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RESEARCH HIGHLIGHT

SRSF1-dependent nuclear export of *C9ORF72* repeat-transcripts: targeting toxic gain-of-functions induced by protein sequestration as a selective therapeutic strategy for neuroprotection

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Microsatellite repeat expansions cause several incurable and lethal neurodegenerative disorders including ataxias, myotonic dystrophy, Huntington's disease and C9ORF72-linked amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Abnormal repeat transcripts generated from the expanded loci are substrates of repeat-associated non-AUG (RAN) translation, an unconventional form of translation leading to the production of polymeric repeat proteins with cytotoxic and aggregating properties. The mechanisms involved in the pathogenesis of microsatellite repeat expansion disorders remain a hotly debated topic. They are shared between toxic loss/gain of functions due to intranuclear RNA foci that sequesters RNA-binding proteins and RAN translation of repeat proteins in the cytoplasm. We recently elucidated the molecular mechanism driving the nuclear export of *C9ORF72* repeat transcripts and showed for the first time that this pathway can be manipulated to confer neuroprotection. Strikingly, we discovered that intron-retaining *C9ORF72* repeat transcripts hijack the physiological NXF1-dependent export pathway by selective RNA-repeat sequestration of SRSF1. Antagonizing SRSF1 and the nuclear export of *C9ORF72* repeat transcripts promoted in turn the survival of patient-derived motor neurons and suppressed neurodegeneration-associated motor deficits in *Drosophila* (Hautbergue et al. Nature Communications 2017; 8:16063). In this invited *Research Highlight* review, we aim to place this work in the context of our previous studies on the nuclear export of mRNAs, provide a summary of the published research and highlight the significance of these findings as a novel therapeutic strategy for neuroprotection in C9ORF72-ALS/FTD. In addition, we emphasize that protein sequestration, often thought as of inducing loss-of-function mechanisms, can also trigger unwanted protein interactions and toxic gain-of-functions.

Keywords: Amyotrophic lateral sclerosis; Frontotemporal dementia; Neurodegeneration; Microsatellite repeat expansions; C9ORF72; RNA nuclear export; SRSF1; NXF1; RAN translation; Therapeutic strategy

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C9ORF72-related amyotrophic lateral sclerosis and frontotemporal dementia

Polymorphic GGGGCC (or G4C2) hexanucleotide-repeat expansions in intron 1 of the *C9ORF72* gene are the most common known cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) [1,2], a spectrum of incurable and fatal adult-onset neurodegenerative diseases. FTD involves loss of neurons in the frontal and temporal lobes of the cerebral cortex leading to cognitive defects and altered personality features such as psychological disinhibition. ALS causes selective death of upper and lower motor neurons in the motor cortex and spinal cord, resulting in muscle atrophy, progressive paralysis and death usually by respiratory failure within 3-5 years from symptom onset. A proportion of patients with ALS develop FTD, and conversely, many patients with FTD will eventually suffer from ALS symptoms [3]. Both ALS and FTD-related disorders have an annual incidence rate of 1 to 2 per 100,000 people. Approximately 220,000 cases of patients with ALS were recorded across the globe in 2015 and this number is projected to increase in an ageing world population up to over 370,000 cases by 2040 [4]. Whilst no treatment is available for FTD, the current standard of care for ALS patients, riluzole, only marginally extends survival and does not relieve the symptomatic motor deficits.

The pathophysiology leading to neuronal injury is complex and potentially involves three non-exclusive mechanisms of pathogenesis which have been extensively studied (reviewed in [5, 6]). These include: (i) RNA toxic gain-of-functions by sequestration of RNA-processing factors onto hexanucleotide-repeat RNAs, (ii) protein toxic gain-of-function due to unconventional repeat-associated non-AUG (RAN) translation of cytotoxic dipeptide-repeat proteins (DPRs) in all frames and in the absence of canonical start codons, and (iii) haploinsufficiency due to reduced expression levels of *C9ORF72* mRNA and protein. The *C9ORF72* locus is bi-directionally transcribed leading to expression of G4C2-sense and C4G2-antisense transcripts which form sense and antisense RNA foci and also lead to the RAN translation of sense and antisense DPRs (sense poly-Glycine-Alanine, poly-Glycine-Proline, poly-Glycine-Arginine and antisense poly-Proline-Arginine, poly-Proline-Glycine, poly-Proline-Alanine) [7].

We identified that approximately 100 RNA-binding factors, including a large proportion of RNA Recognition Motif (RRM) containing proteins, are sequestered onto sense G4C2-repeat RNA sequences [8]. Among these, we noted sequestration of the SR-rich splicing factor (SRSF) and nuclear export adaptor proteins SRSF1, SRSF3 and SRSF7 [9] as well as of the general nuclear export adaptor ALYREF (Aly/RNA export factor also known as REF [10]). SRSF1 had also been identified as a G4C2-repeat RNA binder in a separate study which involved the screening of RNA-processing proteins based on their known or predicted binding to GC-rich sequences [11]. We hypothesized that the sequestration of these nuclear export adaptors might license the inappropriate nuclear export of intron-retaining *C9ORF72* repeat transcripts and subsequently lead to the RAN translation of DPRs in the cytoplasm [8].

The nuclear export of bulk mRNAs

The nuclear export of mRNAs is functionally linked to the co-transcriptional processing of pre-mRNAs synthesized by the RNA polymerase II (RNAPII). RNAPII-dependent transcription involves the assembly of a pre-initiation complex upstream of the transcription start site (TSS) in the promoters of protein-coding genes, following recognition of the TATA box element by the TATA box binding protein (TBP), a subunit of the general transcription factor TFIID, which triggers the recruitment of additional general transcription factors and the hypo-phosphorylated form of the RNAPII (RNAPIIA). The large subunit of the RNAPII exhibits a characteristic carboxyl-terminal domain (CTD) constituted by repetitions of the heptapeptide sequence YSPTSPS (52 repeats in mammals). Differential phosphorylation of the CTD controls the co-transcriptional processing of pre-mRNAs upon selective recruitment of various RNA-processing protein complexes (reviewed in [12]). Serine-5 phosphorylation of the RNAPII CTD by the CDK7 kinase subunit of TFIIH promotes the stable initiation of transcription initiation whilst the interaction of the capping enzyme (CE) with the CTD phosphorylated on serine-5 residues allows for addition of the CAP at the 5'-end of the nascent transcript. Phosphorylation of the RNAPII CTD serine-2 residues by the CDK9 kinase subunit of P-TEFb (positive transcriptional elongation factor b) is further required to maintain the processivity of the hyper-phosphorylated form of the RNAPII (RNAPIIO) during the elongation phase (reviewed in a special issue [13]). Splicing of introns is linked to the deposition of the RNA/ATP-dependent DEAD-box RNA helicase 39B (DDX39B also known as UAP56 for U2AF65-associated protein 56), which promotes the assembly of spliceosomes via interactions with the CTD predominantly phosphorylated

on serine-2. Different combinations of heterogeneous nuclear ribonucleoproteins (hnRNPs) and hyper-phosphorylated SRSF factors are involved in the regulation of alternative splicing. SRSFs and hnRNPs usually play antagonistic roles respectively stimulating and inhibiting the splicing of exons. The termination of transcription involves recruitment of the cleavage and polyadenylation specificity factor (CPSF) at the sequence of core consensus AAUAAA [14] via the RNAPII CTD predominantly phosphorylated on serine-2. Approximately 200 adenosines are added to the 3'-end of human mRNAs.

mRNAs produced from intron-containing reporter constructs are more efficiently exported into the cytoplasm of mammalian cells [15]. In human, the vast majority of mRNA molecules are exported from the nucleus to the cytoplasm in a process that is functionally coupled to splicing via dynamic assembly of the evolutionary conserved transcription-export complex (TREX) [16]. Interactions of TREX with the CAP-binding protein CBP80 at the 5'-end of nascent transcripts provide in turn 5' to 3' directionality to the nuclear export of mRNAs [17]. TREX is composed of the subcomplex THO, DDX39B and the nuclear export adaptor ALYREF [18]. Additional factors are associated to TREX including interactions with other nuclear export adaptors [19, 20]. The interactions of a varied combination of proteins and nuclear export adaptors are thought to form substitutes of TREX complexes with alternative functionalities and/or mRNA selectivity. Direct binding of ALYREF to DDX39B, which promotes spliceosome assembly onto the nascent transcript, stimulate the ATPase and RNA helicase activities of DDX39B leading in turn to the handover of the RNA from DDX39B to ALYREF [21] and recruitment of ALYREF onto spliced transcripts prior to the nuclear export process [22]. Successive rounds of DDX39B-dependent ATP hydrolysis were suggested to facilitate the assembly of TREX by loading of proteins with nuclear export adaptor functions [21]. Mutually exclusive interactions of ALYREF with DDX39B and the nuclear export factor 1 (NXF1, also known as TAP [23]) lead to the displacement of DDX39B and handover of the RNA molecule from the nuclear export adaptor to NXF1 by switching NXF1 to a high RNA affinity mode [24] following remodeling of NXF1 into an open conformation that exposes its RNA-binding domain [25]. The assembly of TREX and remodeling of NXF1 is much more complex than described here, involving additional proteins, such as nuclear export co-adaptors, post-translational modifications and dynamic re-arrangements (recently reviewed in [26, 27]). A TREX-2 complex, which contains NXF1 and a distinct set of proteins, was reported to promote efficient recruitment of NXF1 to the nuclear pore complex (NPC) and couple transcription to nuclear export, potentially providing a link for the nuclear export of intronless transcripts [28, 29]. The TREX complex has also been involved in an alternative RNA export pathway (AREX) for the NXF1-dependent nuclear export of intronless transcripts through direct interactions with the CAP-binding complex [30].

The direct interactions of NXF1 with the FG-repeat regions of some of the nucleoporins, which constitute the NPC and protrude in the central channel, further allow docking of the mature mRNA-protein complexes and transport across the nuclear pore. On the cytoplasmic side of the NPC, activation of the DEAD-box RNA helicase Dbp5 activity (DDX19B in human) by interaction with the nucleoporin GLE1 (RNA export mediator) associated to inositol hexakisphosphate (IP6) leads to recruitment of Dbp5/DDX19B onto the mRNAs promoting the unwinding of secondary RNA structures, the displacement of bound protein factors and the cytoplasmic release of mRNAs (reviewed in [31]). Dissociation of NXF1 from the RNA and the nuclear export adaptor is predicted to revert NXF1 into a low RNA affinity mode that would also contribute to efficient mRNA release [24,32]. It remains unknown whether the CAP-dependent translation of mRNAs is linked or not to the nuclear export process and/or to the cytoplasmic release. Interactions of the eukaryotic initiation translation factor 4F (eIF4F), composed of the CAP-binding protein eIF4E, the scaffold protein eIF4G and the DEAD box RNA helicase eIF4A, in association with the poly(A)-binding protein (PABP) allow for circularization and pre-activation of the mRNA. The pre-assembled 43S complex comprising the 40S small ribosome subunit, the factors eIF1,1A,3,5 and the ternary complex eIF2:Met-tRNA is recruited to the activated mRNA via interactions with eIF4F to promote scanning of the 48S complex through the 5'-untranslated region of the mRNA and eIF5B-dependent recruitment of the 60S large ribosomal subunit at the AUG start codon for the translation of proteins (reviewed in [33, 34]).

The SR-rich splicing factors SRSF1, SRSF3 and SRSF7 have also been reported to play a role as nuclear export adaptors by directly interacting with the amino-terminal domain of NXF1 under a dephosphorylated form upon completion of splicing [35]. Like ALYREF, they promote the remodeling of NXF1 into a high RNA-binding affinity mode that triggers handover of the RNA from the SRSF1,3,7 proteins to NXF1 [24]. The RNA handover is also facilitated by mutually exclusive interactions of the nuclear export adaptors with the RNA and NXF1 which both interact with arginine residues in unstructured low complexity arginine/proline-rich peptides juxtaposed to the RNA recognition motifs of ALYREF [24,36], SRSF1 [37] and SRSF3/SRSF7 [38]. This led to a model in which the role of nuclear export adaptors is to license mRNA nuclear export by remodeling NXF1 to a high affinity

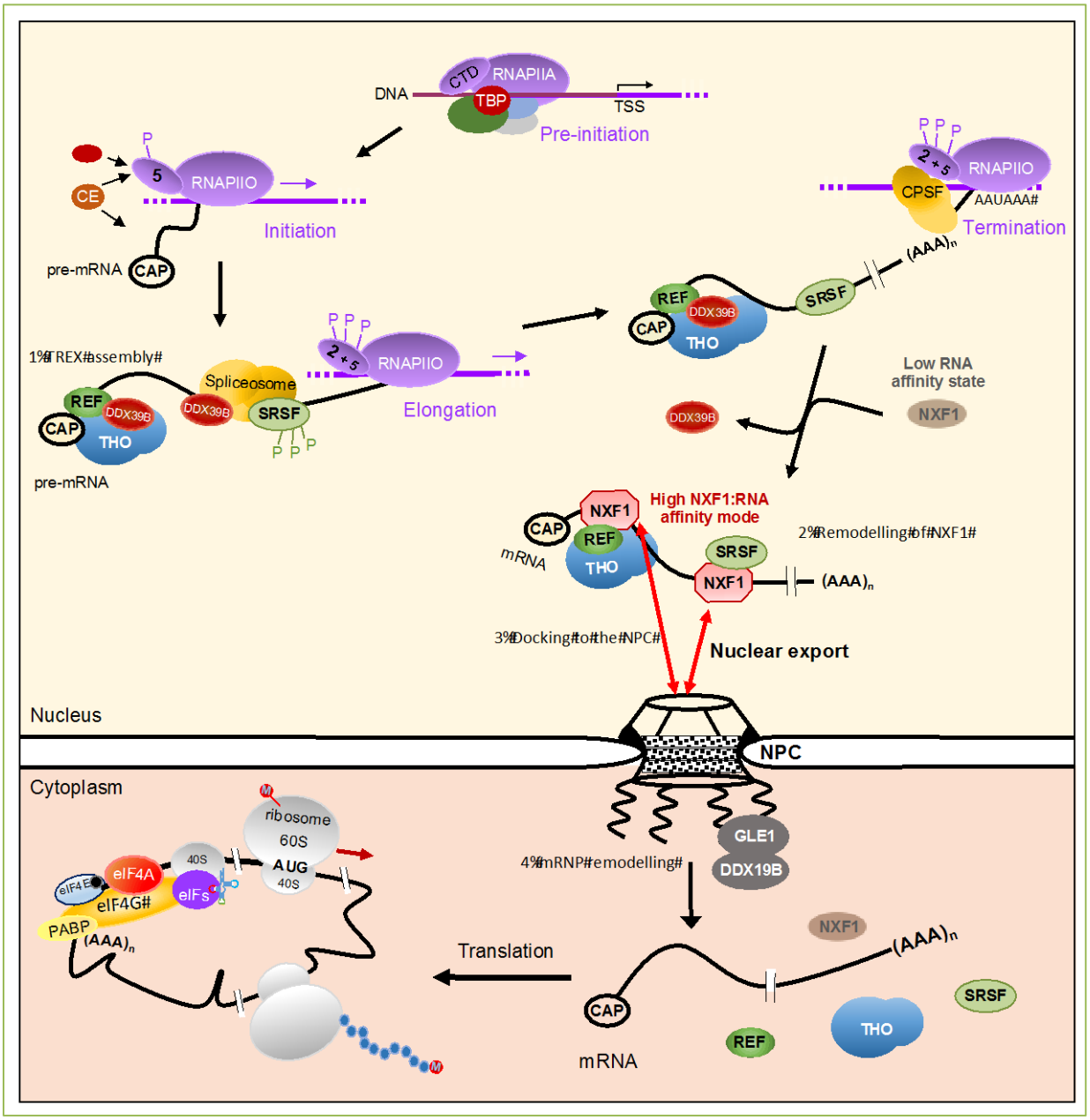

Figure 1. Expression of protein-coding genes. The diagram represents an overview of the molecular mechanisms involved in the biogenesis and the co-transcriptional processing of RNAPII transcripts with a particular emphasis on the bulk nuclear export of human ~~bulk~~ mRNAs which is coupled to splicing via the TREX complex. TREX provides a platform for the high RNA-affinity remodeling of NXF1 prior docking to the nuclear pore through interactions of NXF1 with nucleoporins. Abbreviations: REF for ALYREF; SRSF for SRSF1 or SRSF3 or SRSF7; NPC for nuclear pore complex. Other nuclear export adaptors including UAP56-interacting protein (UIF [19]) and Leucine Zipper Protein 4 (LUZP4 [20]) associate with TREX and are likely to play a similar role as ALYREF.

RNA-binding mode through mutually exclusive interactions with RNA and NXF1 on completion of splicing, providing in turn a mechanism to retain unprocessed transcripts in the nucleus [32]. A simplified diagram represents a summary of the human NXF1-dependent nuclear export of the bulk mRNAs and its functional coupling to the expression of protein-coding genes (**Figure 1**).

Several non-essential proteins with mRNA nuclear export adaptor functions have been characterised in higher eukaryotes. Different adaptors were also found to interact with the same transcripts [19]. Depletion of ALYREF is dispensable to the nuclear export of bulk mRNAs in human and *Drosophila* cells [19, 39] and to the development of nematodes [40]. On the other hand, several of the SR-rich splicing factors play an essential role in the splicing switch that occurs during development to juvenile life. The homozygous deletions of SRSF1 and SRSF3 cause

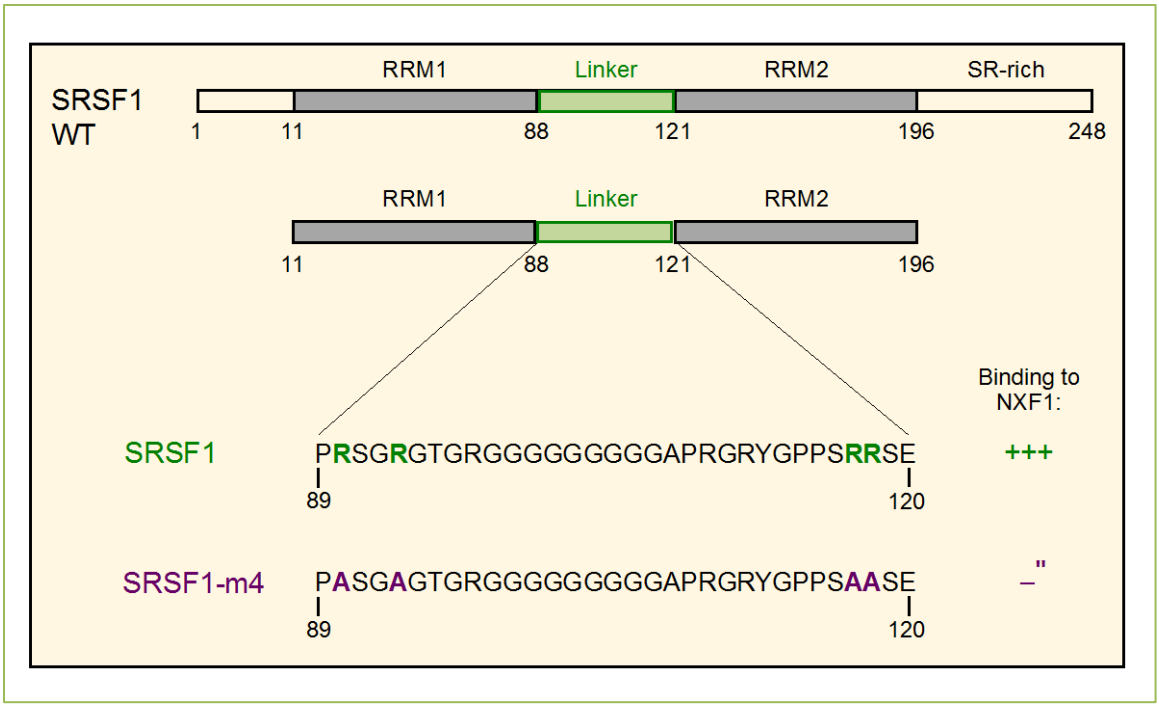

Figure 2. Diagrammatic representations of SRSF1 and the NXF1-binding site. SRSF1 wild type (WT) represents the full length of SRSF1. It is composed of a short amino-terminal region, a linker flanked by two RNA Recognition Motif (RRM) domains and the carboxyl-terminal RS-rich region (Arginine-Serine-rich domain). Amino acids composing the NXF1-binding site are highlighted together with the flanking RNA Recognition Motif (RRM) domains which constitute the SRSF1 and SRSF1-m4 proteins. The four arginine residues required for the interaction with NXF1 are labeled in green whilst alanine substitutions in SRSF1-m4 are shown in purple. Numbers represent amino acid position. SRSF1 binds NXF1 as efficiently as SRSF1-WT (+++) while SRSF1-m4 does not (-).

embryonic lethality due to failure of the developing heart [41] and impaired blastocyst formation [42] respectively. However, the depletion of each of the SRSF1-7 proteins in murine cells only affect the nuclear export of 0.5-2% of mRNAs despite interactions with thousands of transcripts [43]. Taken together, the NXF1-dependent nuclear export adaptor function appears to involve redundancy and/or cooperation in cultured and differentiated cells.

SRSF1-dependent nuclear export of sense and antisense *C9ORF72* repeat transcripts

As described above, sense *C9ORF72* repeat RNA expansions sequester ALYREF and SRSF1,3,7 proteins letting us hypothesize that increased local concentrations of nuclear export adaptors might inappropriately remodel NXF1 into an high RNA-affinity mode which triggers the nuclear export of unspliced *C9ORF72* pre-mRNAs retaining repeat expansions in intron 1 [8]. In support of this, ALYREF and NXF1 have been identified by another group as modifiers of the *C9ORF72* hexanucleotide-repeat mediated neurotoxicity in a *Drosophila* loss-of-function screen [44]. In the *Nature Communications* study [45] which forms the basis for this research highlight review, we showed that recombinantly-purified ALYREF and SRSF1 proteins directly interact with 5 repetitions of G4C2-sense and C4G2-antisense synthetic RNA oligonucleotides. These findings are consistent with the co-localisation of ALYREF [8] and SRSF1 [45] with nuclear RNA foci in human post mortem spinal motor neurons from C9ORF72-ALS cases. To investigate the potential involvement of ALYREF and SRSF1 in the nuclear export of hexanucleotide repeat transcripts which are substrates of cytoplasmic RAN translation, we crossed transgenic ALYREF-RNAi and SRSF1-RNAi *Drosophila* lines with flies expressing 36 uninterrupted G4C2 repeats and exhibiting DPR-mediated neurotoxicity [46]. Strikingly, we observed that depletion of 70-80% SRSF1 mRNAs inhibit the nuclear export of repeat transcripts and the subsequent RAN translation of DPRs, preventing in turn the rough eye phenotype and the neurodegeneration-associated locomotor deficits in both larvae and adult flies. In contrast, similar depletion levels of ALYREF did not show significant rescue of neurodegeneration and motor function. The partial depletion of SRSF1 specifically affects G4C2-repeat transcripts since it had no effect on dampening the neurotoxicity induced by expression of DPRs encoded independently of the hexanucleotide repeat sequence.

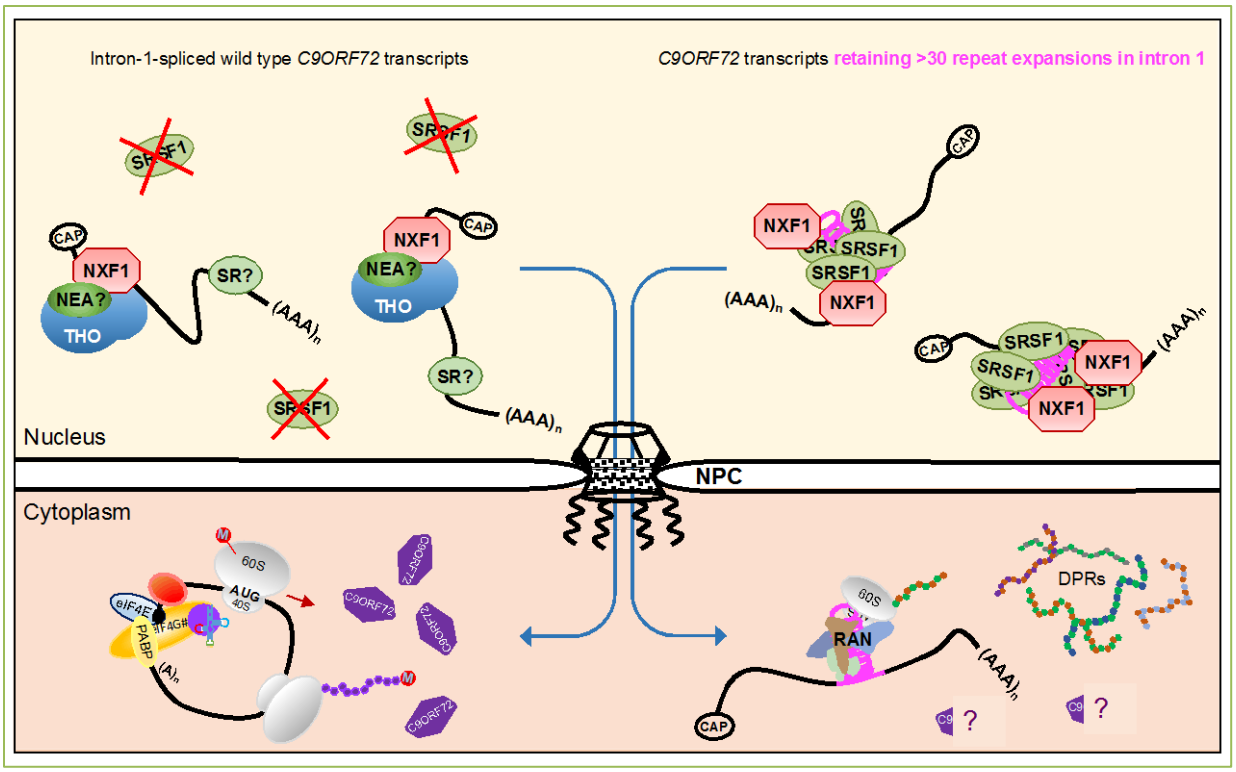

Figure 3. Physiological and pathological nuclear export of *C9ORF72* transcripts. The nuclear export of wild type *C9ORF72* transcripts has not yet been investigated. It might involve recruitment of the TREX complex by uncharacterised nuclear adaptor(s) (NEA) and/or coupling to other SR-rich NEAs such as SRSF3 or SRSF7 (SR) during splicing. Strikingly, it does not involve SRSF1. The nuclear export of pathological *C9ORF72* hexanucleotide-repeat transcripts specifically involves RNA-repeat sequestration of SRSF1 and interaction with NXF1. We proposed that the increased local concentrations of sequestered SRSF1 inappropriately recruit and remodel NXF1 into a high RNA-affinity mode which licenses the nuclear export process.

We next demonstrated that SRSF1 is sequestered in a repeat length dependent manner in neuronal N2A cells using reporter sense G4C2x15 / G4C2x38 and antisense C4G2x15 / C4G2x39 transcripts that lack canonical AUG start codons and Kozak elements to recapitulate the features of RAN translation. Since SRSF1 is involved in both splicing and nuclear export functions, the reporter constructs were also designed without splicing consensus sites or intronic sequences to allow for specific investigation of the SRSF1-dependent nuclear export potential of G4C2 and C4G2 repeat sequences in absence of functional coupling to splicing. Interestingly, the expression of uninterrupted G4C2x38 or C4G2x39 repeat transcripts which lead to the production of DPRs, but not of G4C2/C4G2x15 repeat transcripts which do not, is much more potent at inhibiting neuronal cell proliferation (by approximately 50%, 48 hours post transfection) [45] than transfection (not shown) or stable integration [47] of interrupted transcripts harboring 102 repeats of the G4C2 sequence - in direct correlation with the expression levels of DPRs. Depletion of ~70% of the SRSF1 protein in neuronal N2A cells transfected with the DPR-reporter constructs further led to an efficient inhibition of the nuclear export and subsequent RAN translation of sense/ antisense repeat transcripts which correlated with a suppression of the *C9ORF72* repeat-mediated cytotoxicity in cell proliferation assays. Similar results were obtained in primary mouse cortical neurons. The structural and functional characterisation of the SRSF1-dependent nuclear export function previously showed that 4 arginine residues within the unstructured arginine-rich peptide connecting the two RNA recognition motifs (RRMs) are required for the interaction with NXF1 [37]. Only the de-phosphorylated forms of SRSF1,3,7 proteins interact with NXF1 upon completion of splicing [35] and the SRSF1 region encompassing the two RRMs confers the same NXF1-binding efficiency as wild type SRSF1 - the linker peptide constituting the NXF1-binding site [37]. Substitution of 4 arginines by alanines in the linker region impairs the interaction of SRSF1-m4 with NXF1 in both HEK and N2A cells [37,45] (**Figure 2**). In our recent study, we also showed that SRSF1-m4 directly interact with G4C2x5 and C4G2x5 synthetic RNA probes and is sequestered in a length dependent manner onto G4C2-sense and C4G2-antisense repeat transcripts in neuronal N2A cells. Expression of the SRSF1-m4 mutant protein also resulted in nuclear retention of the repeat transcripts and in reduced DPR production[45],

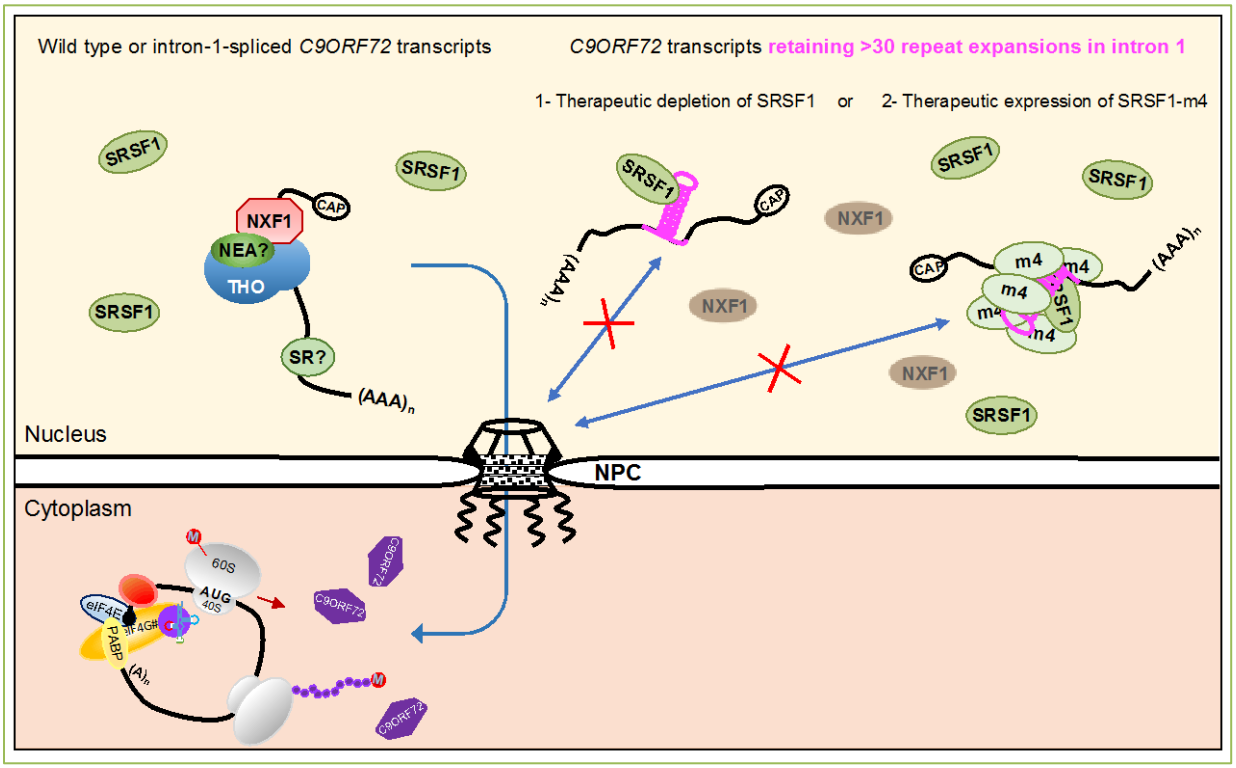

Figure 4. Therapeutic strategies to inhibit the nuclear export of pathological *C9ORF72* repeat transcripts. Our research identified two novel therapeutic strategies for neuroprotection which include the partial depletion of SRSF1 or the expression of the engineered SRSF1-m4 protein which is specifically sequestered onto *C9ORF72* repeat transcripts but fails to efficiently recruit NXF1. Importantly, these interventions do not affect the expression levels, the splicing or the nuclear export of intron-1-spliced *C9ORF72* transcripts which lead to translation of the wild type C9ORF72 protein.

clearly demonstrating that preventing RNA-repeat sequestration of endogenous SRSF1 and interaction with NXF1 specifically inhibits the nuclear export of synthetic reporter repeat transcripts, independently of the splicing function of SRSF1.

To validate these findings in the context of pathological *C9ORF72* repeat transcripts in human disease, we differentiated neurons and motor neurons from induced-neural progenitor cells (iNPCs) reprogrammed using sex/age-matched control and C9ORF72-ALS patient fibroblast biopsies [48]. Pathological *C9ORF72* repeat transcripts retaining intron-1 were indeed found to accumulate in the cytoplasm while unspliced forms of the wild type *C9ORF72* transcripts were normally retained in the nucleus. Our data further showed that the partial depletion of SRSF1 does not affect the physiological unspliced fraction of wild type *C9ORF72* transcripts but selectively inhibits the nuclear export of expanded *C9ORF72* repeat transcripts in correlation with reduced RAN translation of DPRs and an increased survival of motor neurons. In contrast, the depletion of SRSF1 had no effect on the expression levels, splicing of intron-1 or nuclear export of *C9ORF72* transcripts which encode the wild type C9ORF72 protein. The mechanisms involved in the nuclear export of wild type *C9ORF72* transcripts have not been investigated in this study and remain unknown. Significantly, they do not involve SRSF1. In summary, a simplified diagram represents our findings regarding the nuclear export of wild type and pathological *C9ORF72* transcripts (**Figure 3**).

Manipulating the nuclear export pathway as a novel therapeutic strategy for neuroprotection

A growing body of evidence implicate DPRs as one of the primary drivers of pathogenesis in cellular and animal models of C9ORF72-ALS/FTD [46, 49-51]. Our work clearly reinforces this concept using an unbiased approach of investigation which did not rely on modifying expression levels or repeat-length of repeat transcripts but rather on selectively manipulating transport across the nuclear pore to either promote nuclear retention of transcripts or production of DPRs. The increased number of nuclear RNA foci upon SRSF1 depletion did not appear to induce cytotoxicity in neuronal cell models (not shown) or astrocytes in agreement with another study in *Drosophila* [51]. While no detrimental effects of SRSF1 depletion were observed in neuronal cells or patient-derived neurons, the reduced RAN translation of DPRs specifically led to decreased cytotoxicity and to neuroprotection of C9ORF72-ALS *Drosophila* and

patient-derived motor neurons co-cultured with astrocytes. Significantly as a potential therapeutic strategy, the neuroprotection conferred by the depletion of SRSF1 was functionally linked to a suppression of the *C9ORF72* repeat-associated motor deficits in adult *Drosophila*. ALS astrocytes were also reported to be toxic on their own to primary and differentiated mouse motor neurons via non-cell autonomous mechanisms [48]. Interestingly, while the depletion of SRSF1 in astrocytes had no effect in control co-culture systems, it specifically reduced the C9ORF72-ALS patient-derived astrocyte-mediated neurotoxicity promoting in turn the survival of control motor neurons in a mechanism that has not yet been investigated. Nonetheless, antagonizing the SRSF1 function therefore appears to promote survival of motor neurons through two pathways that involve inhibiting the RAN translation of DPRs and dampening the astrocytic-mediated neurotoxicity.

Identifying the detailed mechanisms driving the nuclear export of repeat transcripts and manipulating their transport across the nuclear pore might constitute a valid approach for dissecting the complex mechanisms of pathogenesis and develop therapeutic strategies in other microsatellite repeat expansion disorders. In particular, our study revealed that RNA-repeat sequestration of SRSF1 confers toxic gain-of-functions by tricking a physiological nuclear export adaptor and evading the normal nuclear retention mechanisms of unspliced transcripts, which further lead to RAN translation of cytotoxic DPRs in the cytoplasm. Since the sequestration of SRSF1 is specific to pathological *C9ORF72* repeat transcripts, it provides a rare opportunity for a selective therapeutic strategy using gene therapy approaches that would either aim at depleting SRSF1 or at expressing the engineered SRSF1-m4 protein (**Figure 4**). Both strategies efficiently inhibited the nuclear export of *C9ORF72* repeat transcripts and prevented DPR-mediated cytotoxicity in neuronal cell models. However, the safety of such interventions remains to be tested *in vivo* in wild type animals such as in mice for example. Expression of SRSF1 is essential to the development of the heart during embryonic development and no study so far has been reported on the effects of partial depletion of SRSF1 in the central nervous system during adulthood. The expression of the SRSF1-m4 protein might present an advantage over the partial depletion of SRSF1 as it does not involve altering the physiological levels of SRSF1 and is sequestered onto RNA-repeats in place of the endogenous SRSF1 protein. Other alternative therapeutic strategies also include preventing the de-phosphorylation of SRSF1 by protein phosphatase 1 (PP1) or promoting its SRSF protein kinase 1 (SRPK1)-dependent phosphorylation to inhibit interaction with NXF1.

Perspectives

We are now evaluating the safety and efficacy of SRSF1 depletion and SRSF1-m4 expression in wild type and C9ORF72-ALS/FTD mice using adeno-associated gene therapy approaches through intrathecal or *cisterna magna* delivery in the central nervous system. Antisense oligonucleotide (ASO) therapy to knock down SRSF1 is also considered as a possible therapeutic strategy since controlling the virally induced depletion level of SRSF1 might be challenging particularly in patients. Gene therapy approaches are indeed getting closer and closer to becoming valid therapeutic strategies in the clinic as highlighted by the recent ASO clinical trials to deplete SOD1 in SOD1-related ALS [52] and the very promising replacement of the *SMN* (Survival Motor Neuron) gene using single-dose injection of adeno-associated AAV9 viral vectors in babies with spinal muscular atrophy [53], a juvenile form of motor neuron disease.

Conflicting interests

G.M.H., M.A., A.J.W. and P.J.S. have filed a patent application on the use of SRSF1 antagonists for the treatment of neurodegenerative disorders by gene therapy approaches (PCT/GB2017/051539).

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Author contributions

L.M.C. and G.M.H. wrote the review. Y.H.L. and G.M.H. designed the figures. All authors approved the manuscript and contributed to final editing.

Abbreviations

AAV: adeno-associated virus; ALS: amyotrophic lateral sclerosis; ALYREF: Aly/RNA export factor; AREX: alternative RNA export pathway; C4G2: CCCCGG; C9ORF72: chromosome 9 open reading frame 72; CBP: CAP-binding protein; CE: capping enzyme; CTD: carboxyl-terminal domain; CPSF: cleavage and polyadenylation specificity factor; DDX: DEAD-box RNA helicase; DPRs: dipeptide-repeat proteins; eIF: eukaryotic initiation translation factor; FTD: frontotemporal dementia; G4C2: GGGGCC; GLE1: RNA export mediator; IP6: inositol hexakisphosphate; NPC: nuclear pore complex; NXF1: nuclear export factor 1; hnRNPs: heterogeneous nuclear ribonucleoproteins; PABP: poly(A)-binding protein; P-TEFb: positive transcriptional elongation factor b; RAN: repeat-associated non-AUG; SRSF, Serine/Arginine-rich splicing factor; RRM: RNA recognition motif; RNAPII: RNA polymerase II; SMN: survival motor neuron; TBP: TATA box binding protein; TREX: transcription-export complex; TSS: transcription start site; UAP56: U2AF65-associated protein 56.

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