Motoneuron diseases cause paralysis and death due to loss of motoneurons that innervate skeletal muscle. Spinal muscular atrophy is a human motoneuron disease that is genetically linked to the survival motor neuron gene (SMN). Although SMN was identified more than a decade ago, it remains unclear how decreased levels of the SMN protein cause spinal muscular atrophy. The use of animal models, however, offers a crucial tool in determining the function of SMN in this disease. In this review, we discuss our efforts to develop a zebrafish model of spinal muscular atrophy.

**Keywords:** spinal muscular atrophy; SMN; zebrafish

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**Zebrafish as a Model Organism**

Dr George Streisinger developed zebrafish as a model organism in the 1970s at the University of Oregon. Streisinger saw the potential to develop another vertebrate model system amenable to genetic manipulation. This work was carried forward and expanded by his colleagues at the University of Oregon, who established zebrafish as a superb vertebrate model organism for studying developmental biology. Thus, by the 1980s, the zebrafish was an emerging model for developmental genetics. During the 1990s, genetics and genomics were at the forefront of zebrafish research, including the completion of large genetics screens for mutations that disrupted development, the establishment of an effective antisense approach, introduction of new approaches to mutagenesis, such as viral insertional mutagenesis and transposons, and sequencing of the zebrafish genome (http://www.ensembl.org/Danio_rerio). Now, in the 21st century, with these tools in place, the zebrafish is being used to model human cancers, heart disease, kidney disease, and neurodegenerative disease, as well as for in vivo drug discovery.

The zebrafish is an excellent organism for modeling neuromuscular diseases. Zebrafish have a very stereotyped neuromuscular system that has been well characterized at the cellular level. Because they develop in utero, it can be difficult to analyze early developmental events. When studying neuromuscular diseases, the complexity of the mammalian nervous system may make it difficult to discern effects that contribute to pathology. To circumvent these issues and to add another vertebrate genetic model for studying disease, the freshwater *Danio rerio*, or zebrafish, offers a complementary system. In this review, we discuss the work we have done using zebrafish to model spinal muscular atrophy.

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Animal models are essential tools for addressing the biology that underlies disease. By exploiting different animal models, we can take advantage of their strengths to answer unresolved questions in the field. Mice, as mammals, have long been the animal of choice when modeling human disease because they may closely resemble humans in their pathology. In addition, mice are amenable to genetic manipulations such as transgenesis and gene knockout. In the case of genetic diseases, a larger repertoire of genetic tools increases the likelihood that the disease can be more closely mimicked. However, mice present some challenges, especially when studying diseases that manifest during early stages of development. Because they develop in utero, it can be difficult to analyze early developmental events. When studying neuromuscular diseases, the complexity of the mammalian nervous system may make it difficult to discern effects that contribute to pathology. To circumvent these issues and to add another vertebrate genetic model for studying disease, the freshwater *Danio rerio*, or zebrafish, offers a complementary system. In this review, we discuss the work we have done using zebrafish to model spinal muscular atrophy.
first axons extend and innervate the body muscle in a stereotyped manner. As development proceeds, additional secondary motoneurons are born and extend axons that fasciculate to form nerves. At the end of embryonic development (72 hours postfertilization), each of the approximately 30 zebrafish trunk muscles on either side of the body are innervated by 3 nerves. Because of the stereotyped development of the initial axonal projections and the subsequent nerves, alterations in these nerves can be visualized under the microscope, either by antibody labeling or by using transgenic fish that express green fluorescent protein in motoneurons and axons (such as the transgenic fish with the gata2 promoter driving green fluorescent protein or fish with the HB9 promoter driving green fluorescent protein).14,15

A Zebrafish Model of Spinal Muscular Atrophy

Spinal muscular atrophy is a neurodegenerative disease whose phenotype can manifest in childhood and, in severe cases, at birth or even prenatally.16 It results from low survival motor neuron (SMN) protein levels. All species, except primates, have only 1 SMN gene, SMN1. Chimpanzees have 2 copies of SMN1, presumably due to a gene duplication event.17 The SMN2 gene, which evolved from SMN1 and contains only 5 nucleotide differences compared with SMN1, first appears in humans and is present in no other species.17 Targeted knockout of SMN1 in mice results in early embryonic lethality.18 This finding suggests that SMN has an essential role during development, and it is presumed that this is also the case in other species. In humans, however, SMN2 can generate some full-length protein (approximately 10%).19,20 Thus, when SMN1 is deleted, as it is in approximately 92% of spinal muscular atrophy patients,21 some full-length SMN protein is made from SMN2. As this is an unstable region of the genome, individuals have varying copies of SMN2, and those with more copies have a less severe form of the disease due to the greater amount of SMN protein.20

What is the best way to mimic spinal muscular atrophy in zebrafish? Like other nonhumans, zebrafish only have the smn1 gene. Therefore, 1 approach is to generate mutations in smn that will eliminate the functional protein in combination with an SMN2 transgenic fish, similar to the approach taken in the mouse.22 Although this strategy generates a stable genetic model, targeted gene knockout is not yet established in zebrafish; thus, this approach entails finding mutations in zebrafish smn and then making a transgenic with the human SMN2 gene. Another approach that does not depend on finding a genetic mutation is to decrease Smn levels using strategies that block protein production. These methods are transient, but allow protein production to be “knocked down” during embryogenesis and early larval stages of development. The strategy used most often in zebrafish is an approach that uses modified antisense oligonucleotides referred to as morpholinos.4 These 25-base nucleotides are stabilized by the addition of a morpholine group and are designed either against the translation start site of a ribonucleic acid (RNA) to inhibit protein translation (referred to as translation-blocking morpholinos) or to a splice region of RNA to cause aberrant splicing ultimately leading to a nonfunctional protein (referred to as splice-junction morpholinos).4,23 Because zebrafish mate externally, embryos can be collected immediately after fertilization. Morpholinos are injected into the 1- to 2-cell stage embryo (Figure 1) using a simple pressure injection system and a stereomicroscope. By titrating the amount of smn morpholino added to the embryo, protein levels can be decreased in a dose-dependent manner and quantitated either by Western blotting, for the translation-blocking morpholinos, or by reverse transcriptase-polymerase chain reaction for the splice-junction morpholinos.

To model spinal muscular atrophy in zebrafish, we used a morpholino against zebrafish smn RNA to decrease the level of Smm protein during development. We used a translation-blocking morpholino to decrease Smm to levels
similar to those seen in human patients as assayed by Western blot analysis (approximately 75%). Although morpholinos only transiently bind target RNA, Smn levels were decreased for approximately 2 weeks. Because zebrafish develop so rapidly, during this time frame, the neuromuscular system and all other organs have formed. At this level of knockdown, approximately 55% of the embryos died before 24 hours postfertilization. If we injected higher doses of smn morpholino, we had increased embryonic death consistent with Smn being an essential protein in zebrafish, as has been shown in mouse and suggested in humans (based on the fact that no human has been identified that lacks both SMN1 and SMN2). Because spinal muscular atrophy causes muscle denervation and motoneuron death, we first analyzed the neuromuscular system of smn morpholino-injected embryos (referred to as smn morphants). We found no difference in the number of motoneurons present at 24 hours postfertilization. However, upon examination of motor axon projections, we found that both the early developing primary motoneuron axons and the later developing nerves did not grow normally along the body muscle (Figure 2). The axons were often short and aberrantly branched. Interestingly, the axons still projected to the correct muscle region. For example, identified motoneurons in zebrafish that normally project ventrally still projected ventrally when Smn levels were decreased; however, these axons often were excessively branched and often did not reach the distalmost region of the ventral muscle.

These results suggest that the motoneurons are born, become the right type of motoneuron, and extend their axons out of the spinal cord correctly. However, they are unable to extend along their stereotyped axonal pathway. We also found that the pectoral fin, the evolutionary equivalent of the forelimb in terrestrial vertebrates, was also not innervated correctly in approximately 65% of smn morphants (Figure 3). In addition, we examined the hindbrain cranial nerves and found that the facial nerve did not project normally in 25% of smn morpholino embryos, compared with 11% of control embryos (Figure 4). These defects are relevant because both mouse models of spinal muscular atrophy and human spinal muscular atrophy patients exhibit limb muscle paralysis and denervation. In addition, in both the severe and mild mouse models, there is loss of facial motoneurons, and in type 1 spinal muscular atrophy patients, the facial nerve can be affected. Thus, the motor nerve defects seen in zebrafish when Smn levels are decreased are the same motoneurons affected in mouse models and human patients.

Although motor axons were aberrant, we characterized other axon populations and found them to be normal in smn morphants. These included Rohon-Beard sensory axons that extend peripherally between the skin and muscle, the
lateral line sensory nerve that extends along the body of the fish just under the skin, and the hindbrain Mauthner axons that extend from the hindbrain to the tip of the tail within the spinal cord. Thus, decreasing Smn levels in zebrafish causes motor axon-specific defects in populations of motoneurons that are affected in mouse models and in human patients. We also found no evidence of motoneuron cell death at early (72 hours postfertilization) stages. This finding is consistent with what is seen in mouse models where the severe mice are paralyzed but show only approximately 30%–40% motoneuron loss, suggesting that motoneuron dysfunction leading to muscle denervation may precede motoneuron cell death.

In zebrafish, motor nerve defects are the first manifestation of low Smn levels. What are the consequences of aberrant motor axon outgrowth? It is possible that, because these motor axons are not following their normal pathways, they are not receiving appropriate cues from the muscle, which could have detrimental effects on their subsequent function. We have also shown that synapse formation does occur in these fish. It is possible, however, that these synapses are not maintained, either because of the initial defects in axon outgrowth or additional problems due to low levels of Smn in axons, which compromises synapses. We have observed fish with low Smn levels over time and find that those with more severe motor axon defects have decreased survival. These data indicate that the motor axon defects observed upon Smn knockdown are detrimental to the organism. In humans, presymptomatic spinal muscular atrophy patients have normal motoneuron function; however, decreased motor unit estimation and compound motor action potential amplitude are seen as symptoms manifest. Our data are
consistent with this finding and show that motoneurons are innervating muscle, as least at early stages, even though the motor axons are abnormal. We predict that over time the synapses established under these aberrant nerves may be lost. Examination of the smn morphants at later developmental stages will address this issue.

It was previously shown in mouse and human that smn RNA and protein are expressed in all cell types examined.\(^\text{19,30}\) We also found in zebrafish that smn RNA and protein are expressed ubiquitously.\(^\text{24}\) This finding raises the question of whether Smn in motoneurons or in the surrounding tissues, perhaps muscle, is needed for proper motor axon outgrowth. To address this issue, we took advantage of the fact that zebrafish embryos are optically transparent and the earliest developing motoneurons can be visualized under a compound microscope.\(^\text{10}\) We iontophoretically introduced smn morpholino into single motoneurons at a time when they were undergoing axon outgrowth, thus knocking down Smn specifically in these cells.\(^\text{24}\) This strategy resulted in aberrant motor axons, indicating that Smn is needed cell-autonomously for proper motor axon outgrowth. These experiments do not rule out a role for Smn in muscle, but show that Smn in motoneurons is necessary for their normal development.

### An In Vivo Motoneuron Assay to Analyze SMN Function

To understand how the Smn protein functions in motor axon outgrowth, we asked what forms of the human SMN protein could rescue these defects. We first designated criteria for nerve classification to enable us to quantify the nerve defects.\(^\text{27}\) For example, a motor axon was classified as severe if it was truncated or truncated followed by excessive branching. If a fish had 10%–20% severely affected motor axons, it was considered a severe fish. If 10%–20% of the defects were moderate (no truncation, but excessively branched, for example), then the fish was considered moderately affected. Using these criteria and scoring close to 4000 motor nerves, we found that 16% of the smn morphants were severe, 22% moderate, 36% mild, and 26% unaffected.\(^\text{27}\) To prove that low levels of Smn protein caused these defects, we coinjected smn morpholino with full-length human SMN (hSMN) RNA, which is not recognized by the morpholino, and found that the motor nerve defects were partially rescued. For example, the percentage of severe fish went from 16% (smn morpholino) to 0% (smn morpholino + hSMN), and fish with no defects went from 27% (smn morpholino) to 60% (smn morpholino + hSMN). We did not expect to see complete rescue, because both the RNA and morpholino injections can be mosaic (i.e., different cells may take up different amounts of RNA or morpholino). Because the zebrafish and human Smn proteins are so similar (52% identity, 75% similarity at the amino acid level), we predict we would get similar rescue with the zebrafish smn RNA. The predominant protein made from human SMN2 lacks the C-terminal 16 amino acids encoded by exon 7. This protein, referred to as hSMN\(^\Delta 7\), is unstable\(^\text{31}\) and cannot compensate for full-length SMN in mouse models or human.\(^\text{20,32}\) Therefore, we asked whether hSMN\(^\Delta 7\) RNA could rescue the motor axon defects caused by low Smn levels in zebrafish. When we coinjected smn morpholino and hSMN\(^\Delta 7\) RNA, we found that the motor axon defects were not rescued and that the defects observed were the same as in smn morphants alone. If these motor axon defects are relevant to spinal muscular atrophy, at least in this model system, then we predict that point mutations in human SMN1 that cause spinal muscular atrophy would not rescue these motor axon defects. Therefore, we asked whether human patient mutations SMN A111G, Y272C, and G279V could rescue the motor axon defects in zebrafish. We found that none of these human mutations rescued the motor axon defects in zebrafish when coinjected with smn morpholino. These data show that the motor axon defects caused by low levels of Smn follow the genetics of spinal muscular atrophy in that neither SMN\(^\Delta 7\) nor SMN point mutations that cause spinal muscular atrophy can rescue these defects. Thus, we can use these motor axon defects as a readout of SMN function to test what properties of SMN are required for normal motor axon outgrowth.

The first function ascribed to SMN was as an assembler of small nuclear ribonuclear proteins.\(^\text{30}\) These are RNA:protein complexes that are important for RNA splicing. SMN was shown to increase the fidelity and specificity of splicing by facilitating Sm protein binding onto U1 RNAs.\(^\text{33}\) The mechanism of this function of SMN has been well characterized in cultured cells; how it relates to spinal muscular atrophy, however, remains unclear. It is possible there are motoneuron-specific splicing defects that occur when SMN is decreased; however, this finding has not yet been reported. The role of SMN as an essential splicing factor could explain the fact that complete lack of SMN causes early embryonic lethality in mice, and presumably in human as well, as no patient has ever been found to lack both SMN1 and SMN2. It is possible, however, that, at low levels, SMN is still capable of facilitating snRNP assembly but is deficient in some other functional property. SMN has also been shown to interact biochemically with numerous proteins not involved in snRNP assembly. These proteins include hnRNP-R and Q, profilin, Fibrillarin, and Gar1.\(^\text{34-38}\) The significance of these complexes to spinal muscular atrophy remains to be determined, but it does suggest that the SMN protein is most likely multifunctional. SMN also possesses a Tudor domain, a protein domain first identified in Drosophila that binds dimethylated arginines.\(^\text{39}\) Sm proteins, important for splicing, bind to this region of SMN, but it is likely that other dimethylated arginine-containing proteins bind here as well.\(^\text{39}\) It has been suggested that, at
decreased SMN levels, low-affinity complexes would be lost first (eg, motoneuron complexes), whereas higher-affinity complexes (eg, snRNP complexes) would remain.25 Important questions then are whether SMN has other functions independent of small nuclear ribonucleoprotein assembly, and if so, whether at low levels these functions are disrupted in motoneurons.

To address what function of SMN is crucial for motor axon outgrowth, we used zebrafish motor axons as a readout and asked what forms of human SMN could rescue the motor axon defects in smn morphants. To start, we asked if adding the exon 7 amino acid motif QNQKE could rescue the motor axon defects. This motif was interesting to us based on work by Zhang and colleagues showing that tagged SMN moved down axons and into growth cones when expressed in cultured neurons.40 Of interest, SMN lacking exon 7 (SMNΔ7), the predominant protein form from SMN2, failed to do so. However, when the exon 7 motif QNQKE was added back to SMNΔ7, it was now transported down the axon. We had already shown that hSMNΔ7 could not rescue the axon defects; however, when we added RNA encoding hSMNΔ7-VDQNQKE we found that the motor axons were rescued to levels similar to when we injected full-length hSMN RNA.27 To look at this in more detail, we made an hSMN RNA with an altered sequence so that the first Q in the QNQKE motif, which is highly conserved across animal species, was changed to an alanine (hSMN Q282A). Upon injecting this RNA along with the smn morpholino, we observed no rescue, suggesting that this amino acid is critical for normal motor axon outgrowth. These experiments indicated that the hSMN exon 7 QNQKE motif is important. Other studies, however, had suggested that adding any amino acid sequence onto hSMNΔ7 would stabilize this protein.41,42 To test whether random amino acids could restore motor axons caused by low Smn levels, we used another hSMNΔ7 RNA that had an additional 9 amino acids due to read-through of a stop codon at the beginning of exon 8. This construct (hSMNΔ7 read-through) failed to rescue the motor axon defects in zebrafish.27 Therefore, whereas this hSMN read-through form was transported down axons when tested in the cell-culture assay, it was not able to rescue motor axons. We concluded from these experiments that the QNQKE motif present in exon 7 was important for hSMN function in motor axons and that random amino acids could not substitute, further suggesting that this motif was functionally relevant. Its exact function is currently under investigation.

Now that we had forms of human SMN that rescue motor axon defects (hSMNΔ7-VDQNQKE) and forms that do not (hSMNΔ7, hSMN Q282A, and human mutant hSMN G279V, hSMN Y272C, and hSMN A111G), we wanted to know whether these forms retained properties needed for small nuclear ribonucleoprotein biosynthesis. If they did not retain these functions but could still rescue motor axons or visa versa, then we could rule out small nuclear ribonucleoprotein function as relevant to motor axon outgrowth. Two properties of SMN reported to be crucial for small nuclear ribonucleoprotein biogenesis are oligomerization and binding to Sm proteins.43-45 Using in vitro binding assays, we asked whether the different constructs tested above in the motor axon assay were able to perform functions needed for small nuclear ribonucleoprotein assembly.27 hSMNΔ7, hSMN G279V, and hSMN Y272C were not able to oligomerize or bind Sm proteins, but they also did not rescue motor axons. However, hSMNΔ7-VDQNQKE, which rescued motor axons caused by low levels of Smn, was not capable of oligomerization or Sm binding in vitro. Conversely, both the synthetic mutant hSMN Q282A and the human patient mutation hSMN A111G, although unable to rescue motor axons, retained small nuclear ribonucleoprotein assembly properties. These data show that SMN properties important for small nuclear ribonucleoprotein biogenesis are not necessary for proper motor axon outgrowth, indicating that the SMN protein is multifunctional and that the originally identified small nuclear ribonucleoprotein assembly function of SMN may not be the function that leads to motoneuron dysfunction in spinal muscular atrophy (see Table 1 for summary).

### Table 1. Summary of Human SMN Constructs Tested in Zebrafish

<table>
<thead>
<tr>
<th></th>
<th>Rescues motor axon defects</th>
<th>Self-associates</th>
<th>Binds Sm protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>hSMN</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>hSMNΔ7</td>
<td>–</td>
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<tr>
<td>hSMNΔ7-VDQNQKE</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>hSMN Q282A</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>hSMNΔ7 read-through</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>hSMN G279V</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>hSMN Y272C</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>hSMN A111G</td>
<td>–</td>
<td>+</td>
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</tr>
</tbody>
</table>

a. The ability of the SMN forms to rescue the motor axon defects caused by low Smn in zebrafish, their capacity to self-associate, and their ability to bind Sm protein are listed.

### Gemin2 and Motor Axons

To further explore the role of small nuclear ribonucleoprotein biogenesis in spinal muscular atrophy, we asked whether knocking down other components of the SMN complex also causes defects in motor axons, similar to what we found with Smm knockdown. We reasoned that, if SMN's function in small nuclear ribonucleoprotein biogenesis was crucial for motor axon outgrowth, then decreasing other proteins involved in small nuclear ribonucleoprotein biogenesis should yield a similar phenotype. Thus, we chose to knock down Gemin2. Gemin2
is a novel protein and was identified because it bound
SMN.46,47 In addition, knockdown of Gemin2 in cells
causes reduced small nuclear ribonucleoprotein assembly.48
Knockout mice for this protein, however, are embryonic-
lethal and thus do not lend insight into whether low levels
of Gemin2 can cause motor axon defects.49 When we
knocked down Gemin2 in zebrafish, we found that most of
the embryos died or were developmentally delayed and mor-
phologically abnormal (M.L.M. and C.E.B., manuscript
submitted for publication). We know from human patients,
mouse models, and the zebrafish model that decreasing
SMN does not affect overall development and body shape.
So this finding was not similar to a spinal muscular atrophy
phenotype.

We further showed that, while the embryos with defect-
tive bodies had motor axon defects, those with normal bod-
ies (approximately 30% of those that lived to 24 hours
postfertilization) had normal motor axons. This finding sug-
gests that misshapen bodies can secondarily cause aberrant
motor axon projections. This suggestion is not surprising, as
motor axons extend directly along the body wall muscle, and
any malformations or developmental delays in the body
development can cause secondary effects on motor axons.
To test this directly, we created morpholino-based genetic
mosaics. In these experiments, cells are taken from 1 geno-
type, in this case gemin2 morphants, and transplanted into
fish of another genotype, in this case wild-types. These
experiments can be done in both directions and are the
standard, accepted method for testing where a particular
gene function is needed (ie, cell autonomy).50 We had
already demonstrated that Smn is needed in motoneurons
for proper motor axon outgrowth.24 When we tested cell
autonomy for Gemin2, we found that it was not needed cell-
autonomously in motoneurons for motor axon outgrowth.
Indeed, we found that the only situation involving Gemin2
that gave aberrant motor axons is when the body was mal-
formed. Thus, Gemin2, unlike Smn, is not needed in motor
neurons for axon outgrowth.

Because these 2 proteins share their small nuclear
ribonucleoprotein assembly properties yet affect motor
axons differently, these findings strongly suggest that Smn
has a function independent of small nuclear ribonucleo-
protein biogenesis that is important in motor axons and per-
haps spinal muscular atrophy. This conclusion is in conflict
with that published by Winkler et al.51 In this study, the
authors attest that Gemin2 knockdown in zebrafish causes
motor axon defects; however, they did not take into consid-
eration the defective body morphology or test cell autonomy.
Once the defective embryos are taken into account, the
numbers do not support a role for Gemin2 in motor axon
outgrowth. A crucial point has emerged from these data:
When examining motor axon phenotypes, it is essential to
take into consideration the body morphology. If there are
defects in body morphology, then cell-autonomy tests such
as the genetic mosaics described above need to be per-
formed to determine whether the motor axon defects are a
primary deficit or are secondary to the body defects.

A Function for SMN in Axons?

Zebrafish is the only organism reported to have motor axon
defects in response to low Smn levels. It is possible that
zebrafish motor axons are more sensitive or less able to com-
promise for low Smn levels. Other data do support a role for
SMN in axons. Immunohistochemistry analyzing rat spinal
cord tissue52 and cultured PC12 cells53,54 revealed that
SMN is present in axon branch points and growth cones.
More importantly, Rossoll and colleagues showed that,
when motoneurons from the severe spinal muscular atrophy
mouse (SMN-/-; hSMN2 2 copies)22 were cultured in vitro,
they had shorter axons, smaller growth cones, and reduced
levels of growth cone β-actin RNA compared with cultured
motoneurons from wild-type mice.55 Thus, mouse motor
axons in culture and zebrafish motor axons in vivo act simi-
larly under low Smn conditions. They further showed that
hnRNP-R, an RNA-binding protein, interacts with SMN
and that both are needed for normal axonal outgrowth in
vitro. As mentioned earlier, tagged SMN moves down axons
and into growth cones when expressed in cultured neu-
rons.50 It has also been shown that knockdown of SMN in
PC12 cells results in shorter neurites and changes in the ratio
of globular and filamentous actin, and adding back just the
C-terminal region of SMN (exons 6 and 7) can rescue these
SMN knockdown effects.56 Together, these data suggest
that SMN is present in axons and growth cones consistent
with it functioning in this part of the motoneuron.

The C-terminal region of SMN may play a role in this
process, as may other proteins such as hnRNP-R. One
hypothesis that has emerged from this collection of data is
that SMN is involved in a protein complex that transports
RNAs to the growth cone to be used for localized protein
translation.57 This explanation would suggest that SMN is
an assembler, not just of small nuclear ribonucleoprotein
complexes, but also of other RNA protein complexes, such
as messenger RNA-protein complexes. Studies are ongoing
in several systems to test this hypothesis.

Summary

Using zebrafish to model spinal muscular atrophy has
provided insight into how SMN is functioning in this dis-
ease. Our finding that decreasing Smn in zebrafish results
in motor axon defects indicates that SMN plays a role in
motoneuron development. This phenotype is consistent
with cultured motoneurons from the severe spinal mus-
cular atrophy mice, which also show short, aberrant

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axons. The zebrafish model is not only providing insight into where SMN function is needed, but is also serving as an assay to test SMN function. Indeed, we have found that the motor axon defects follow the genetics of the human disease in that forms of SMN that cause spinal muscular atrophy also fail to rescue the motor axon defects in zebrafish. Also, using this assay, we have data suggesting that the small nuclear ribonucleoprotein function of SMN can be uncoupled from the role of SMN in motor axon outgrowth. This finding indicates that other functions of SMN may be more important for motoneuron development and perhaps spinal muscular atrophy. It is possible that SMN is functioning in more than 1 manner to cause spinal muscular atrophy. Certainly one can imagine that, at very low SMN levels, many functions of SMN, including small nuclear ribonucleoprotein assembly, would be compromised. At slightly higher (but still below normal) levels, however, only those SMN protein complexes with low affinity would be lost. A low-affinity SMN complex that has a function in axons could explain the motor axon outgrowth defects in zebrafish and cultured motoneurons from spinal muscular atrophy mice. The challenge now is to determine whether these complexes exist and what function they play in facilitating normal motoneuron development and maintenance. Research using zebrafish and other animal models will continue to provide opportunities to address these crucial questions regarding the function of SMN in spinal muscular atrophy.

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