both mutant and wild-type Smn protein due to the presence of both transcripts, smn-1(ok355) larvae contain only wild-type smn-1 transcript. Nevertheless, even though the smn-1(ok355) mutant relates to the vast majority of SMA cases showing depletions in the SMN2 gene, the situation in this nematode model of SMA differs from SMA in several facets. Caenorhabditis elegans produces full-length SMN-1 protein from only one gene encoding an SMN ortholog, smn-1. In contrast, in addition to SMN1, humans possess SMN2 which in SMA patients acts as a phenotypic modifier gene producing low levels of functional SMN protein. As a consequence, SMA is caused by insufficiency rather than a complete loss of SMN protein. Furthermore, unlike the case for C. elegans and Drosophila, there is no maternally contribution of SMN mRNA in mammals. For example, homozygous loss of Smn in mice is embryonic lethal (23), which has been overcome by expressing human SMN2 in Smn−/− mice (9,24). Thus, the absence of a C. elegans SMN2 ortholog combined with maternally contributed SMN-1 causes smn-1(ok355) mutants to exhibit a gradual decline in SMN-1 levels ultimately leading to a complete loss of the protein as opposed to the low remaining SMN levels seen in SMA patients. Nevertheless, even though maternal transmission may mask possible functions of SMN-1 during development, the smn-1(ok355) mutant provides the opportunity to study postembryonic roles of SMN-1 in motor neurons and at neuromuscular junctions.

To further understand the pathological events leading to SMA, it is critical to elucidate whether the SMN reduction in motor neurons is a primary defect in the disease or whether there are independent muscle defects as well. The answer to this question is particularly important for the development of therapeutic strategies aiming to up-regulate the SMN levels in selected cell types. Whether the smn-1(ok355) phenotype originates due to defects in muscles or neurons has been addressed here by rescue experiments in which smn-1 was delivered locally to either muscle cells or neurons. It emerged that neuronal-directed, but not muscle-directed, expression of smn-1 partially rescued the smn-1(ok355) phenotype. When assessing the rescue experiments, it has to be taken into account, however, that even though the unc-119 promoter used here exhibits pan-neuronal activity we cannot exclude the possibility of promoter activity levels below the detection limit in cell types other than neurons. Furthermore, we cannot rule out the possibility that the structure and composition of the extrachromosomal arrays may be different thus producing variability in the extent of mosaicism and transgene expression levels.

In the light of our findings, it is of interest to note that neuronal expression of full-length human SMN by the Prion protein (PrP) promoter rescues severe SMA mice containing two copies of human SMN2 and lacking mouse Smn (Smn−/−; SMN2+/−) (14). These SMA mice normally have an average lifespan of 5 days (9), which could be extended to 210 days in one line homozygous for the PrP-SMN transgene (Smn−/−; SMN2+/−; PrP-SMN). When SMN was targeted only to skeletal muscle using the human skeletal actin (HSA) promoter neither improvement of the SMA phenotype nor any extension of survival was seen. One HSA–SMN line showing low expression of SMN in the spinal cord did affect the SMA phenotype with mice living for 160 days on average. Thus, a small increase of SMN in neurons was sufficient to extend the survival of SMA mice considerably whereas high SMN levels in mature skeletal muscle alone had no effect. In comparison to these data we observed only partial as opposed to full rescue when expressing smn-1 in the nervous system of smn-1(ok355) animals. This difference may be explained by the complete loss of maternally contributed SMN-1 activity in the non-neuronal tissues of rescued smn-1(ok355); Ex[Punc-119::smn-1] animals in contrast with the low remaining levels in rescued Smn−/−; SMN2+/−; PrP-SMN mice.

In conclusion, the C. elegans mutant smn-1(ok355) provides a disease model mimicking aspects of SMA such as reduced SMN-1 protein levels throughout the organism. Mutant

Figure 5. Progressive development of movement defects in C. elegans smn-1(ok355) animals, balanced smn-1(ok355)/hT2[qls48] heterozygotes and N2 wild-type animals. For each group, n = 20. Asterisks: P < 0.001. The graphs depict mean ± SEM.

Figure 6. Pharyngeal pumping analysis of C. elegans smn-1(ok355) mutants, balanced smn-1(ok355)/hT2[qls48] heterozygotes and N2 wild-types as well as muscle-expressing smn-1(ok355); Ex[Prm-3::smn-1] and neuronal-expressing smn-1(ok355); Ex[Punc-119::smn-1] animals. Asterisks: P < 0.0001. Numbers at the base of each bar indicate number of animals used. The graphs depict mean ± SEM.
animals proceed through embryogenesis but arrest as late larvae indicating that, in *C. elegans*, SMN-1 has functions later during development and in adult life. In future studies, *smn-1(ok355)* mutants may serve as a starting point for the identification of modifier genes in genetic or genome-wide RNAi screens that enhance or suppress the *smn-1(ok355)* phenotype and for developing novel therapeutic strategies in drug screens.

**MATERIALS AND METHODS**

**Strains**

Strains were grown and maintained as described previously (25). The following strains were used in this study: N2, NW1229 [evls111[F25B3.3::GFP; dpy-20(+)]]; RM1872 [mdEx72[punc-17::GFP;pha-1(+)]]; LM97 [smn-1(ok355)/+ I]; LM99 [smn-1(ok355) I/hT2[bla-4(e937) let-7(q782) qls48] (I;III)]; LM111 [smn-1(ok355) I/hT2[bla-4(e937) let-7(q782) qls48] (I;III); evls111], LM112 [smn-1(ok355) I/hT2 [bla-4(e937) let-7(q782) qls48] (I;III); mdEx72] and VC1005 [klp-16(ok1505) I].

**Isolation of the smn-1(ok355) deletion**

The *smn-1(ok355)* deletion allele of *smn-1* located on chromosome I was obtained from the *C. elegans* Gene Knockout Consortium. From the genetically heterogeneous population, we cloned individual animals heterozygous for the deletion and out-crossed them six times to wild-type (N2) nematodes. The unbalanced *smn-1(ok355)/+ I* strain (LM97) was maintained by picking heterozygous animals and confirming the presence of the deletion by PCR using primers 5'-GACTTCAGATTTGATCAACGGTC-3' and 5'-ACACCAATTACCAAACGATCAAC-3'. Stable maintenance of the deletion was achieved by introducing the genetic balancer hT2 (I;III), which is a reciprocal translocation of chromosomal segments between chromosomes I and III, generating strain LM99. To distinguish homozygous from heterozygous
animals visually, we used the hT2[bli-4(e937) let-?(q782) qIs48] (I,III) derivative of the chromosomal balancer, where qIs48 is an insertion of extrachromosomal ccEx9747 carrying the promoter::GFP fusions myo-2::GFP (expressed in the pharynx), pex-10::GFP (expressed in 4–60 cell embryos) and a gut-specific enhancer fused to GFP (26,27). Heterozygous smn-1(ok355)/hT2[qIs48] (I,III) (referred to as ‘smn-1(ok355)/hT2[qIs48]’) segregate smn-1(ok355) homozygotes, hT2[qIs48] homozygotes, smn-1(ok355)/hT2[qIs48] heterozygotes and arrested aneuploid progeny. Since hT2[qIs48] is homozygous lethal, all animals expressing GFP are homozygous for smn-1(ok355). Animals homozygous for the smn-1(ok355) deletion can be identified due to absence of GFP fluorescence in the pharynx.

Physiological assays
Embryos were released by subjecting gravid N2 and LM99 hermaphrodites to bleach and were subsequently left in M9 overnight for obtaining hatched synchronized smn-1(ok355) homozygotes, balanced smn-1(ok355)/hT2[qIs48] heterozygotes and N2 wild-types at the L1 larval stage. Synchronized L1 larvae were placed on nematode growth medium (NGM) plates seeded with E. coli OP50, and left for 1 day at 22°C. Motility was then assessed every day by placing animals in a 1 min period. A single thrash was defined as a change in the direction of bending at the mid-body. Animals were left in M9 for at least 5 min prior to measurement. For quantification of pharyngeal pumping synchronized smn-1(ok355) homozygotes, smn-1(ok355)/hT2[qIs48] heterozygotes, N2 wild-type and tissue-specific rescue animals (see below) aged 1 day post-L1 were placed on OP50-seeded plates at 22°C. Terminal bulb pumps were measured each day under a dissection microscope for periods of 15 s. Between 2 and 10 periods of 15 s were counted for each animal.

Lifespan and brood size assays
For the lifespan assay synchronized smn-1(ok355) homozygotes, smn-1(ok355)/hT2[qIs48] heterozygotes and N2 wild-types aged 1 day post-L1 were placed on OP50-seeded plates (5 cm diameter) at 22°C. Survival was monitored daily, and animals were scored as dead when they no longer responded to head or tail touch using a platinum wire pick. To avoid crowding due to progeny production and to ensure the availability of sufficient food, animals were transferred to new plates when deemed necessary. Animals that crawled off the NGM agar were excluded from the data. For brood size assays, klp-16(ok1505) and wild-type N2 animals were synchronized and their brood size determined over a 5 day period post-L4 at 20°C. PCR analysis was used to confirm the presence of the klp-16(ok1505) deletion using primers recommended in WormBase (http://www.wormbase.org/).

Tissue-specific rescue of the smn-1(ok355) mutant
To rescue the smn-1(ok355) phenotype in a tissue-specific manner, smn-1(ok355)/hT2[qIs48] heterozygotes were microinjected with the promoter::smn-1 fusion constructs Pmyo-3::smn-1 or Punc-119::smn-1 expressing smn-1 in body wall muscle or neuronal tissue, respectively. These rescuing constructs contain smn-1 from the ATG start to the TAA stop codon (amplified from N2 genomic DNA) placed under transcriptional control of 2 kb upstream of myo-3 for Pmyo-3::smn-1 (generating muscle-directed expression) or 2.2 kb upstream of unc-119 in Punc-119::smn-1 (for pan-neuronal expression). To visualize promoter activity, smn-1 was replaced by GFP to generate the reporters Punc-119::GFP and Pmyo-3::GFP. For neuronal rescue, Punc-119::smn-1 and Punc-119::GFP were co-injected at 10 ng/µl each together with pRF4 at 200 ng/µl encoding the marker rol-6(su1006) the expression of which induces a ‘rolling’ phenotype (28). Analogously, for muscle-directed
rescue attempts, Pmyo-3::smn-1 was co-injected with Pmyo-3::GFP and pRF4.

For body length measurements, photomicrographs were taken of mobile but growth-arrested smn-1(ok355) animals, which were identified by their starved, pale appearance, and adult smn-1(ok355)/ht2[qls48] animals from mixed-stage populations. For body length analysis of transgenic rescue nematodes, individual animals were monitored daily when deemed necessary due to variability in the extent of rescue observed. Photomicrographs of transgenic rescue animals were taken when they appeared starved, i.e. growth-arrested, or immobile. Images of animals were obtained using a Nikon SMZ1000 stereomicroscope and analyzed using ImageJ software from NIH (http://rsb.info.nih.gov/ij/) to measure the body length along the midline from the tip of the head to the base of the tail. Lifespan of rescue animals was assessed by picking synchronized animals aged 3 days post-L1 and monitoring their survival as described above.

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