Non-nuclear Pool of Splicing Factor SFPQ Regulates Axonal Transcripts Required for Normal Motor Development

Highlights

- SFPQ splicing factor is present in motor axons
- Non-nuclear SFPQ is able to drive axon maturation and connectivity
- Loss of axonal SFPQ affects axonal morphology
- Coiled-coil domain of the protein is important for non-nuclear localization

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In Brief

Thomas-Jinu et al. demonstrate that a non-nuclear pool of the splicing factor SFPQ is necessary for normal motor development, through local mRNA maintenance or processing. SFPQ’s coiled-coil domain is required for axonal localization. Their findings may have an important impact in understanding human motor neuron disorders.
Non-nuclear Pool of Splicing Factor SFPQ Regulates Axonal Transcripts Required for Normal Motor Development

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SUMMARY

Recent progress revealed the complexity of RNA processing and its association to human disorders. Here, we unveil a new facet of this complexity. Complete loss of function of the ubiquitous splicing factor SFPQ affects zebrafish motoneuron differentiation cell autonomously. In addition to its nuclear localization, the protein unexpectedly localizes to motor axons. The cytosolic version of SFPQ abolishes motor axonal defects, rescuing key transcripts, and restores motility in the paralyzed sfpq null mutants, indicating a non-nuclear processing role in motor axons. Novel variants affecting the conserved coiled-coil domain, so far exclusively found in fALS exomes, specifically affect the ability of SFPQ to localize in axons. They broadly rescue morphology and motility in the zebrafish mutant, but alter motor axon morphology, demonstrating functional requirement for axonal SFPQ. Altogether, we uncover the axonal function of the splicing factor SFPQ in motor development and highlight the importance of the coiled-coil domain in this process.

INTRODUCTION

Post-transcriptional regulation of gene expression plays a fundamental role in the temporal and spatial modulation of development and has been recently found at the core of many disease mechanisms. In recent years, much progress has been made in unraveling the players in this process, revealing a daunting complexity in the mechanisms of RNA processing and transport. This complexity is not only created by the many players involved and their intricate interactions in multiple aspects of RNA regulation (transcription termination, non-coding RNA interaction, RNA processing, and transport), but is also generated by the complex role of long non-coding RNAs (Derrien et al., 2012; Mercer and Mattick, 2013; Okazaki et al., 2002).

Our zebrafish forward-genetic screen for embryos displaying abnormalities in neural development led to the isolation of a null mutation in the gene encoding splicing factor proline/glutamine-rich (SFPQ, a.k.a. PSF). SFPQ is a multifunctional protein (Shav-Tal and Zipori, 2002), known to be involved in pre-mRNA splicing (Rosonina et al., 2005), transcriptional repression, and DNA repair, originally isolated from the spliceosome. It is associated with nuclear paraspeckles (Fox et al., 2002) involved in nuclear retention of defective RNAs into subnuclear sequestration of SFPQ complexes (Barry et al., 2014; Johnson, 2012) and with small cytoplasmic RNA granules, the function of which is not yet understood (Kanai et al., 2004).

The structure of SFPQ is conserved and closely related to NONO and PSPC1 proteins, with which it forms heterodimers. They all contain tandem RNA recognition motif domains, a family-specific NOPS (Nono, Pspc1, and Sfpq) domain and a coiled-coil region (Passon et al., 2012). SFPQ additionally contains a DNA-binding domain (DBD) and an N-terminal proline/glutamine-rich low-complexity region (Lee et al., 2015; Patton et al., 1993; Peng et al., 2002; Shav-Tal and Zipori, 2002; Urban et al., 2009). Its RNA-binding activity has been implicated in a variety of cellular processes (Chanas-Sacré et al., 1999; Figueroa et al., 2009; Lukong et al., 2009; Rosonina et al., 2005;
Shav-Tal et al., 2001; Shav-Tal and Zipori, 2002; Sutherland et al., 2005). Of interest for our study, SFPQ has recently been shown to regulate signal-induced alternative splicing (Ray et al., 2011) and to be required, directly or indirectly, for transport of an RNA regulator that promotes sensory axon viability (Cosker et al., 2016). SFPQ is present in cytoplasmic aggregates in brains of patients with a variety of neurodegenerative disorders and is misregulated in autism and dyslexia (Chang et al., 2015; Ke et al., 2012; Kubota et al., 2010; Stamova et al., 2013; Tapia-Páez et al., 2008). The human SFPQ gene lies in a region on chromosome 1p34-p36 linked to speech disorders and language impairment (Grigorenko et al., 2001; Kubota et al., 2010; Miscimarra et al., 2007; Rabin et al., 1993; Tzenova et al., 2004).

Phenotypic characterization of our zebrafish sfpq homozygous mutants revealed a restricted set of defects, unexpected for a ubiquitously expressed multifunctional protein. The CNS was prominently affected, showing brain boundary and axonal defects associated with absence of motility. We assessed the specificity of SFPQ functional targets by microarray RNA profiling analysis, comparing the transcriptome of the sfpq homozygous mutants to its wild-type and heterozygous siblings at the earliest stage at which the phenotype is robustly recognizable. An unexpectedly narrow set of transcripts was affected by the loss of SFPQ, with a specific enrichment in alternatively spliced neuronal and synaptic transcripts. The use of a tagged version of the protein revealed that SFPQ is abundant in the axons of a subset of neuronal populations including all motor neurons (MNs), an observation confirmed by antibody staining of endogenous SFPQ. We demonstrated that these axons also contain intron-retaining transcripts and spliceosome core components, suggesting a possible involvement in non-canonical cytoplasmic RNA processing. We demonstrate that cytoplasmic SFPQ is functionally relevant to MN differentiation, showing that a non-nuclear version of the protein is able to restore a specific set of neuronal transcripts and rescue the axonal and motility defects in the zebrafish null mutant. The severe motor phenotype of the zebrafish null mutant and presence of SFPQ in aggregates in human degenerative disorders encouraged us to screen ALS patients for SFPQ mutations. From these patients, we identified two novel SFPQ missense variants affecting the coiled-coil domain of the protein and absent from ~66,000 normal human exomes. In double-blind zebrafish null mutant rescue experiments, the ALS-linked SFPQ variants uniquely led to motor axon morphological abnormalities in the rescued mutants and showed a much-reduced cytoplasmic localization. These findings uncover the importance of the non-nuclear axonal function of SFPQ protein in motor development and indicate the importance of the coiled-coil domain in this process.

**RESULTS**

**Loss of Zebrafish sfpq Affects Development of Brain Boundaries and Motor Function**

A recessive zebrafish mutant identified during a small-scale ENU mutagenesis screen was selected for its early brain boundary defects and was named coma due to its vegetative state and its later comma-shaped body (Figure S1A). Preceding any morphologically visible defect, homozygous mutants fail to twitch their tail at 15–16 somite stage (ss). By 24 hr post-fertilization (hpf), all homozygous mutant larvae fail to undergo the morphogenetic changes required to form the posterior mesencephalic wall and a mature isthmic organizer (Figures 1A and 1B), and 22% have asymmetric ectopic mesencephalic neuro-pitithelial folds (Figures 1C and 1D). The expression of midbrain-hindbrain boundary (MHB) markers, pax2.1 and fgf8, is normal at early stages, but reduced or lost at later stages, indicating that the midhindbrain boundary is established, but not maintained (Figures 1E, 1F, and S1B). The mutant also lacks rhombomere boundaries, failing to express early boundary markers such as rfnf (Figures 1G and 1H), and undergoes ectopic neurogene-sis in boundary regions (Figures 1I–1L). These boundary defects are neither due to a general loss of apical-basal polarity, nor to specific cell death in these areas (Figures S2E and S2H).

Most other aspects of CNS patterning appear normal in the mutant apart from a loss of Wnt signaling activity observed in the hypothalamus (Figure S1D). No significant difference in cell proliferation was found during the two first days of development (Figures S2A–S2F and S2I). All mutant embryos develop further defects such as lack of heart looping, pericardial edema, short curved body, and kinky tail (Figure S1A); never acquire motility (Movie S1); and die by 4 days post-fertilization (dpf).

The genetic characterization of coma−/− by fine mapping and candidate gene analysis indicated that the genetic lesion was in sfpq. This was confirmed by complementation test with an existing allele of the gene (whitesnake, ws$^{2241}$) (Lowery et al., 2007). The point mutation in coma was then identified by sequencing as a C-to-T transition at +604 position from the ATG start codon, changing CAA to TAA (glutamine [Q] at aa. 203 to STOP codon) in the proline (P)/glutamine (Q)-rich domain of the protein (Figure 1M). The rescue of the mutant phenotype by microinjection of the zebrafish or human sfpq full-length mRNA (Figures S1C and 7) establishes that coma is a novel null allele of the gene sfpq (named sfpq$^{kn41}$) located at 56.2 cM on zebrafish chromosome 19 and that the protein has a conserved function in zebrafish and human.

**Sfpq Is Cell-Autonomously Required for Motor Axon Development**

As the earliest observable sfpq$^{kn41}$ defect is its lack of early motility at 16 ss, we investigated its neuronal differentiation. From 24 hpf onward, homozygous sfpq−/− embryos show defects in axonal tracts in discrete areas of CNS and peripheral nervous system (PNS), with lack of posterior and supra-optic commissures and reduced inter-rhombomeric commissural axons (Figures 2A–2D). These also show defects in the axonal projections of both cranial (Figures 2E and 2F) and spinal cord MNs (Figures 2G–2P).

The sfpq$^{kn41}$ anterior spinal motor axons do occasionally manage to form and exit normally the spinal cord, neuronal behavior more readily observed in the Tg(Xla.Tubb2b:Hsa.MAPT-GFP)$^{kn2}$ background, expressing a GFP-tagged microtubule-associated Tau protein in neurons, indicating that Tau over-expression has some beneficial effect in sfpq$^{kn41}$ neurons. In this transgenic background, around 15% of homozygous mutants form anterior axons with neuro-muscular junctions (NMJs) and...
can generate some movements (Movie S2). In all other backgrounds, most anterior MNs do not progress, stalling at the horizontal myoseptum and either stop there or branch excessively with poor progression along the somitic mass (Figures S2 G–S2 N). Immunostaining with α-bungarotoxin (α-btxn; data not shown) and pre-synaptic SV2 shows normal pre- and post-synaptic pre-patterning in the mutant (Figures S2 O and S2 P; data not shown). However, the mutants never form mature NMJs. The motor axon defect of the mutant is not due to cell death, as healthy MN cell bodies are found until day 4 (GFP+ in Figures S2 G–S2 N) and the mutants lacking p53 (thereby devoid of cell death; Sakhi et al., 1994) still exhibit severe axogenesis defects (Figures S2 J–S2 Q).

Somatic muscle differentiation occurs normally in the sfpq null mutant, with typical V-shaped somitic boundaries, proper differentiation of both slow and fast muscle, and formation of sarcomeres (Figures S2 R–S2 Y). The absence of axons in the vast majority of MNs, without initial defects in muscle differentiation, indicates that the primary defect is in the MNs.

Mosaic experiments (Figure 3) further demonstrate that sfpq is cell-autonomously required in neurons for their maturation.

**Figure 1. SFPQ Is Required for Brain Boundaries**

Dorsal (A–D and I–L) and lateral (E–H) views of 32 hpf zebrafish brain with anterior to the top (A and B) or left (C–L).

(A and B) Immunostaining of sfpq; Tg(dlx4.6 Gfp) embryos. Anti-acetylated tubulin staining (red) reveals asymmetrical folds in the midbrain (white arrowheads) and thickening of the isthmic organizer (IsO) in coma mutant (B; n = 8) compared to its wild-type sibling (A; n = 24). GFP staining (green) reveals disorganized neuronal distribution in the cerebellum.

(C and D) Dorsal views, anterior to the top, of sibling (C) and mutant (D) sfpq; Tg(b-actin: HRAS gfp) embryos showing failure of morphological thinning of the isthmus (white arrows in D, n = 9/9) in the homozygous mutant.

(E and F) Expression of pax2.1 in the MHB, greatly reduced in all sfpq mutants (F; n = 10) compared to siblings (E; n = 30) at 36 hpf.

(G and H) Expression of boundary marker, rfgq, in siblings (G; n = 21) and mutant (H; n = 7, reduced or absent) at 24 ss.

(I and J) Expression of zash1a in the hindbrain at 15 ss, in siblings (I; n = 19) and mutant (J; n = 6).

(K and L) HuC staining at 32 hpf, in siblings (K; n = 17) and mutant (L; n = 5) Scale bar, 100 μm.

(M) Schematic of the sfpq gene and the zebrafish and human mutations described in this report and, below, the sequence altered in the zebrafish coma mutant. PO, proline (P) glutamine (Q) rich; RRM, RNA recognition motif; NOPS, NONA/paraspeckle domain; NLS, nuclear localization signals.
Figure 2. Axonogenesis Is Affected in sfpq−/− Embryos
Lateral (A, B, and G–P) and dorsal (C–F) views, anterior to the left, of zebrafish brain at 32 hpf (A and B) and spinal cord at 24–56 hpf. (A and B) sfpq;Tg(dlx4.6:GFP) embryos. Lack of supra-optic commissure (white arrow) and posterior commissure (white arrowhead) in coma (n = 8/32) is revealed by acetylated tubulin staining. (C and D) Dorsal view, anterior to the left of acetylated tubulin staining showing hindbrain disorganized axonal tracks in sfpq mutant (D; green arrowhead, n = 8) compared to siblings (C; n = 26). (E and F) Dorsal view, anterior to the left of GFP+ motor neurons in sfpq; Tg(isl1:gfpl) siblings (E) and mutant (F), showing cranial motor neuronal clusters lacking axonal projections in the mutant (red arrowhead, n = 7). (G–N) Lateral view, anterior to the left, of confocal live imaging, showing temporal defect in axogenesis in the majority of spinal motor neurons in a mutant (H, J, L, and N; n = 14) compared to a sibling (G, I, K, and M; n = 42) in the Tg(mnx1:GFP) background. (O and P) Lateral view, anterior to the left, of SV2 antibody staining of siblings (O) and sfpq null mutant (P), showing pre-synaptic protein in the few axons formed in sfpq mutants (n = 9). Scale bar, 100 μm.

sfpq−/− ventral neuro-progenitors do not show significant change in motor to interneuron ratio (Figure 3M; isl1/isl2a staining; data not shown). Conversely, donor wild-type MNs form functional axons (Figures 3 C, 3D, and 3N; n = 7), eliciting muscle twitching, in 30 hpf sfpq−/− mutant embryos. The donor neurons show some expected late axonal deformation in the strongly affected 3 dpf mutants (Figures 3 E and 3F).

SFPQ Regulates a Specialized Set of Transcripts
To uncover the molecular complexity driving loss of motility in sfpq−/−, we analyzed the mutant transcriptome at onset of the phenotype. Nimblegen microarray transcriptome analysis, on triplicate samples of total RNA extracted from 50 sfpq−/−;Tg(mnx1:GFP) and sibling embryos at the 22 ss, shows 571 of the 38,489 probes differentially expressed (99% confidence level). The vast majority of these transcripts (566/571) were downregulated in the homozygous sfpq mutant, and only five were overexpressed (Figure 4A). Validation of the microarray data was achieved by qPCR on randomly picked downregulated transcripts (Figure 4B).

Cell transplantations in spinal cord territory of the neural plate at ~70% epiboly show that sfpq−/− MNs transplanted into wild-type embryos fail to show motor axon projections in 30 hpf host embryos (Figures 3G–3L and 3N; n = 8). Transplanted
ArrayStar analysis with the ZFIN gene annotation file provided annotation with gene ontology (GO) terms for only 8% (3,043 probes) of the whole chip and 7% (41 probes) of the SFPQ differentially expressed set (DES). With the customized gene annotation file generated (see STAR Methods), the 81% (29,364 probes) of the chip was mapped to gene identities and 70% (26,798 probes) provided GO terms for the total set on the array. Of the DES, 81% (461 of the 571 probes) were mapped to gene symbols (Table S1) and 74% (424 probes) of these are associated with GO terms (Table S2). Of the five upregulated in the DES, only three are mapped to gene symbols. Out of the 26,798 total annotated probes, a total of 16,745 whole chip were annotated with GO terms. In total, 14 of the 59 GO terms at level 2 of the GO term hierarchy were enriched in the annotated array (p < 0.05 after multiple testing correction; Figure 4C). Considering all levels of hierarchy, only 192 of the 8,624 GO terms represented in the total annotated array set were found enriched in the DES. These identified an unambiguously specific enrichment for molecules involved in cell adhesion, cell junctions, neuronal/synaptic structures, glutamate, and Wnt signaling (Figure 4C; Table S2). Moreover, three upregulated transcripts (pard3, arvcbf, and dgt1) are involved in cell adhesion (Kausalya et al., 2004), cell polarity (Shi et al., 2003), adherens junction complexes (Gladden et al., 2010; Mariner et al., 2000), and dendritic spine morphogenesis (Zhang and Macara, 2006). Finally, 21% of the SFPQ transcriptome code for proteins identified as part of the synaptic proteome. The mutants undergo a substantial downregulation of neuronal-specific transcripts (genes listed in Figure S4) despite other possibilities, we assessed whether SFPQ-regulated transcripts were present in any unprocessed form in wild-type neurites. We chose to screen for the first alternative intron of a series of SFPQ-dependent neuronal transcripts. Introns were detected in neurites of wild-type embryos for 11 of 36 neuronal transcripts tested (Figures 5G–5M; Table S3; at least 20 embryos per probe), but undetectable in homozygous sfpq mutants, suggesting that unprocessed transcripts may be abundant in specific neuronal populations and degraded, or not produced, in sfpq mutants. The axonal presence of intronic sequences is coincidental with detection of the core spliceosome protein snRNP70 (U1 snRNP), expressed in neurites across the whole of the CNS, including motor axons (Figures 5N and 5O).Non-nuclear SFPQ Rescues the Motor Axon Defect of the sfpq Mutant

To assess whether the presence of SFPQ in axons is functionally relevant to MN function, we tested whether a cytoplasmic form of the protein was able to rescue the neuronal defect in the mutant. We made use of the human truncated protein lacking the C-terminal non-canonical nuclear localization signal (NLS), previously shown to prevent SFPQ nuclear import without affecting the structure and functional domains (Dye and Patton, 2001). We found that injection of the RNA coding for this cytoplasmic form is able to restore both motor axon growth and movement in the homozygous sfpq mutant (Figures 6E–6L; Movie S3; n = 37 homozygous embryos). Deletion of a second putative minor NLS sequence sitting in the coiled-coil domain (see Figure 1E) affected the protein structure and function (Lee et al., 2015; data not shown). The motor rescue can’t be
Figure 4. Sfpq Loss of Function Affects a Specific Set of Transcripts

(A) Scattered plot of the expression level of the total probe sets in the array. The 38,489 dots represent total individual probes for genes or expressed sequence tags (ESTs). The gene expression level is expressed in relative arbitrary units of fluorescence intensity on a logarithmic scale. Genes whose expression level is identical between the two groups lie on the diagonal line and the non-identical ones lie outside of the two lines parallel to the central diagonal purple line. The dots positioned above the upper diagonal line represent probe sets that are upregulated and the dots appearing below represent probe sets that are downregulated in the mutants as compared to siblings. Black dots represent those 571 probes with expression level at least 2-fold significantly different (p < 0.05, moderated t test) between sfpq+/-;C0/+ and sfpq-/-;C0/C0 (Tg:mnx1 gfp) embryos. A high correlation ($R^2 = 0.98$) was observed for the linear regression performed on the gene set.

(B) Validation of microarray results by qRT-PCR performed on randomly picked downregulated transcripts. The gene expression data from microarray and qPCR exhibited high correlation (Spearman’s rho test; $r(6) = 1, p = 0.01$). Statistical comparison was performed using paired t test with Bonferroni correction for multiple

| GO Terms                        | GO ID   | P-Value      | Fold enrichment |
|---------------------------------|---------|--------------|-----------------|
| Regulation of signalling        |         |              |                 |
| Positive regulation of non-canonical Wnt signalling | 2000052 | 0.00000202 | 19              |
| Ephrin receptor signalling      | 48013   | 0.0102       | 11              |
| Glutamate receptor signalling   | 7215    | 0.0000282    | 9.9             |
| Negative regulation of canonical Wnt signalling | 90090 | 0.00000512 | 9               |
| Regulation of Ras protein signal transduction | 46578 | 0.0183       | 2.5             |
| Negative regulation of response to stimulus | 48585 | 0.000531    | 2.26            |
| Negative regulation of cell communication | 10648 | 0.00276      | 2.1             |
| Membrane                        |         |              |                 |
| anchored to membrane            |         |              |                 |
| outer membrane-bounded periplasmic space | 31225 | 0.0069       | 15.25           |
| cell surface                    |         |              |                 |
| integral to plasma membrane     |         |              |                 |
| Receptor activity               |         |              |                 |
| glutamate receptor activity     |         |              |                 |
| transmembrane receptor protein tyrosine kinase activity | 5321 | 0.000952 | 5.2             |
| Gated channel activity          |         |              |                 |
| excitatory extracellular        |         |              |                 |
| ligand-gated ion channel activity | 5231 | 0.000952 | 5.2             |
| ligand-gated ion channel activity | 15276 | 0.00359 | 4.8             |
| ion gated channel activity      |         |              |                 |
| Synaptic part                   |         |              |                 |
| postsynaptic membrane           |         |              |                 |
| Regulation gene expression      |         |              |                 |
| regulation of RNA splicing      |         |              |                 |
| Growth                          |         |              |                 |
| developmental growth            |         |              |                 |
| involved in morphogenesis       |         |              |                 |
| axis elongation                 |         |              |                 |
| Cell junction                   |         |              |                 |
| Biological Adhesion             |         |              |                 |
| Cell adhesion                   |         |              |                 |
| Synapse                         |         |              |                 |

(legend continued on next page)
explained by residual nuclear localization of hsfpq\(\Delta\text{NLS}\), as the threshold required for rescue by injection of the wild-type form is well above detection level (Table S4) and mosaic co-injection of pmnx1:GFP and pCS2:hsfpq\(\Delta\text{NLS}\) DNA in the mutant shows axonal expression of SFPQ in restored axons, with nuclei lacking detectable HSFPQ protein (Figures 6E and 6F; \(n = 14\); Figure S3A).

The motor rescue obtained with the hsfpq\(\Delta\text{NLS}\) is accompanied by a strong morphological phenotype (short body axis and variable cyclopia) likely induced by cytoplasmic accumulation of SFPQ in the developing embryo. This phenotype is observed in homozygous, but not in heterozygous and wild-type siblings (Figures 6A–6D). The absence of phenotype in siblings is explained by the presence of GFP-hsfpq\(\Delta\text{NLS}\) in their nuclei, due to correct transport elicited through complex with endogenous SFPQ (inset in Figure 6D). Finally, qPCR done on extracts from control and hsfpq\(\Delta\text{NLS}\)-injected sfpq\(+/-\) progeny for ten randomly picked neuronal SFPQ-dependent transcripts shows rescue of expression level for six of these by the SFPQ null background, while still enabling NMJ formation and normal motor axonal development. Perturbation of motor axon morphology in sfpq null mutants may suggest a causal link between these two mutations and the human motor degenerative disease. However, segregation data and additional cases carrying mutations in the same residues are needed to support this possibility further.

**DISCUSSION**

Our study shows that the ubiquitously expressed multi-functional splicing factor SFPQ is functionally required for expression of a restricted set of transcripts during development and carries an essential non-nuclear function in developing motor axons. Moreover, we identify two human sfpq point mutations affecting the protein function in zebrafish MNs. We show that these human mutated proteins are affected in their ability to localize in motor axons and to elicit normal motor axon morphology in an endogenous SFPQ null background, while still enabling NMJ formation and normal motility, indicating a likely role for axonal SFPQ in normal motor axonal development, connectivity, and thereby motility in zebrafish. Furthermore, two fALS-linked mutations, lying within the coiled-coil domain of SFPQ, significantly impede its distribution into the distal axon and affect motor axonal branching in zebrafish, showing the importance of the coiled-coil domain in axonal localization and normal motor axon development. Perturbation of motor axon morphology in sfpq mutant embryos rescued by fALS-linked variants may suggest a causal link between these two mutations and the human motor degenerative disease. However, segregation data and additional cases carrying mutations in the same residues are needed to support this possibility further.
human motor function and a possible developmental origin to some forms of motor degeneration.

**SFPQ in RNA Transport and Cytoplasmic Processing**

SFPQ has been previously described as associated with transport granules in cell culture (Kanai et al., 2004) and very recently shown to be required directly or indirectly for transport of RNA regulon in sensory neurons (Cosker et al., 2016). Our study provides the first in vivo evidence that the cytoplasmic pool of SFPQ is required for axon development through regulation of RNA transport and/or processing in MNs. As transport granules are indeed found to be essential for neuronal function (Kanai et al., 2004), one possible requirement of SFPQ in axon may be transport, as suggested by Cosker and colleagues in cultured sensory neurons.

Figure 5. SFPQ Protein Is Found in Axons, with Unprocessed RNA Targets and Spliceosome Component U1 snRNP70

Transverse single confocal sections of 24 hpf (A, N, and O) and 48 hpf (B–M) forebrain (A and I–K) or spinal cord dorsal to the top (G, H, L, and M) or to the left (B–D), and lateral view of head, anterior to the left (E and F).

(A–F) SFPQ protein expression shown by antibody staining (A–D) or GFP-tagged ectopically expressed protein (E and F). Identity of red, green, and blue staining is labeled on bottom right corner of each picture. Arrow shows axonal localization.

(G–K) Localization of first alternative introns (in red) of SFPQ-dependent transcripts, bcas3 (G and H), and gnao1a (I–K). Axons are stained by acetylated tubulin antibody in green.

(L and M) High-resolution imaging of bcas3 intron detection (L; red) in motor axons stained with acetylated tubulin antibody (M; green).

(N and O) Double antibody staining of U1 snRNP70 proteins (N) with acetylated tubulin (O; merged channels) in spinal axons. Arrows are pointing to motor axons. Scale bar, 25 μm.
Figure 6. Non-nuclear Sfpq Rescues Loss of Transcripts and Axonal Phenotype in the coma Mutant
Lateral view of 30 hpf zebrafish (A–D) and spinal cord (G–L), anterior to the left. (A–D) General morphology of $sfpq^{-/-}$ (A); $sfpq^{-/-}$ siblings (B); $sfpq^{-/-}$ injected with GFP-hsfpq\text{D\text{NLS}} (C) and $sfpq^{+/-}$ injected with GFP-hsfpq\text{D\text{NLS}} (D). Insets showing localization of the human GFP-tagged protein in early ss embryos, CAAX-Cherry in red.
neurons. However, we found that complete loss of SFPQ function, such as the one they induced in sensory neurons, severely affects transcripts coding for transport proteins such as dynein chains (Table S1), making it difficult to distinguish direct from indirect requirement in transport. More compelling is the ability of our cytoplasmic version of SFPQ to rescue motor activity and restore a subset of neuronal transcripts in the zebrafish mutant, strongly demonstrating a cytoplasmic role in transcript maintenance. One such function may be to protect transcripts (intron retaining or not) from nonsense-mediated decay (Yap et al., 2012). Intron retention has been previously reported in neurons (Bell et al., 2010). Our study shows intron retention in developing axons, and detection of U1 snRNP70 spliceosome protein, opening a third possibility: SFPQ may be involved in some form of extra-nuclear processing. Tissue-specific alternative splicing has long been recognized as a powerful source of molecular diversity. This mechanism is particularly important in the nervous system, where isoform variation is involved in synaptic formation, neurotransmission, synaptic plasticity, cell recognition, and ion channel function (Grabowski and Black, 2001; Kiebler et al., 2013; Norris and Calarco, 2012). While post-transcriptional regulation commonly occurs in the nucleus, recent studies (Bell et al., 2010; Buckley et al., 2014; Glanzer et al., 2005; König et al., 2007) indicate that modification of 3′ UTR sequences, alternative polyadenylation, and even splicing may be in some situation cytoplasmic, which opens the possibility of sophisticated local axonal and dendritic RNA processing occurring within developing and mature neurons.

The possibility that a subset of transport granules may be specialized for local processing of specific types of introns is a very attractive idea in the context of neurons. Many of the neuronal populations controlling our behaviors have the cell nucleus tens of centimeters away from the site of active synapses. The option of local splicing would be an advantageous mechanism to generate alternative transcripts known to be required for the rapid modulation of synaptic connectivity and other neuronal functions. Our SFPQ mutants are ideal models to now address these possibilities.

**SFPQ and Neurodegenerative Disorders**

SFPQ plays a key role in processing pre-mRNA coding for proteins that are central to several neurodegenerative conditions. It regulates tau pre-mRNA splicing by suppressing Tau exon 10 inclusion and thus regulating the balance of Tau4R/Tau3R ratio (Ray et al., 2011), associated with tauopathies such as frontotemporal dementia (FTD; Cairns et al., 2007; Connell et al., 2001; Grover et al., 1999; Hasegawa et al., 1998; Hasegawa et al., 1999; Hutton et al., 1998; Mackenzie et al., 2010; Spillantini et al., 1998). SFPQ is expressed at higher levels in the hippocampus and cortex of human brain, the regions principally affected by tauopathy (Ray et al., 2011). The nucleo-cytoplasmic redistribution of SFPQ under pathological conditions is similar to that reported for the RNA-binding proteins, TDP-43 and FUS, in ALS and FTLD (Neumann et al., 2006; Urwin et al., 2010; Vance et al., 2009). Microarray data analysis of transcripts in cortical neurons of Alzheimer’s disease (AD) patients identified upregulated SFPQ expression (Guttula et al., 2012). In addition to neurodegenerative diseases, SFPQ is also associated with other neurodevelopmental disorders. Recent studies have associated SFPQ and its partner NONO to autism spectrum disorders and intellectual disability, and found SFPQ upregulated in the frontal cortex of bipolar disorder patients (Nakatani et al., 2006; Le-Niculescu et al., 2008).

Our work showed U1-snRNP70 and SFPQ together in motor axons. Intriguingly, two very recent publications showed that this U1 spliceosome protein functionally interacts with the ALS-causative RNA processing protein FUS (Yu et al., 2015; Sun et al., 2015). In these studies, the expectation is that these interact inside the nucleus. However, we find partial co-localization of SFPQ with FUS in axons and FUS has now been found enriched in synapses, suggesting that the synaptic dysfunction observed in FUS mutant may be due to local axonal/synaptic loss of function.

Our work puts a new light on these recent findings, shifting the focus onto cytoplasmic interaction of these proteins and RNAs in the normal context and potential dysregulation of these interactions in neurodegenerative disorders. Neurodegeneration has mostly been understood as caused by sequestration of splicing factors and/or other proteins in cytoplasmic aggregates that induce loss of essential protein function in the nucleus and/or detrimental toxicity in the cytoplasm. However, our data support the intriguing possibility that some neurodegenerative and neurodevelopmental disorders may arise from progressive loss of a cytoplasmic RNA processing function in axons and synapses, triggered during development. Such a mechanism is suggested by a very recent study showing that HNRNP2B1 is shaping the splicing landscape of neurons, a role specifically affected by ALS-associated mutations in this gene (Martinez et al., 2016). Neuronal homeostasis may be dependent upon a complex balance between nuclear and extra-nuclear RNA processing events controlled by proteins such as SFPQ, HNRNP2A, U1 snRNP70, and FUS, a balance that, when disturbed, may contribute mechanistically to a wide range of neurological disorders.

[E] Whole-mount detection of SFPQ (red) in a 28 hpf sfpq-/- mutant, injected at one-cell stage with pmx1:GFP and pCS2:hSFPQΔNLS DNA, expressing these mosaically. Arrow shows motor axon. Note the negative nuclei (arrowhead).

(F) Measurement of ventral motor axon length in siblings and homozygous mutant uninjected or injected with RNA (DNLS) or DNA (MOSAIC DNLS) coding for hSFPQΔNLS. For the controls and RNA injections, the ventral motor axons were measured in five embryos over five–somite length upstream of the cloaca. For mosaic DNA-injected samples, measures were done only in seven homozygous mutants, in the same trunk area for the rare SFPQ- neurons.

(G–L) Lateral view, anterior to the left of spinal cords, showing rescue of the axonal defect both in the anterior (G and J) and posterior (H and K) trunk of sfpq-/- : Tg (mmx1:GFP) by injection of the ΔNLS human SFPQ. Sibling axons are unaffected by the injection (I and L).

(M) qPCR results for six out of ten DES transcripts from 32 hpf siblings and sfpq-/- mutant embryos uninjected or injected with human ΔNLS sfpq RNA. Bars show expression fold changes compared to the expression level of the transcript in uninjected sibling embryos taken as reference. The four transcripts not plotted did not show any improvement after ΔNLS rescue.
Figure 7. Human Mutations Affect SFPQ Localization and Motor Development in the Zebrafish
(A–K) Lateral (A–F) and transverse (G–K, N, and O) views of the spinal cord in sfpq−/− mutant (A, B, and H), siblings (C and G), and homozygous mutant rescued by wild-type human Sfpq (D and I) or L534 (E, J, and O) or N533 (F and K) mutant human Sfpq.

(L) Distribution of SFPQ variants in all exome-sequenced human individuals. In red, the position of the variants only found in FALS patients.

(M) Schematic of the wild-type hSFPQ protein and the seven variants cloned and tested in double-blind rescue experiments in the Tg(mnx1:GFP) background. S8N (c.23G>A) is present in 4 SALS and 24 ExAC samples, showing a modest enrichment in SALS (p = 0.029, two-tailed Fisher’s test). P51L (c.152C>T) is private to a single SALS patient and absent from all controls. M469V (c.1405A>G) is in a single SALS patient and twice in ExAC (p = 0.071). A515V (c.1544C>T) and A515T (c.1543G>A) are in two and one ExAC normal individuals, respectively, and are located in the coiled-coil domain close to the two FALS variants we identified.

(N and O) Close-up of the motor axon and its environment, stained for SFPQ in red and GFP in green in the sfpq−/−; Tg(mnx1:GFP) rescued by injection of the human (N and N0) or L-mutated form (O and O0). Arrowheads show localization of SFPQ in motor axons.

(P) Quantification of the α-SFPQ red fluorescent signal in motor axons on confocal stacks, for five pairs of motor axons per 48 hpf embryo stained with SFPQ antibody. Measurement was done in no less than 30 (maximum, 46) embryos per variant injected. Injection is done in sfpq−/−; Tg(mnx1:GFP) incross progeny. The embryos showing poor signal intensity were genotyped and were all homozygous mutants.

(Q) Quantification of the length of ventral motor axons. Measures were made for five segments per 48 hpf embryo (five somites anterior to the cloaca) on confocal stacks, using Fiji Single Neurite Tracer in no less than 28 (maximum, 41) embryos per variant injected. Injection is done in sfpq−/−; Tg(mnx1:GFP) incross progeny. The embryos showing shorter axon length were genotyped and were all homozygous mutants. Asterisks indicate highly significant reduction in axon lengths compared to wild-type (pairwise ANOVA, ***p < 0.0001). The two significantly different variants show same difference when comparing pairwise to the other variants. All other variants are not significantly different from wild-type.

(legend continued on next page)
STAR METHODS

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SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures, four tables, and three movies and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2017.03.026. A video abstract is available at http://dx.doi.org/10.1016/j.neuron.2017.03.026#mmc6.

AUTHOR CONTRIBUTIONS

C.H. designed the research project and supervised and secured funding for the zebrafish team. S.T.-J. and C.H. wrote the manuscript. S.T.-J., T.F., P.M.G., R.T., V.S., H.B., and A.P.-V. performed and conceived experiments in zebrafish, and E.B. and S.T. provided the computational analysis of zebrafish protein structure, and W.S.T. performed the genetic mapping of the zebrafish mutant. We thank James Patton (Vanderbilt) for the human wild-type and NLS deletion SFPQ constructs, and Hazel Sive (MIT) for providing the whitesnake zebrafish mutant. We thank Michael Simpson, Division of Genetics and Molecular Medicine, Kings College London, for the assembly and variant calling of the KCL exome data. This work is supported by the Medical Research Foundation (MRF-060-0003-RG) to B.N.S.; the Medical Research Council (G0901525) and the BBSRC (BB/P001599/1) to C.H.; the Wellcome Trust to C.H. and C.E.S. (MC G 1000733); MRC (G0900688) and Motor Neuron Disease Association and Heaton Ellis Trust to C.E.S.; NHMRC of Australia (APP1095215) and MND Australia Leadership grant to I.P.B.; and the MND Research Institute of Australia Grants-in-Aid to I.P.B., K.L.W., and G.A.N.

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REFERENCES

Allende, M.L., and Weinberg, E.S. (1994). The expression pattern of two zebrafish achaete-scute homolog (ash) genes is altered in the embryonic brain of the cyclops mutant. Dev. Biol. 166, 509–530.

Amoyel, M., Cheng, Y.C., Jiang, Y.J., and Wilkinson, D.G. (2005). Wnt1 regulates neurogenesis and mediates lateral inhibition of boundary cell specification in the zebrafish hindbrain. Development 132, 775–785.

Barry, G., Briggs, J.A., Vanichkina, D.P., Poth, E.M., Beveridge, N.J., Ratnu, V.S., Nayler, S.P., Nones, K., Hu, J., Bredy, T.W., et al. (2014). The long non-coding RNA Gomafu is acutely regulated in response to neuronal activation and involved in schizophrenia-associated alternative splicing. Mol. Psychiatry 19, 486–494.

Bell, T.J., Miyashiro, K.Y., Sul, J.Y., Buckley, P.T., Lee, M.T., McCullough, R., Jochems, J., Kim, J., Cantor, C.R., Parsons, T.D., and Eberwine, J.H. (2010). Intron retention facilitates splice variant diversity in calcium-activated big potassium channel populations. Proc. Natl. Acad. Sci. USA 107, 21152–21157.

Brand, M., Granato, M., and Nueslein-Volhard, C. (2002). Keeping and raising zebrafish. In Zebrafish, A Practical Approach, C. Nueslein-Volhard and R. Dahm, eds. (Oxford University Press), pp. 7–37.

Buckley, P.T., Khaladkar, M., Kim, J., and Eberwine, J. (2014). Cytoplasmic intron retention, function, splicing, and the sentinel RNA hypothesis. Wiley Interdiscip. Rev. RNA 5, 223–230.

Cains, N.J., Bigio, E.H., Mackenzie, I.R., Neumann, M., Lee, V.M., Hatanpaa, K.J., White, C.L., 3rd, Schneider, J.A., Grinberg, L.T., Halliday, G., et al.; Consortium for Frontotemporal Lobar Degeneration (2007). Neuropathologic diagnostic and nosologic criteria for frontotemporal lobar degeneration: consensus of the Consortium for Frontotemporal Lobar Degeneration. Acta Neuropathol. 114, 5–22.

Carl, M., Bianco, I.H., Bajoghli, B., Aghaallaei, N., Czerny, T., and Wilson, S.W. (2007). Wnt/Axin1/beta-catenin signaling regulates asymmetric nodal activation, elaboration, and concordance of CNS asymmetries. Neuroreport 18, 393–405.

Chanas-Sacre’, G., Mazy-Servais, C., Wattiez, R., Pirard, S., Register, B., Patton, J.G., Belachew, S., Malgrange, B., Moonen, G., and Leprince, P. (1999). Identification of PSF, the polypyrimidine tract-binding protein-associated splicing factor, as a developmentally regulated neuronal protein. J. Neurosci. Res. 57, 62–73.

Chang, J., Gilman, S.R., Chiang, A.H., Sanders, S.J., and Vitkup, D. (2015). Genotype to phenotype relationships in autism spectrum disorders. Nat. Neurosci. 18, 191–198.

Cheng, Y.C., Amoyel, M., Qiu, X., Jiang, Y.J., Xu, Q., and Wilkinson, D.G. (2004). Notch activation regulates the segregation and differentiation of rhombomere boundary cells in the zebrafish hindbrain. Dev. Cell 6, 539–550.
exon 10. FEBS Lett.

FTDP-17 mutations N279K and S305N in tau produce increased splicing of Hasegawa, M., Smith, M.J., Iijima, M., Tabira, T., and Goedert, M. (1999). under the control of the islet-1 promoter/enhancer. J. Neurosci.

ulon to promote axon viability. Nat. Neurosci.

Cosker, K.E., Fenstermacher, S.J., Pazyra-Murphy, M.F., Elliott, H.L., and Cooper, M.S., Szeto, D.P., Sommers-Herivel, G., Topczewski, J., Solnica-Krezel, L., Kang, H.C., Johnson, I., and Kimelman, D. (2005). Visualizing morphogenesis in transgenic zebrafish embryos using BODIPY TR methyl ester dye as a vital contrastant for GFP. Dev. Dyn. 232, 359–368.

Casper, K.E., Fenstermacher, S.J., Pazyra-Murphy, M.F., Elliott, H.L., and Segal, R.A. (2016). The RNA-binding protein SFPQ orchestrates an RNA regulon to promote axon viability. Nat. Neurosci. 19, 690–696.

Derrien, T., Johnson, R., Bussotti, G., Tanzer, A., Djebali, S., Tilgner, H., Guernec, G., Martin, D., Merkel, A., Knowles, D.G., et al. (2012). The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. Genome Res. 22, 1775–1789.

Dorsky, R.I., Shieldahl, L.C., and Moon, R.T. (2002). A transgenic Lef1/beta-catenin-dependent reporter is expressed in spatially restricted domains throughout zebrafish development. Dev. Biol. 241, 229–237.

Dye, B.T., and Patton, J.G. (2001). An RNA recognition motif (RRM) is required for the localization of PTB-associated splicing factor (PSF) to subnuclear speckles. Exp. Cell Res. 263, 131–144.

Figuerola, A., Fujita, Y., and Gorospe, M. (2009). Hacking RNA: Hakai promotes tumorigenesis by enhancing the RNA-binding function of PSF. Cell Cycle 8, 3648–3651.

Flanagan-Steet, H., Fox, M.A., Meyer, D., and Sanes, J.R. (2005). Neuronal morphogenesis in transgenic zebrafish embryos using BODIPY TR methyl ester dye as a vital contrastant for GFP. Dev. Dyn. 232, 359–368.

Kubota, M., Kasahara, T., Iwamoto, K., Ishiwata, M., Miyauchi, T., and Kato, T. (2010). Therapeutic implications of down-regulation of cyclophilin D in bipolar disorder. Int. J. Neuropsychopharmacol. 13, 1355–1368.

Kwan, K.M., Fujimoto, E., Grabber, C., Mangum, B.D., Hardy, M.E., Campbell, D.S., Parant, J.M., Yost, J.H., Kanki, J.P., and Chien, C.B. (2007). The Tol2kit: a multisite gateway-based construction tool for Tol2 transposon transgenesis constructs. Dev. Dyn. 236, 3088–3099.

Lagier-Tourenne, C., Polymenidou, M., Hutt, K.R., Yu, A., Baughn, M., Huelga, S.C., Citrario, K.M., Ling, S.C., Liang, T.Y., Mazur, C., et al. (2012). Divergent roles of ALS-linked proteins FUS/TLS and TDP-43 intersect in processing long pre-mRNAs. Nat. Neurosci. 15, 1488–1497.

Lee, M., Sadowska, A., Bekere, I., Ho, D., Gully, B.S., Lu, Y., Iyer, K.S., Trethewia, J., Fox, A.H., and Bond, C.S. (2015). The structure of human SFPQ reveals a coiled-coil mediated polymer essential for functional aggregation in gene regulation. Nucleic Acids Res. 43, 3826–3840.

Lowery, L.A., Rubin, J., and Sive, H. (2007). Whitesnake/spq is required for cell survival and neuronal development in the zebrafish. Dev. Dyn. 236, 1347–1357.

Lukong, K.E., Hu, and Richardson, S. (2009). BPK phosphorylates PSF promoting its cytoplasmic localization and cell cycle arrest. Cell. Signal. 21, 1415–1422.

Mackenzie, I.R., Neumann, M., Bigio, E.H., Cairns, N.J., Alafuzoff, I., Kril, J., Kovacs, G.G., Betti, H., Halliday, G., Holm, I.E., et al. (2010). Nomenclature and nosology for neuropathologic subtypes of frontotemporal lobar degeneration: an update. Acta Neuropathol. 119, 1–4.

Mariner, D.J., Wang, J., and Reynolds, A.B. (2000). ARVCF localizes to the nucleus and adherens junction and is mutually exclusive with p120(ctn) in frontotemporal lobar degeneration: an update. Acta Neuropathol. 119, 1–4.

Martinez, F.J., Pratt, G.A., Van Nostrand, E.L., Batra, R., Huelga, S.C., Kapell, K., Freese, P., Chun, S.J., Ling, K., Gelbion-Burkhart, C., et al. (2016). Protein-RNA networks regulated by normal and ALS-associated mutant HNRNPA2B1 in the nervous system. Neuro 92, 780–795.

Mercer, T.R., and Mattick, J.S. (2013). Structure and function of long non-coding RNAs in epigenetic regulation. Nat. Struct. Mol. Biol. 20, 300–307.

Hutton, M., London, C.L., Rizzu, P., Baker, M., Froelich, S., Houlden, H., Pickering-Brown, S., Chakraverty, S., Isaacs, A., Grover, A., et al. (1998). Association of missense and S’-splice-site mutations in tau with the inherited dementia FTD-P17. Nature 393, 702–705.

Johnson, R. (2012). Long non-coding RNAs in Huntington’s disease neurodegeneration. Neurobiol. Dis. 46, 245–254.

Kanai, Y., Dohmae, N., and Hirokawa, N. (2004). Kinesin transports RNA: isolation and characterization of an RNA-transporting granule. Neuro 43, 513–525.

Kaulsa, P.J., Phua, D.C., and Hunziker, W. (2004). Association of ARVCF with zona occludens (ZO-1 and ZO-2: binding to PDZ-domain proteins and cell-cell adhesion regulate plasma membrane and nuclear localization of ARVCF. Mol. Biol. Cell 15, 5503–5515.

Krauss, S., Johansen, T., Korch, V., and Fjose, A. (1991). Expression of the zebrafish paired box gene pax[(cf-b) during early neurogenesis. Development 113, 1193–1206.

Köning, H., Matter, N., Bader, R., Thiele, W., and Müller, F. (2007). Splicing segregation: the minor spliceosome acts outside the nucleus and controls cell proliferation. Cell 131, 718–729.

Kubota, M., Kasahara, T., Iwamoto, K., Ishiwata, M., Miyauchi, T., and Kato, T. (2010). Therapeutic implications of down-regulation of cyclophilin D in bipolar disorder. Int. J. Neuropsychopharmacol. 13, 1355–1368.

Lee, M., Sadowska, A., Bekere, I., Ho, D., Gully, B.S., Lu, Y., Iyer, K.S., Trethewia, J., Fox, A.H., and Bond, C.S. (2015). The structure of human SFPQ reveals a coiled-coil mediated polymer essential for functional aggregation in gene regulation. Nucleic Acids Res. 43, 3826–3840.

Lowery, L.A., Rubin, J., and Sive, H. (2007). Whitesnake/spq is required for cell survival and neuronal development in the zebrafish. Dev. Dyn. 236, 1347–1357.

Lukong, K.E., Hu, and Richardson, S. (2009). BPK phosphorylates PSF promoting its cytoplasmic localization and cell cycle arrest. Cell. Signal. 21, 1415–1422.

Mackenzie, I.R., Neumann, M., Bigio, E.H., Cairns, N.J., Alafuzoff, I., Kril, J., Kovacs, G.G., Betti, H., Halliday, G., Holm, I.E., et al. (2010). Nomenclature and nosology for neuropathologic subtypes of frontotemporal lobar degeneration: an update. Acta Neuropathol. 119, 1–4.

Mariner, D.J., Wang, J., and Reynolds, A.B. (2000). ARVCF localizes to the nucleus and adherens junction and is mutually exclusive with p120(ctn) in E-cadherin complexes. J. Cell Sci. 113, 1481–1490.

Martinez, F.J., Pratt, G.A., Van Nostrand, E.L., Batra, R., Huelga, S.C., Kapell, K., Freese, P., Chun, S.J., Ling, K., Gelbion-Burkhart, C., et al. (2016). Protein-RNA networks regulated by normal and ALS-associated mutant HNRNPA2B1 in the nervous system. Neuro 92, 780–795.

Mercer, T.R., and Mattick, J.S. (2013). Structure and function of long non-coding RNAs in epigenetic regulation. Nat. Struct. Mol. Biol. 20, 300–307.
Miscimarra, L., Stein, C., Millard, C., Kluge, A., Cartier, K., Freebairn, L., Hansen, A., Shriberg, L., Taylor, H.G., Lewis, B., and Iyengar, S.K. (2007). Further evidence of pleiotropy influencing speech and language: analysis of the DYX8 region. Hum. Hered. 63, 47–58.

Nakada, C., Satoh, S., Tabata, Y., Arai, K., and Watanabe, S. (2006). Transcriptional repressor foxl1 regulates central nervous system development by suppressing shh expression in zebrafish. Mol. Cell. Biol. 26, 7246–7257.

Nakatani, N., Hattori, E., Ohnishi, T., Dean, B., Iwayama, Y., Matsumoto, I., Kato, T., Osumi, N., Higuchi, T., Niwa, S., and Yoshikawa, T. (2006). Genome-wide expression analysis detects eight genes with robust alterations by suppressing shh expression in zebrafish. Mol. Cell. Biol., 26, 7246–7257.

Kato, T., Osumi, N., Higuchi, T., Niwa, S., and Yoshikawa, T. (2006). Mutations in the tau gene in familial multiple system tauopathy with presenile dementia. Proc. Natl. Acad. Sci. USA 96, 7701–7706.

Nakada, C., Satoh, S., Tabata, Y., Arai, K., and Watanabe, S. (2006). Transcriptional repressor foxl1 regulates central nervous system development by suppressing shh expression in zebrafish. Mol. Cell. Biol. 26, 7246–7257.

Passon, D.M., Lee, M., Rackham, O., Stanley, W.A., Sadowska, A., Filipovska, A., Fox, A.H., and Bond, C.S. (2012). Structure of the heterodimer of human spliceosome Sm proteins: a novel family of apoptosis modulators? J. Cell. Biochem. 94, 5–24.

Passon, D.M., Lee, M., Rackham, O., Stanley, W.A., Sadowska, A., Filipovska, A., Fox, A.H., and Bond, C.S. (2012). Structure of the heterodimer of human NONO and paracaspase protein component 1 and analysis of its role in subnuclear body formation. Proc. Natl. Acad. Sci. USA 109, 4846–4850.

Passon, D.M., Lee, M., Rackham, O., Stanley, W.A., Sadowska, A., Filipovska, A., Fox, A.H., and Bond, C.S. (2012). Structure of the heterodimer of human NONO and paracaspase protein component 1 and analysis of its role in subnuclear body formation. Proc. Natl. Acad. Sci. USA 109, 4846–4850.

Paton, J.G., Porro, E.B., Galceran, J., Tempst, P., and Nadal-Ginard, B. (1993). Cloning and characterization of PSF, a novel pre-mRNA splicing factor. Genes Dev. 7, 393–406.

Peng, R., Dye, B.T., Pérez, I., Barnard, D.C., Thompson, A.B., and Patton, J.G. (2002). PSF and p54nrb bind a conserved stem in U5 snRNA. RNA 8, 1334–1347.

Postlethwait, J.H., Johnson, S.L., Midson, C.N., Talbot, W.S., Gates, M., Ballinger, E.W., Africa, D., Andrews, R., Carl, T., Eisen, J.S., et al. (1994). A linkage group framework for the zebrafish genome. Science 260, 699–703.

Rabin, M., Wen, X.L., Hepburn, M., Lubs, H.A., Feldman, E., and Duria, R. (1993). Suggestive linkage of developmental dyslexia to chromosome 13q34-p36. Lancet 342, 178.

Ray, P., Kar, A., Fushimi, K., Havioglu, N., Chen, X., and Wu, J.Y. (2011). PSF suppresses tau exon 10 inclusion by interacting with a stem-loop structure downstream of exon 10. J. Mol. Neurosci. 45, 453–466.

Reifers, F., Böhl, H., Welsh, E.C., Crossley, P.H., Stainier, D.Y., and Brand, M. (1998). Fgf8 is mutated in zebrafish acerebellar (ace) mutants and is required for motor neuron outgrowth in zebrafish. Nucleic Acids Res. 26, 393–406.

Shav-Tal, Y., Cohen, M., Laper, S., Dye, B., Patton, J.G., Vandeerkovijhe, J., and Zipori, D. (2001). Nuclear relocalization of the pre-mRNA splicing factor PSF during apoptosis involves hyperphosphorylation, masking of antigenic epitopes, and changes in protein interactions. Mol. Biol. Cell 12, 2328–2340.

Shav-Tal, Y., Cohen, M., Laper, S., Dye, B., Patton, J.G., Vandeerkovijhe, J., and Zipori, D. (2001). Nuclear relocalization of the pre-mRNA splicing factor PSF during apoptosis involves hyperphosphorylation, masking of antigenic epitopes, and changes in protein interactions. Mol. Biol. Cell 12, 2328–2340.
# STAR★METHODS

## KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Antibodies          |        |            |
| acetylated α-tubulin| Sigma, Mouse IgG2b | Cat#T6793; RRID: AB_477585 |
| GFP                 | Amersham, Rabbit IgG | Cat# TP401; RRID: AB_10013661 |
| F59                 | DSHB, Mouse IgG1 | Cat# 159; RRID: AB_528373 |
| F310                | DSHB, Mouse IgG1 | Cat# F310; RRID: AB_531863 |
| phosho-Histone H3   | Millipore UK, Rabbit-IgG | Cat#06-570; RRID: AB_310177 |
| ZO-1                | Invitrogen, mouse IgG1 | Cat#339100; RRID: AB_87181 |
| HuC/D               | Molecular Probes | Cat#A21271; RRID: AB_221448 |
| α-bungarotoxin      | Invitrogen, Alexa Fluor 488 conjugated | Cat#B13422; RRID: AB_2636927 |
| snRNP70             | AvivaSysBio | N/A |
| SFPQ                | AbCam38148 | Cat#ab38148; RRID: AB_945424 |
| FastDigest Clal     | Thermo Scientific | Cat#FD0144 |
| CAAX-mcherry RNA    | Sean Megason (Scott Fraser) Caltech, CA 91125 | N/A |
| TRIZOL RNA isolation reagent | Invitrogen | Cat#10296010 |
| DNaseI              | Ambion TURBO DNase Free kit | Cat#AM1907 |
| Bacterial and Virus Strains |        |            |
| Zebrafish sfpq complete cDNA | Source Bioscience | IMAGE: 6962536 |
| pDONR221 entry clone | Gateway, Invitrogen | Cat#12536017 |
| Critical Commercial Assays |        |            |
| mMessage Sp6 kit    | Ambion | Cat#AM1340 |
| The Gateway cloning system | Invitrogen | Cat#18248013 |
| Quickchange II Site-Directed Mutagenesis Kit | Agilent Technologies | Cat#200523 |
| TOPO TA Expression Kit | Invitrogen | Cat#K4810-01 |
| High pure RNA isolation kit | Roche | Cat#11828665001 |
| SuperScript Double-Stranded cDNA Synthesis Kit | Invitrogen | Cat#11917020 |
| First strand CDNA synthesis kit | Thermo Scientific (Molecular Biology) | Cat#K1612 |
| Qiaquick PCR purification kit | QIAGEN | Cat#28104 |
| SYBR Green I Master kit | Roche | Cat#04707516001 |
| NimbleGen 12 x 135 k zebrafish gene-expression arrays | Roche Diagnostics Limited | Cat#05545862001 |
| Deposited Data |        |            |
| Microarray Data     | ArrayExpress | ArrayExpress: E-MTAB-5559 |

### Experimental Models: Organisms/Strains

- **Zebrafish:** Tg(isl1:GFP)rw0 | Higashijima et al., 2000 | ZFIN ID: ZDB-TGCONSTRCT-070117-161
- **Zebrafish:** Tg(1.4dlx5a-dlx6a:gfpiot1 | Ghanem et al., 2003 | ZFIN ID: ZDB-TGCONSTRCT-070117-17
- **Zebrafish:** Tg(TOP:gfp)w25 | Dorsky et al., 2002 | ZFIN ID: ZDB-TGCONSTRCT-070117-137
- **Zebrafish:** Tg(Bactin:HRAS-EGFP)vu119 | Cooper et al., 2005 | ZFIN ID: ZDB-TGCONSTRCT-070117-75
- **Zebrafish:** Tg(Xla.Tubb2b:Hsa.MAPT-GFP)zc1 | Tilton and Tanguay, 2008 | ZFIN ID: ZDB-TGCONSTRCT-070117-135

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Zebrafish Embryos Maintenance
Zebrafish (Danio rerio) were maintained at 28°C on a 14 hr light/10 hr dark cycle (Brand et al., 2002). Embryos collected were cultured in fish water containing 0.003% 1-phenyl-2-thiourea to prevent pigmentation and 0.01% methylene blue to prevent fungal growth. ENU mutagenesis was performed as previously described (Solnica-Krezel et al., 1994). The sfpq heterozygous mutant was crossed with a set of GFP transgenic lines (Tg(isl1:GFP)rw0 (Higashijima et al., 2000), Tg(1.4dlx5a-dlx6a:gfp) (Ghanem et al., 2003), Tg(TOP:gfp)w25 (Dorsky et al., 2002), Tg(Bacalin:HRAS-EGFP)vu119 (Cooper et al., 2005), Tg(Xla.Tubb2b:Hsa.MAPT-GFP))zc1 (Tilton and Tanguay, 2008) and Tg(mnx1:GFP)ml2/+(AB) (also known as Tg(HB9: gfp); Flanagan-Stee et al., 2005). Embryos collected from incross of heterozygous mutant carriers from these transgenic mutant lines were selected for the transgene based on GFP fluorescence.

The animal experimentations have been authorized by KCL Ethic Review Committee and under the HO license 70/7577.

Recruitment of Human Subjects
Patients were recruited from the Local ALS Clinic of Kings College London, Denmark Hill, UK and through neurogenetic clinics at Concord Hospital, Sydney, Australia, under informed written consent. Patients were diagnosed with definite or probable ALS according to El Escorial criteria, and had a family history of ALS and/or FTD.

METHOD DETAILS
Genotyping of sfpq mutant
The homozygous mutant embryos were selected from offspring of incross of heterozygous carriers based on the brain and motility phenotype at 28 hpf. Genomic DNA was separately extracted from 300 homozygous embryos and same number of siblings by standard protocol. The mutation was mapped by PCR amplification with random amplified polymorphic DNA (RAPD) and simple sequence length polymorphism (SSLP) markers on a section of zebrafish chromosome 19. Linkage analysis and subsequent candidate gene approach indicated ‘sfpq’ as the gene mutated in the ENU mutant under study (Postlethwait et al., 1994).

Whole mount in situ hybridization and Immunohistochemistry
Whole-mount in situ hybridization was performed as described elsewhere (Thomas-Jinu and Houart, 2013). The following antisense RNA probes were used: pax2.1 (Krauss et al., 1991), fgf8 (Reifers et al., 1998), foxb1.2 (Amoyel et al., 2005; Nakada et al., 2006), EphA417, zash1a (Allende and Weinberg, 1994), ring (Cheng et al., 2004), lhx5 (Toyama et al., 1995) and axin2 (Carl et al., 2007). TUNEL (terminal deoxynucleotide (TdT) dUTP nick labeling) assay was performed using ApopTag peroxidase In situ Detection Kit (Chemicon Cat# S7100) to identify apoptotic cells. Digoxigenin-labeled DNA fragments in the assay were detected by antibody conjugated to alkaline phosphatase (instead of anti digoxigenin-HRP recommended by manufacturer’s protocol) and color reaction was carried out using NBT/BCIP.

Transgenic GFP embryos used for in situ hybridization with digoxigenin labeled antisense RNA probes were stained with Fast Red (Roche) prior to GFP immunostaining. Immunostaining was carried out as previously described (Shannugalingam et al., 2000). Primary antibodies and dilutions used were as follows: acetylated α-tubulin (Sigma, Mouse IgG2b, 1:1000), GFP (Amsbio, Rabbit IgG,
for in vitro transcription of wild-type full length GFP-PSF and the GFP-PSF D cDNA to generate the expression constructs UAS-zSFPQ-GFP and Hs-zSFPQ-GFP. The GFP tagged human constructs, containing a minimum of 180 pg/embryo is required for full phenotypic rescue. The extent of the rescue was determined by assessing the brain phenotype, motility and the motor neuron expression at 36hpf. A sfpqORF5 pcDNA3.1 NT-GFP using the TOPO TA Expression Kit (Invitrogen K4810-01), by PCR amplification from pDonr221 with the primers sfpq/ORF5: ATGTCTCGGGATCGGTTCCGG and sfpqORF3 0: CTAAAATCGGGGTTTTTTGTTTGG and the Q5 High-Fidelity proof-reading DNA Polymerase (NEB M0491S).

Live imaging

Time-lapse study was performed to investigate the mutant axonogenesis defects by live imaging a sfpq+/−; Tg(mnx1:GFP) embryos from 20hpf until 72hpf. The primary motor axon initiates exit at 17hpf and reaches the target during the second day of embryonic development in WT. Images were taken every 10 min for up to 2h at different stages of growth on a Nikon E800 confocal microscope. The embryos were anesthetized in 0.02% tricaine, embedded in 1% low melting point agarose dissolved in E2 embryo medium in a 35 mm tissue culture plastic dish and filled with E3 medium for confocal imaging. The embryos were removed from the agarose after imaging, maintained in E2 embryo media at 28°C.

Rescue experiments and localization of GFP-tagged SFPQ proteins

The IMAGE clone (IMAGE: 6962536) of zebrafish sfpq complete cDNA sequence in pCMVsport6.1 (Source Bioscience) was sequence verified and stored at −20°C. The plasmid DNA was linearized with FastDigest ClaI (ThermoScientific) and used as template for in vitro synthesis of capped sfpq mRNA using the mMessage Sp6 kit (Ambion). The mutant rescue was performed by injecting z-sfpq mRNA into one-cell stage embryos derived from the sfpq−/−; Tg(mnx1:GFP) incross and sfpq−/−; Tg(isl1:GFP) incross. The extent of the rescue was determined by assessing the brain phenotype, motility and the motor neuron expression at 36hpf. A minimum of 180 pg/embryo is required for full phenotypic rescue.

The Gateway cloning system (Invitrogen, Carlsbad, CA) and Tol2 kit (Kwan et al., 2007) was used to clone the full-length zsfpq cDNA to generate the expression constructs UAS-zSFPQ-GFP and Hs-zSFPQ-GFP. The GFP tagged human constructs, containing full length GFP-PSF and the GFP-PSF Δ1-702 (Dye and Patton, 2001), obtained from Prof. James Patton were subcloned into pc2S+ for in vitro transcription of wild-type hsfq and ΔNLShsfq. The human SFPQ L534I and N533H mutant forms were made by site directed mutagenesis according to the manufacturer’s protocol (Quickchange II Site-Directed Mutagenesis Kit, Stratagene) using a Gateway pDONR221 entry clone containing WT full length coding SFPQ (Gateway, Invitrogen). These were then subcloned in pcDNA3.1 NT-GFP using the TOPO TA Expression Kit (Invitrogen K4810-01), by PCR amplification from pDon’t221 with the primers sfpqORF5: ATGTCTCAGATTCCGTCCAG and sfpqORF3: CTAAAATCGGGGTTTTTTTGTGG and the Q5 High-Fidelity proof-reading DNA Polymerase (NEB M0491S).

Mutation screening of Human SFPQ

Exome sequencing was performed on 151 index cases from the UK (n = 87) and Australia (n = 64), with an average age-of-onset of 59 years and an average disease duration of 34 months. UK FALS cases were all Caucasian individuals from the South-East of London and Kent. FALS cases from Australia were predominantly of European ancestry. All samples were free from currently known ALS-associated coding mutations and the intronic C9orf72 GGGGCC repeat expansion. Samples were captured using either Nimblegen V3, Illumina TruSeq Exome Enrichment or Agilent SureSelectXT Human All Exon-V5-UTR probes and 100bp paired-end reads sequenced on an Illumina HiSeq-2000. Reads were assembled to the hg19 reference genome achieving an average of 115x coverage across all Refseq coding bases, and variants were called with ‘SAMtools Mpileup’. Novel SFPQ variants which were absent from all public variant databases were confirmed by PCR from original stock DNA and directly sequenced using Big-Dye Terminator v1.1 chemistry and an ABI3130 genetic analyzer (Applied Biosystems Pty Ltd, Warrington, UK). Sequence chromatograms were analyzed for mutations using Sequencher 4.10 (Gene Codes Corporation, Ann Arbor, Michigan, USA).

Spinal motor axon mosaic experiment

Progenies from incrosses of sfpq+/−; Tg(mnx1:mGFP) were injected with CAAX-mcherry RNA at one cell stage (donor embryos). Mosaic embryos were produced by homotopic and homochronic transplantation into wild-type hosts. Around 20 dorso-posterior epiblast cells collected from late gastrula stage donor embryo (80%-100% epiboly; collected by capillary suction around the posterior midline) were placed at the equivalent location in the same stage wild-type host embryo. The donor and host embryo were identified as sfpq−/− or WT sibling based on the MHB and axonogenesis phenotype at 24hpf and identity confirmed by PCR at 36hpf after cloned analysis. Embryos with the transplants that lead to the incorporation of the donor cells (mcherry+) in the ventral spinal cord and GFP-positive motor axons were selected for further analysis. Only 25% of the donor clones are homozygous for sfpq. We transplanted more than 120 embryos across different days. To assess the behavior of wild-type neurons in mutants, the same approach was taken with Tg(mnx1:mGFP) wild-type donors into host embryos from sfpq+/− crosses.
DNA Microarray

A total of six sets of embryos (three pools of 50 homozygous sfpq;Tg(mnx1:GFP) and WT sibling embryos) were fast-frozen in liquid nitrogen and stored at − 80 °C until RNA extraction. All homozygous sfpq embryos exhibit impaired motility at 22 hpf and this feature was used to segregate mutant embryos from WT siblings for the study. Total RNA was isolated using high purity RNA isolation kit (Roche). Quality and purity was assessed by Nanodrop absorbance ratios and by Agilent Bioanalyzer (BRC genomics core facility, KCL) and used for microarray analysis and subsequent quantitative RT-PCR experiments.

Six RNA pools of 10 μg of total RNA each were used to make double stranded cDNAs, synthesized using the SuperScript Double-Stranded cDNA Synthesis Kit (Invitrogen). These were hybridized separately on NimbleGen 12 × 135 k zebrafish gene-expression arrays. The array contains 38,489 probe sets with up to 3 probes of 60-mer oligonucleotides per gene. The arrays represented at least 24,000 genes plus additional ESTs from multiple zebrafish tissues. The design of the array relied on gene and EST information from several sources; Ensembl 46 (August 2007, Zv7), RefSeq (September 2007), TIGR (Release 14.0), UniGene (Build 54), Vega 27, and ZGC (August 2007).

RT-qPCR

Total RNA was extracted from pools of sfpq+/−;Tg(mnx1:GFP) and sfpq+/-;Tg(mnx1:GFP) embryos at 24 hpf using TRIZOL RNA isolation reagent (Invitrogen). cDNA was synthesized (First strand CDNA synthesis kit, GE Healthcare) after treating the RNA isolated with DNaseI (Ambion TURBO DNase Free Kit). Products were purified using Qiagruc PCR purification kit (QIAGEN). Reactions were performed using SYBR Green I Master kit (Roche) on a Stratagene MX3005P and data analyzed using MxPro software. Three independent biological replicates of the WT sibling and mutant pools were included in the analysis and all reactions were carried out in triplicates. Cycling parameters were as follows, 95 °C 10 min for 1 cycle then 95 °C 30 s, 60 °C 30 s, 72 °C 30 s for 40 cycles then 95 °C 60 s, 60 °C 30 s, 95 °C 30 s for all primer sets. Primer sequences used are as follows: bcas3 (F − 5′ GCATGTTGAGATAATGGCCA3′, R − 5′ CAGTCCCTCGCACCAGAATGCCA3′); fsdtb (F − 5′ TCTCTCAACTGCCTGAACC3′, R − 5′ TCCCTCAACTGCCTGAACC3′); dig1 (F − 5′ TTGGCTGTGTAATGTATTTTG3′, R − 5′ GCCTCCTCCTGATCTCCT3′); crimi (F − 5′ CCACACTGTCAGATGATC3′, R − 5′ CTCGTCATGACGTCTCCT3′); nbeaa (F − 5′ GTGTATAACGTTTGTTGGA3′, R − 5′ CTCCTAAGACTGAGAGAC3′); trn (F − 5′ GCTCAGTCAACAGAAAT3′, R − 5′ TCTCTGGCTCTCCTCCT3′); smyd3 (F − 5′ AAACCTGCCTGAAATGTGC3′, R − 5′ TCCGCTTCTCCTCCTCCT3′); gopc3 (F − 5′ GCAAAATGGAGGAGGTTAGC3′, R − 5′ GCATCGAGGCGCAAGATA3′); edli3 (F − 5′ CCAGGAGATAAAGCCCT3′, R − 5′ TCTTCCCTCATCGCTTAG3′) and efla (F − 5′ TGGAGAGAAA TCCTGGTGTGCT3′, R − 5′ GAGGAGGTTGATTTGAGGAACT3′). The expression levels of the genes selected were normalized to an endogenous control gene ef1a.

Intronic whole mount in situ hybridization

Antisense probes against the sequences of intron 2 of the following twenty genes (ncam1b, igsf21a, nbeaa, gria3b, Hpca, gnao1a, bcas3, gripi3, bmp3, Tncn2, bmis3, fut8, Chrm3a, dbn1, ctnna2) were made from PCR amplified fragments (5′ primers containing the sp6 promoter sequence) using zebrafish genomic DNA as template. RNA probes of the genes were then made using the standard protocols. Whole mount in situ hybridization for the intronic genes were performed on 24 h and 48 h embryos using an InSituPro automated system (Intavis, Germany).

QUANTIFICATION AND STATISTICAL ANALYSIS

Gene Expression and Ontology Analysis

The gene expression level and folds changes calculated from six separate samples were normalized by quantile normalization and the gene expression values were generated by RMA (Robust Multichip Average) algorithm. The processed microarray data files received from the Nimblegen were subsequently analyzed using ArrayStar4 software (DNASTAR, Inc. Madison, USA). Technical replicates were all highly correlated (R² > 0.97 on all replicate pairs; cross R² test) and the replicate sets were created for sfpq+/-;Tg(mnx1:GFP) and sfpq+/-;Tg(mnx1:GFP) by mean method for subsequent gene expression analyses. The scattered plot of the gene expression levels showed a high linear correlation (R² = 0.98) between the two groups. Genes were considered differentially expressed when the level of expression change was at least two fold (upregulation or downregulation) in the homozygous sfpq embryos as compared to its WT siblings and the difference between the two groups was significant (p < 0.01 moderated t test, in the false discovery rate (FDR) was controlled by the Benjamini Hochberg correction method).

Ontology analysis conducted for the biological interpretation of the differentially expressed genes using ZFIN gene annotation file gave only few annotations as the microarray design is based on Zv7 release. Hence more recent gene annotations were downloaded from ZFIN (release 9, April 24th 2013), and the probe sequences were aligned against the D. rerio genome from ENSEMBL release 71. The GO terms and hierarchy was downloaded from Gene Ontology (http://www.geneontology.org) on May 7th 2013. All genes and functional annotations were imported in ArrayStar4 for further analysis, The GO terms with p < 0.05 (after Benjamini Hochberg correction) and with percentage of genes per GO term above the percentage of the genes of its major first level GO classification is considered as enriched among the differentially expressed genes.

The number of variant transcripts per gene was obtained using the genome provided by ENSEMBL release 75 (Zfin v9, February 2014) (Allende and Weinberg, 1994). The plots and computations were done using R version 3.1.0 (http://www.R-project.org/).
Validation of microarray results
Validation of microarray results by qRT-PCR was performed on six randomly picked transcripts downregulated according to transcriptome analysis. The correlation of the gene expression data from microarray and qPCR was tested using Spearman’s Rho test. Statistical comparison was performed using paired t test with Bonferroni correction for multiple comparisons.

Splice variant analysis
Analysis of number of splice variants in whole transcriptome and DES transcriptome was performed using one-sided Wilcoxon-Mann-Whitney rank sum test with continuity correction based on the number of transcripts for each gene.

Quantification of neuronal morphological parameters
Quantification of the α-SFPQ fluorescent signal in motor axons was done in double-blind on confocal stacks, for 5 pairs of motor axons per embryo using Fiji. Measurement was done in no less than 30 (max. 46) embryos per variant injected. Quantification of ventral motor axon lengths and branching complexity were made for 5 segments per embryo (always 5 somites anterior to the cloaca) on confocal stacks, using FIJI Single Neurite Tracer in no less than 28 (max. 41) embryos per variant injected. The variants significantly different from wild-type show same difference when comparing pairwise to the other variants (pairwise ANOVA, p < 0.0001 and p < 0.001 respectively).

DATA AND SOFTWARE AVAILABILITY
The accession number for the microarray datasets reported in this paper is ArrayExpress: E-MTAB-5559.