STARs in the CNS

Ingrid Ehrmann*[1], Philippe Fort† and David J. Elliott*

*Institute of Genetic Medicine, Newcastle University, Central Parkway, Newcastle NE1 3BZ, U.K.

†Université Montpellier, UMR 5237, Centre de Recherche de Biologie Cellulaire de Montpellier, CNRS, Montpellier, France

Abstract

STAR (signal transduction and activation of RNA) proteins regulate splicing of target genes that have roles in neural connectivity, survival and myelination in the vertebrate nervous system. These regulated splicing targets include mRNAs such as the *Neurexins (Nrxn)*, *SMN2* (survival of motor neuron) and *MAG* (myelin-associated glycoprotein). Recent work has made it possible to identify and validate STAR protein splicing targets *in vivo* by using genetically modified mouse models. In this review, we will discuss the importance of STAR protein splicing targets in the CNS (central nervous system).

Introduction

Alternative splicing expands the coding capacity of the genome. More than 90% of human genes are alternatively spliced. Each individual pre-mRNA can be spliced in different ways involving cassette exons, mutually exclusive exons, alternative 3′ splice sites, alternative 5′ splice sites, intron retention, alternative promoters and alternative polyadenylation [1]. The most common form of alternative splicing involves the exclusion or inclusion of a cassette exon. Either the function or the amount of protein encoded by an mRNA maybe changed by alternative splicing. Firstly, the cell can vary protein function by introducing or deleting cassette exons from the mRNA. If the exon is located in an important domain of the protein, the function of the protein may change dramatically. Secondly, the cell can change the quantity of protein produced by splicing exons into the message which introduces stop codons leading to nonsense mediated decay. Finally, splicing can also affect non-coding areas of the RNA such as the 5′ or 3′ UTR which in turn regulate the expression of the protein. For example, the choice of translational control elements in the 5′ UTR may change the amount of protein that is made by the cell [2]. Alternative splicing is controlled in part by RNA-binding proteins, which in vertebrates are often comprised of evolutionarily related families that have similar structures.

The STAR (signal transduction and activation of RNA) proteins are an important group of splicing regulator proteins [3–6]. There are five STAR protein family members in vertebrates which are both evolutionarily related to each other and have similar protein modular structures. These STAR proteins are SAM68 (Src-associated in mitosis, 68 kDa), T-STAR (testis-signal transduction and activator of RNA), SLM-1 (Sam68-like mammalian protein 1), QKI (Quaking) and SF1 (splicing factor 1) (Figure 1). With the exception of SF1, each of these STAR proteins contain a KH domain flanked by two QUA domains. The KHDRBS (KH domain RNA binding and signal transduction) branch of this family is made up of SAM68, T-star and SLM-1, and diverged from QKI in early metazoans: this was 800–880 million years ago. The ancestor of KHDRBS/QKI diverged from SF1 in early Opisthokonts, i.e. approximately 1.6 billion years ago (Figure 2). Although the domain organization of the different STAR proteins is similar, recent atomic structures revealed important differences. Sam68 and T-star bind RNA utilizing a novel dimerization interface consisting of the QUA1 and KH domains, whereas the QUA2 is not involved in either dimerization or RNA binding [7]. In contrast, QKI and SF1 bind RNA through their QUA2 and KH domains and the QUA2 is further involved in the dimerization of QKI. Consequently, SAM68 and T-star bind a bipartite motif (A/U)AA-$N_{>15}$-(A/U/AA) [7], whereas QKI and SF1 bind a 7 nucleotide RNA consensus motif consisting of (U/C)ACU(C/A)A(C/U) [8,9]. Homodimerization which occurs through the QUA1 domain, is thought to increase the RNA specificity of all the STAR proteins, apart from SF1 which does not dimerize because it has no QUA1 domain [7,10].

There is great interest in identifying the functional targets of STAR proteins, to reveal the pathways they control and why they have been conserved as a family of RNA-binding proteins. Genetically modified animals are very informative *in vivo* models to answer these questions. Currently, there are viable homozygous knockout mouse models for all the STAR proteins except SF1 which can be exploited to study alternative splicing in an intact organism (the *Sf1* null mouse is lethal) [11–17].

The brain has been shown to have the largest amount of alternative splicing in the body [18], perhaps because of its complex function. One role of the STAR proteins in the brain is to control alternative splicing. In this review, we will discuss

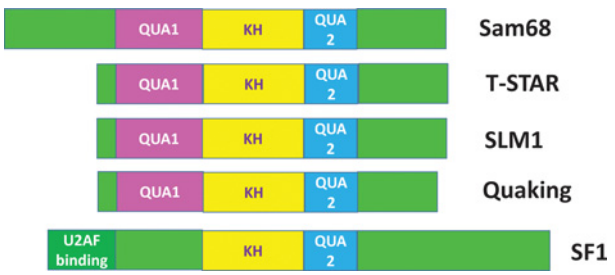
Key words: alternative splicing, CNS (central nervous system), KHDRBS (KH domain RNA binding and signal transduction), neurexin, RNA-binding proteins, STAR (signal transduction and activation of RNA).

Abbreviations: AS4, alternative splice site 4; CLIP, cross-linking and immunoprecipitation; CNS, central nervous system; hGH, human growth hormone; KHDRBS, KH domain RNA binding and signal transduction; MAG, myelin-associated glycoprotein; *Nrxn, Neurexin*; PLP, proteolipid protein; QKI, Quaking; *qk*^{v}, quakingviable; Sam68, Src-associated in mitosis, 68 kDa; SF1, splicing factor 1; Slm1, Sam68-like mammalian protein 1; SMA, spinal muscular atrophy; SMN2, survival of motor neuron; STAR, signal transduction and activation of RNA; T-star, testis-signal transduction and activator of RNA.

[1] **To whom correspondence should be addressed (email ingrid.ehrmann@ncl.ac.uk).**

©2016 The Author(s). Published by Portland Press Limited on behalf of the Biochemical Society.

Figure 1 | The domain structure of the vertebrate STAR proteins
The KH domain has homology to hnRNPK; QUA1 and QUA2 are domains with homology to Quaking. All of the STAR proteins have a similar domain structure. SF1 is the sole example of a STAR protein with only a KH and QUA2 domain.



alternative splicing targets regulated by STAR proteins that have been identified or validated in the brains and spinal cords of genetically modified animals. These investigations have involved both basic and translational research: where a disease is known to have a splicing defect such as spinal muscular atrophy (SMA), the study of the STAR proteins that affect this splice may lead to a better understanding of the disease.

T-star

T-star (also known as Slm2 and Khdrbs3) is expressed mainly in the brain and the testis [19,20]. Knockout mouse models missing *T-star* exon 2 have been generated [12,13]. In these models, loss of exon 2 shifts the reading frame, to create a null allele. *T-star* null mice display no gross phenotypic abnormalities [12,13]. However, using a PCR platform to screen for splicing differences between the wild-type and knockout mouse brains [21], it was found that the absence of T-star leads to increased splicing inclusion of an exon called alternative splice site 4 (AS4) in mRNAs from all three *Neurexin (Nrxn)* genes [12]. Neurexins are important proteins located on the presynaptic membranes of neurons where they determine connectivity with ligands on the postsynaptic membrane. Each *Nrxn* gene consists of a long α and short β form which is dependent on promoter usage. *Nrxn* pre-mRNAs undergo extensive alternative splicing [22]. The particular splice which is regulated by T-star, AS4, is important for the function of the neurexin proteins and is in both the α and β forms of the neurexins. The AS4 cassette exon encodes a 30 amino acid peptide which influences synaptic activity [23]. AS4 peptide negative neurexin proteins interact with neuroligin 1 and LRRTM protein partners, and AS4 peptide positive neurexins interact with cerebellins and other neuroligin protein partners. By switching the postsynaptic receptor that binds to the neurexin protein, a neuron can shift the balance of excitatory and inhibitory activity at the synapse [23]. For instance, Aoto and colleagues [24] demonstrated that when the AS4 exon of *Nrxn3* is constitutively included using a conditional knockin mouse, postsynaptic AMPA receptor (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, mediates fast excitatory neurotransmission) levels go down, NMDA receptor (*N*-methyl-D-aspartate, also involved in excitatory neurotransmission) levels are unaffected and there is more post-synaptic AMPA receptor endocytosis. Synaptic strength is decreased, demonstrating that the inclusion or exclusion of the AS4 exon in presynaptic Neurexin 3 trans-synaptically exerts control on postsynaptic characteristics [24]. Splicing of the *Nrxn* isoforms is correlated with levels of T-star protein in all regions of the mouse brain suggesting that T-star is an important regional regulator of *Nrxn* AS4 splicing [12].

The other splicing change which was identified in the knockout *T-star* mouse brain is the inclusion of a 171 base pair exon (exon 22) in the *Tomosyn-2* mRNA [12]. Tomosyn-2 protein is a syntaxin-1A binding protein that has an inhibitory effect on exocytosis [25]. In the central nervous system (CNS), Tomosyn-2 is expressed mainly in the hippocampus, cerebellum [26] and the spinal cord [27]. Analysis of *Tomosyn-2* knockout mice demonstrated that deletion of *Tomosyn-2* does not affect synaptic transmission in cultured hippocampal neurons. However, further experiments conducted in the *Tomosyn-2* knockout, showed that not only is spontaneous release of acetylcholine from the axons of motor neurons increased in the absence of Tomosyn-2, but also repetitive stimulation of motor neurons leads to more synaptic fatigue than in the wild-type mice. Additionally *Tomosyn-2* null mice do not perform as well at motor tests as the wild-type mice [27].

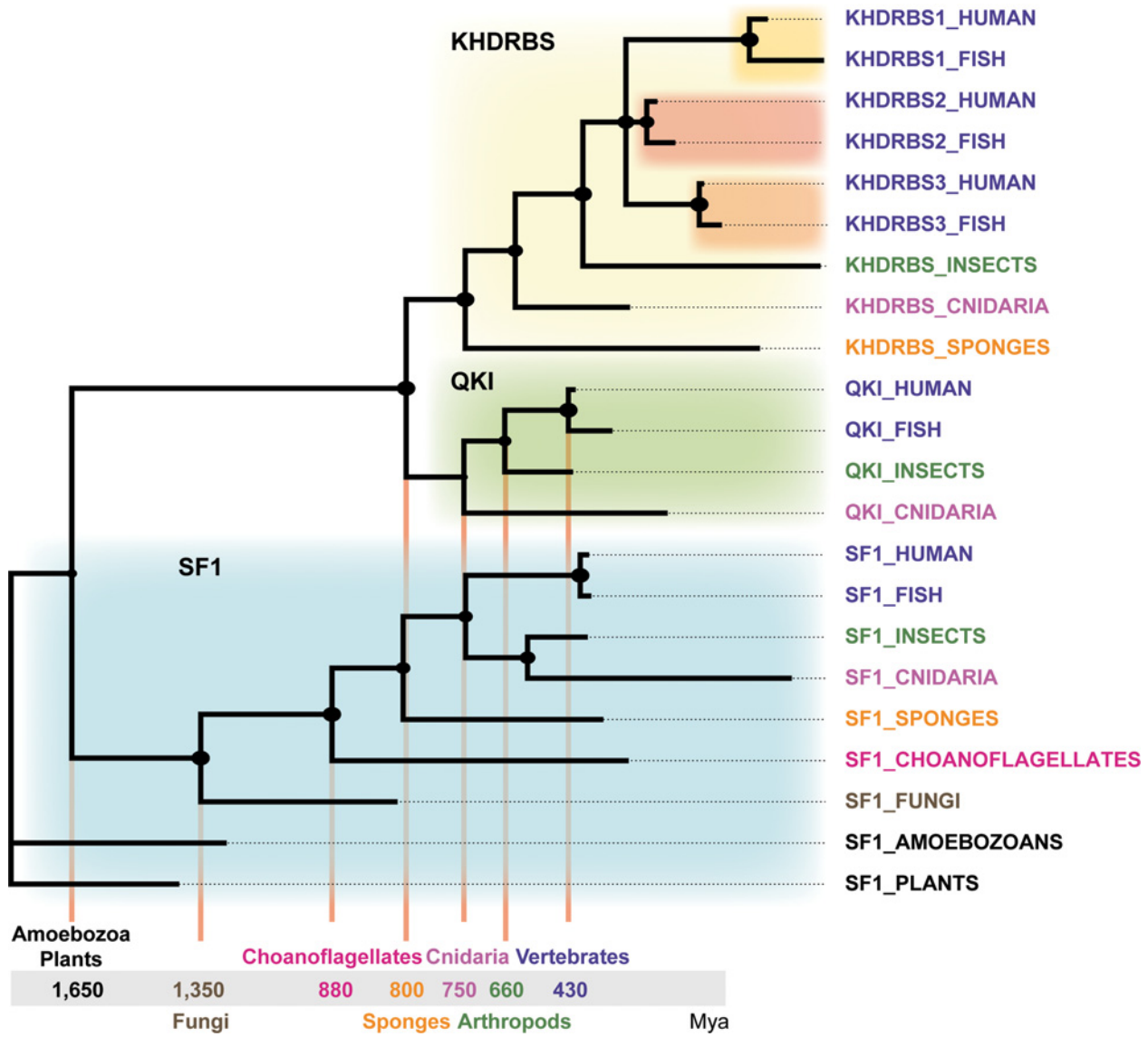
There are four splice isoforms of *Tomosyn-2* [26], and T-star promotes exon skipping of exon 22 which forms the splice isoform *m-tomosyn-2* [12]. Williams et al. [28] found that the basal level of hGH (human growth hormone) secretion is increased when *m-tomosyn-2* is overexpressed in PC12 cells (a neuroendocrine secretory cell model). However, if hGH secretion in PC12 cells is induced by potassium ions both *m-tomosyn-2* and the isoform which includes exon 22, called *xb-tomosyn*, inhibit this secretion. Additionally, the expression of *m-tomosyn-2* in HEK293 cells is higher than *xb-tomosyn-2*. Therefore, it appears that the *m-tomosyn-2* isoform under T-star control may encode a more stable protein and promote an increase in basal secretion in some cell lines. If the protein made from *m-tomosyn-2* behaves similarly in the intact animal as it does in the cell lines, then it is possible that lower levels of *m-tomosyn-2* in the *T-star* knockout mouse could affect neurotransmitter release at the neuromuscular junction.

Since T-star is highly expressed in the hippocampus, which controls spatial learning, Barnes maze tests were performed to determine whether the splicing changes in Neurexin AS4 isoforms between the wild-type and knockout mice could cause a difference in spatial learning and memory. Analysis of these Barnes maze tests showed that *T-star* null mice have no detectable learning deficits as judged by this test [12]. It would also be interesting to assess the motor function of

©2016 The Author(s). Published by Portland Press Limited on behalf of the Biochemical Society.

Figure 2 | Cladogram to demonstrate the evolutionary relationship between the STAR proteins

The duplications of the QKI/KHDRBS ancestor from SF1 and of QKI and KHDRBS occurred between choanoflagellates and sponges, i.e. 800–880 million years ago. Khdrbs1 is Sam68, Khdrbs2 is Slm1 and Khdrbs3 is T-star.



T-star knockout mice because these mice have lower levels of the Tomosyn-2 splice isoform, *m-tomosyn-2*, than wild-type mice. Since T-star is expressed in all regions of brain and in the spinal cord [12], it is also possible that the *T-star* knockout mice will exhibit behavioural changes other than spatial learning and motor function.

Sam68

Sam68 (also known as Khdrbs1) is the best studied member of the KHDRBS family. Sam68 is ubiquitously expressed in the body, and involved in multiple cellular processes such as polysomal recruitment of mRNAs [29] and alternative splicing [5]. The phenotypes displayed by a *Sam68* knockout mouse include decreased motor coordination assessed by beam walking and a rotorod test which points to a neural defect [30]. In 2011, Sam68 was shown to mediate activity-dependent RNA splicing of *Nrxn1* (*Neurexin1)* in the brain [31]. Depolarization of cerebellar (granule cells) neurons in the mouse brain causes AS4 skipping in all three *Neurexins*. Neuronal activity caused by addition of kainic acid (to stimulate glutamate receptors), or electrical stimulation, causes an increase in exon exclusion of *Nrxn1* AS4. Not only did minigene experiments demonstrate that Sam68 can mediate splicing of *Nrxn1* AS4, but RT-PCRs (reverse transcription polymerase chain reaction) performed with the endogenous *Nrxn1* mRNA from the brains of *Sam68* wild-type and knockout mice also show different patterns of *Nrxn1* AS4 splicing, consistent with control of *Nrxn1* AS4 splicing by Sam68.

©2016 The Author(s). Published by Portland Press Limited on behalf of the Biochemical Society.

In order to determine whether Sam68 can control a specific activity-dependent splice in the nervous system, experiments linking Sam68 activity to *Nrxn1* AS4 splicing were performed [31]. Unlike the situation in wild-type mice, *Nrxn1* AS4 splicing patterns in neurons from Sam68 knockout mice do not change in response to depolarization. Kainic acid was injected into the cerebellum of *Sam68* wild-type and knockout mice to stimulate neuronal activity and *Nrxn1* AS4 splicing was monitored. Stimulation of the glutamate receptor induces a change in *Nrxn1* AS4 splicing in patterns in wild-type mice but not in the *Sam68* knockouts demonstrating that Sam68 is responsible for activity-dependent splicing of *Nrxn1* AS4. Activity-dependent *Nrxn1* AS4 splicing is mediated by a phosphorylation event in Sam68 protein at serine 20 in depolarized neurons.

Another splicing target of Sam68 in the brain is the *SMN2* (*survival of motor neuron 2*) gene. SMA is an autosomal recessive neuromuscular disease caused by deletion of *SMN1*. SMA is the most common genetic cause of infant mortality [32], even though a second copy of the gene called *SMN2* exists that could replace its expression. However, a base change from a C to a T in *SMN2* exon 7 causes exon skipping and not much SMN2 protein is produced as a result [33,34]. The C to A mutation in *SMN2* creates a possible Sam68-binding site. Pedrotti et al. [35] showed that Sam68 and hn RNPA1 (a splicing repressor protein) can bind together in this *SMN2* RNA region and enhance exon skipping of exon7 in a cell line [35]. In 2015, the same research group extended their findings into an intact animal [36]. Using brains from the SMA mouse model, *SMN2Δ7;SMN2;Smn −/−* [37], they demonstrated that Sam68 and hn RNPA1 bind to the *SMN2* exon 7 sequence using CLIP (cross-linking and immunoprecipitation). Next, the *SMN* mouse model was bred with the *Sam68* knockout mouse and they looked for an improvement in several SMA parameters. They found that the mice lived longer, weighed more, could right themselves faster and had less motor neuron loss. Together these data demonstrate that lack of Sam68 causes an amelioration of the SMA phenotype [36].

Slm1

Slm1 (also known as Khdrbs2) is the third member of the KHDRBS family. *Slm1* knockout mice are fertile, and have no gross behavioural or phenotypic abnormalities. Slm1 is expressed across the brain in all regions that were tested – these were the olfactory bulb, striatum, cortex, cerebellum, brain stem with higher expression in dentate gyrus, thalamus and hypothalamus and midbrain [14]. Experiments performed by Iijima et al. [14] in the *Slm1* knockout mouse, have demonstrated that Slm1 is important for the splicing of the AS4 exon in all three *Nrxn* genes in the midbrain. Further studies by the same group, have also demonstrated that an up-regulation of Slm1 in certain cell types in the *T-star* knockout mouse partially makes up for the lack of T-star [13]. Although there have been no behavioural defects reported yet for the *Slm1* knockout mouse, it is possible that a Barnes Maze learning test could reveal a phenotype in *Slm1/T-star* double knockout mice.

Quaking

Quaking is a STAR protein first identified in the qk^{v} (*quakingviable*) mouse [38]. Quaking is expressed in a number of tissues including brain, heart, lung, testis, muscle, prostate, colon, stomach and myeloid cells [38,39]. The mutation in the qk^{v} mice, which have tremors in their hind region and a severe deficit in myelin [15], is not in the coding region of *quaking*, but lies within the 5′ regulatory region. Consequently, levels of Quaking are diminished in qk^{v} mice compared with wild-type mice [38]. Myelin, made by oligodendrocytes in the CNS, is important to increase electrical conductance in the nervous system and protects neurons from damage [40]. Quaking has three protein isoforms: QKI-5, QKI-6 and QKI-7 (Quaking 5, 6 and 7) which are named based on the length of their mRNAs. QKI-5 protein is nuclear. QKI-6 and QKI-7 proteins are cytoplasmic.

qk^{v} mice have aberrant splice isoform ratios of several myelin-specific genes such as PLP (proteolipid protein), MBP (myelin-basic protein) and *MAG* (myelin-associated glycoprotein) compared with wild-type mice [41,42]. MAG has an important role in neurite outgrowth [40]. Wu et al. [42] demonstrated that the nuclear form of Quaking protein, QKI-5, regulates *MAG* exon 12 splicing in a cell-based minigene assay. However, Zhao et al. [43] also showed that QKI-6 protein, a cytoplasmic form of Quaking, rescues *MAG* splicing in oligodendrocytes from qk^{v} mice without an increase in QKI-5. These latter experiments suggest that QKI-6 indirectly controls *MAG* splicing by altering the translation of the splicing suppressor hn RNPA1. Earlier experiments had already demonstrated that hypo-myelination and tremor in the qk^{v} mice can be rescued by expressing a transgene encoding QKI-6 in oligodendrocytes [44]. More evidence for QKI-6 effects on splicing come from Mandler et al. [45] who demonstrated that QKI-6 represses two splicing factors called hnRNP F/H *in vivo*. Minigene experiments suggest that hnRNP F/H also regulate *MAG* exon 12 splicing. This group also identified several new splicing targets in the qk^{v} mouse that are part of the HnRNPF/H pathway [45]. Hence, these experiments show that Quaking may regulate splicing in the brain directly and indirectly.

With the advent of a *quaking* conditional knockout mouse by Darbelli et al. [17], the *quaking* gene can be deleted in specific cell types. Crossing the $QkI^{FL/+}$ mice with *Olig2-Cre* to specifically delete *quaking* in oligodendrocytes results in mice with considerable hypomyelination in the CNS, tremors at postnatal day 10 and death at 3 weeks. In order to identify alternative splices regulated by quaking protein in mice, postnatal day 14 brains from wild-type and $QkI^{FL/FL,Olig2\text{-}cre}$ mice were tested on a PCR platform [21]. The investigators found significant changes in 31 alternative splicing events including *NFasc*. *NFasc* makes cell adhesion proteins termed Nfasc155 and Nfasc186. RT-PCRs show that exon 17 which

©2016 The Author(s). Published by Portland Press Limited on behalf of the Biochemical Society.

is specific to the neuronal Nfasc186 is included in the brains of the $QKI^{FL/FL,Olig2\text{-}cre}$ mice and exons 21/22 that are specific for oligodendrocyte Nfasc155 are excluded in the $QKI^{FL/FL,Olig2\text{-}cre}$ mouse brains. Since Nfasc155 protein was absent from the brains of the knockout mice, these authors focused on exons 21/22 in the Nfasc155 isoform. Minigene experiments demonstrated that QKI-5 is the major Quaking isoform that promotes inclusion of Nfasc exons 21 and 22. Nfasc155 is known to be present at axoglial junctions in the spinal cord. By crossing $QkI^{FL/FL}$ with *PLP-CreERT* and inducibly deleting the quaking gene in adult mice, the investigators found defects in axoglial junctions of the spinal cords and notable loss of Nfasc155 staining [17]. Therefore, these conditional mutant mice have been used to uncover a splicing change which results in a phenotypic defect.

SF1

SF1 is widely expressed in human and mouse tissues [46] and is an early component of the assembling spliceosome. SF1 together with the protein U2AF recognize distinct sequences at the 3′ splice site and form part of the E complex: subsequently SF1 is replaced by U2 snRNP to form the A complex [47]. There have been suggestions that SF1 might not be absolutely essential in early spliceosome complex formation. For instance, when SF1 was depleted from human or yeast splicing extracts, splicing complexes formed more slowly but splicing still proceeded [48,49].

Roles for SF1 in alternative splicing in mammals, yeast and plants have been reported. SF1 interacts with the β-catenin/TCf4 complex to influence its pre-mRNA splicing activities in colon cancer [50]. A mutation in SF1 from fission yeast leads to exon skipping [51], and finally a mutation in the *Arabidopsis* homologue of SF1 leads to altered alternative splicing patterns of several genes including heat shock transcription factor *HsfA2* [52]. Corioni et al. [53] explored the function of SF1 in alternative splicing in HeLa cells using CLIP and siRNA knockdown of SF1. They identified novel mRNAs with CLIP tags which were differentially spliced between wild-type HeLa cells and HeLa cells depleted of SF1 by siRNA. These data demonstrate an alternative splicing role for SF1 in cultured cells. However, to date there is no data demonstrating a direct role for SF1 in alternative splicing in an intact animal. Perhaps future experiments could make use of the *Sf1* genetrap mice to explore alternative splicing of *Sf1* in the brain, where it is known to be expressed [46]. Even though *Sf1* homozygous genetrap mice die before embryonic day 8.5, it may be possible to use the heterozygotes to analyse splicing as they are viable [16].

Summary

All of the STAR proteins are expressed in both neuronal and non-neuronal tissues and they have been implicated in a number of human diseases including neurological disorders and cancer. Cancers that show SAM68 up-regulation include breast, prostate and colorectal cancer [54]. On the other hand, QUAKING is down-regulated in lung cancer. The mechanism for this may be because QUAKING has a role in modulating NUMB splicing in the lung. This in turn leads to less proliferation and prevents notch signalling [55]. An amplified region of DNA which includes *T-star* has been found in medulloblastoma cell lines, and there is an increase in *T-star* transcripts in primary medulloblastoma tumours suggesting that T-star is important in this type of childhood cancer [56]. Non-cancerous neurological disorders can also be linked to the splicing changes mediated by STAR proteins in the brain and spinal cord. The KHDRBS proteins T-star, Sam68 and Slm1 are important regulators of *Nrxn1* which has been implicated in human diseases such as autism and schizophrenia. Heterozygous deletions of *NRXN1* α have been found in several studies of patients with schizophrenia and in some autistic patients. A deletion which is common to *NRXN* α and β has also been reported in twins with childhood-onset schizophrenia. The AS4 exon is in the region of *NRXN1* which is common to both the α and β forms [57]. *QUAKING* may be also be associated with schizophrenia as expression of *QKI* mRNA was found to be decreased in brain regions such as the cortex and hippocampus of schizophrenic patients [58]. Additionally, *QUAKING* has also been associated with ataxias and degeneration of Purkinje cells [6]. A family with a severe neurological disease seen in infancy which includes axonal neuropathy, cognitive delay and optic atrophy has been shown to have a homozygous mutation in the gene for *TOMOSYN-2* (*STXBP5L*) emphasizing the importance of this gene in brain development [59]. And finally, the partial abrogation of the SMA phenotype in the SMA mouse model in the absence of Sam68, points to the importance of these RNA-binding proteins as tools for future therapies of some neurological diseases.

Acknowledgements

We thank Cyril Dominguez for his advice and explanation of the structure of STAR proteins.

Funding

This work was supported by the Biotechnology and Biological Sciences Research Council [grant numbers BB/D013917/1 and BB/1006923/1].

References

1 Rajan, P., Elliott, D.J., Robson, C.N. and Leung, H.Y. (2009) Alternative splicing and biological heterogeneity in prostate cancer. Nat. Rev. Urol. **6**, 454–460 CrossRef PubMed

2 Barrett, L.W., Fletcher, S. and Wilton, S.D. (2012) Regulation of eukaryotic gene expression by the untranslated gene regions and other non-coding elements. Cell. Mol. Life Sci. **69**, 3613–3634 CrossRef PubMed

©2016 The Author(s). Published by Portland Press Limited on behalf of the Biochemical Society.

3 Ehrmann, I. and Elliott, D.J. (2010) Expression and functions of the star proteins Sam68 and T-STAR in mammalian spermatogenesis. Adv. Exp. Med. Biol. **693**, 67–81 CrossRef PubMed

4 Sette, C. (2010) Post-translational regulation of star proteins and effects on their biological functions. Adv. Exp. Med. Biol. **693**, 54–66 CrossRef PubMed

5 Vogel, G. and Richard, S. (2012) Emerging roles for Sam68 in adipogenesis and neuronal development. RNA Biol. **9**, 1129–1133 CrossRef PubMed

6 Artzt, K. and Wu, J.I. (2010) STAR trek: an introduction to STAR family proteins and review of quaking (QKI). Adv. Exp. Med. Biol. **693**, 1–24 CrossRef PubMed

7 Feracci, M., Foot, J.N., Grellscheid, S.N., Danilenko, M., Stehle, R., Gonchar, O., Kang, H.S., Dalgliesh, C., Meyer, N.H., Liu, Y. et al. (2016) Structural basis of RNA recognition and dimerization by the STAR proteins T-STAR and Sam68. Nat. Commun. **7**, 10355 CrossRef PubMed

8 Teplova, M., Hafner, M., Teplov, D., Essig, K., Tuschl, T. and Patel, D.J. (2013) Structure–function studies of STAR family Quaking proteins bound to their *in vivo* RNA target sites. Genes Dev. **27**, 928–940 CrossRef PubMed

9 Liu, Z., Luyten, I., Bottomley, M.J., Messias, A.C., Houngninou-Molango, S., Sprangers, R., Zanier, K., Kramer, A. and Sattler, M. (2001) Structural basis for recognition of the intron branch site RNA by splicing factor 1. Science **294**, 1098–1102 CrossRef PubMed

10 Beuck, C., Qu, S., Fagg, W.S., Ares, Jr, M. and Williamson, J.R. (2012) Structural analysis of the quaking homodimerization interface. J. Mol. Biol. **423**, 766–781 CrossRef PubMed

11 Richard, S., Torabi, N., Franco, G.V., Tremblay, G.A., Chen, T., Vogel, G., Morel, M., Cleroux, P., Forget-Richard, A., Komarova, S. et al. (2005) Ablation of the Sam68 RNA binding protein protects mice from age-related bone loss. PLoS Genet. **1**, e74 CrossRef PubMed

12 Ehrmann, I., Dalgliesh, C., Liu, Y., Danilenko, M., Crosier, M., Overman, L., Arthur, H.M., Lindsay, S., Clowry, G.J., Venables, J.P. et al. (2013) The tissue-specific RNA binding protein T-STAR controls regional splicing patterns of neurexin pre-mRNAs in the brain. PLoS Genet. **9**, e1003474 CrossRef PubMed

13 Traunmuller, L., Bornmann, C. and Scheiffele, P. (2014) Alternative splicing coupled nonsense-mediated decay generates neuronal cell type-specific expression of SLM proteins. J. Neurosci. **34**, 16755–16761 CrossRef PubMed

14 Iijima, T., Iijima, Y., Witte, H. and Scheiffele, P. (2014) Neuronal cell type-specific alternative splicing is regulated by the KH domain protein SLM1. J. Cell Biol. **204**, 331–342 CrossRef PubMed

15 Sidman, R.L., Dickie, M.M. and Appel, S.H. (1964) Mutant mice (Quaking and Jimpy) with deficient myelination in the central nervous system. Science **144**, 309–311 CrossRef PubMed

16 Shitashige, M., Satow, R., Honda, K., Ono, M., Hirohashi, S. and Yamada, T. (2007) Increased susceptibility of Sf1(+/−) mice to azoxymethane-induced colon tumorigenesis. Cancer Sci. **98**, 1862–1867 CrossRef PubMed

17 Darbelli, L., Vogel, G., Almazan, G. and Richard, S. (2016) Quaking regulates neurofascin 155 expression for myelin and axoglial junction maintenance. J. Neurosci. **36**, 4106–4120 CrossRef PubMed

18 Yeo, G., Holste, D., Kreiman, G. and Burge, C.B. (2004) Variation in alternative splicing across human tissues. Genome Biol. **5**, R74 CrossRef PubMed

19 Venables, J.P., Vernet, C., Chew, S.L., Elliott, D.J., Cowmeadow, R.B., Wu, J., Cooke, H.J., Artzt, K. and Eperon, I.C. (1999) T-STAR/ETOILE: a novel relative of SAM68 that interacts with an RNA-binding protein implicated in spermatogenesis. Hum. Mol. Genet. **8**, 959–969 CrossRef PubMed

20 Di Fruscio, M., Chen, T. and Richard, S. (1999) Characterization of Sam68-like mammalian proteins SLM-1 and SLM-2: SLM-1 is a Src substrate during mitosis. Proc. Natl. Acad. Sci. U.S.A. **96**, 2710–2715 CrossRef PubMed

21 Venables, J.P., Klinck, R., Koh, C., Gervais-Bird, J., Bramard, A., Inkel, L., Durand, M., Couture, S., Froehlich, U., Lapointe, E. et al. (2009) Cancer-associated regulation of alternative splicing. Nat. Struct. Mol. Biol. **16**, 670–676 CrossRef PubMed

22 Ullrich, B., Ushkaryov, Y.A. and Sudhof, T.C. (1995) Cartography of neurexins: more than 1000 isoforms generated by alternative splicing and expressed in distinct subsets of neurons. Neuron **14**, 497–507 CrossRef PubMed

23 Reissner, C., Runkel, F. and Missler, M. (2013) Neurexins. Genome Biol. **14**, 213 CrossRef PubMed

24 Aoto, J., Martinelli, D.C., Malenka, R.C., Tabuchi, K. and Sudhof, T.C. (2013) Presynaptic neurexin-3 alternative splicing trans-synaptically controls postsynaptic AMPA receptor trafficking. Cell **154**, 75–88 CrossRef PubMed

25 Bhatnagar, S., Oler, A.T., Rabaglia, M.E., Stapleton, D.S., Schueler, K.L., Truchan, N.A., Worzella, S.L., Stoehr, J.P., Clee, S.M., Yandell, B.S. et al. (2011) Positional cloning of a type 2 diabetes quantitative trait locus; tomosyn-2, a negative regulator of insulin secretion. PLoS Genet. **7**, e1002323 CrossRef PubMed

26 Groffen, A.J., Jacobsen, L., Schut, D. and Verhage, M. (2005) Two distinct genes drive expression of seven tomosyn isoforms in the mammalian brain, sharing a conserved structure with a unique variable domain. J. Neurochem. **92**, 554–568 CrossRef PubMed

27 Geerts, C.J., Plomp, J.J., Koopmans, B., Loos, M., van der Pijl, E.M., van der Valk, M.A., Verhage, M. and Groffen, A.J. (2015) Tomosyn-2 is required for normal motor performance in mice and sustains neurotransmission at motor endplates. Brain Struct. Funct. **220**, 1971–1982 CrossRef PubMed

28 Williams, A.L., Bielopolski, N., Meroz, D., Lam, A.D., Passmore, D.R., Ben-Tal, N., Ernst, S.A., Ashery, U. and Stuenkel, E.L. (2011) Structural and functional analysis of tomosyn identifies domains important in exocytotic regulation. J. Biol. Chem. **286**, 14542–14553 CrossRef PubMed

29 Paronetto, M.P., Messina, V., Bianchi, E., Barchi, M., Vogel, G., Moretti, C., Palombi, F., Stefanini, M., Geremia, R., Richard, S. and Sette, C. (2009) Sam68 regulates translation of target mRNAs in male germ cells, necessary for mouse spermatogenesis. J. Cell Biol. **185**, 235–249 CrossRef PubMed

30 Lukong, K.E. and Richard, S. (2008) Motor coordination defects in mice deficient for the Sam68 RNA-binding protein. Behav. Brain Res. **189**, 357–363 CrossRef PubMed

31 Iijima, T., Wu, K., Witte, H., Hanno-Iijima, Y., Glatter, T., Richard, S. and Scheiffele, P. (2011) SAM68 regulates neuronal activity-dependent alternative splicing of neurexin-1. Cell **147**, 1601–1614 CrossRef PubMed

32 Monani, U.R. (2005) Spinal muscular atrophy: a deficiency in a ubiquitous protein; a motor neuron-specific disease. Neuron **48**, 885–896 CrossRef PubMed

33 Monani, U.R., Lorson, C.L., Parsons, D.W., Prior, T.W., Androphy, E.J., Burghes, A.H. and McPherson, J. D. (1999) A single nucleotide difference that alters splicing patterns distinguishes the SMA gene SMN1 from the copy gene SMN2. Hum. Mol. Genet. **8**, 1177–1183 CrossRef PubMed

34 Lorson, C.L., Hahnen, E., Androphy, E.J. and Wirth, B. (1999) A single nucleotide in the SMN gene regulates splicing and is responsible for spinal muscular atrophy. Proc. Natl. Acad. Sci. U.S.A. **96**, 6307–6311 CrossRef PubMed

35 Pedrotti, S., Bielli, P., Paronetto, M.P., Ciccosanti, F., Fimia, G.M., Stamm, S., Manley, J.L. and Sette, C. (2010) The splicing regulator Sam68 binds to a novel exonic splicing silencer and functions in SMN2 alternative splicing in spinal muscular atrophy. EMBO J. **29**, 1235–1247 CrossRef PubMed

36 Pagliarini, V., Pelosi, L., Bustamante, M.B., Nobili, A., Berardinelli, M.G., D'Amelio, M., Musaro, A. and Sette, C. (2015) SAM68 is a physiological regulator of SMN2 splicing in spinal muscular atrophy. J. Cell Biol. **211**, 77–90 CrossRef PubMed

37 Le, T.T., Pham, L.T., Butchbach, M.E., Zhang, H.L., Monani, U.R., Coovert, D.D., Gavrilina, T.O., Xing, L., Bassell, G.J. and Burghes, A.H. (2005) SMNDelta7, the major product of the centromeric survival motor neuron (SMN2) gene, extends survival in mice with spinal muscular atrophy and associates with full-length SMN. Hum. Mol. Genet. **14**, 845–857 CrossRef PubMed

38 Ebersole, T.A., Chen, Q., Justice, M.J. and Artzt, K. (1996) The quaking gene product necessary in embryogenesis and myelination combines features of RNA binding and signal transduction proteins. Nat. Genet. **12**, 260–265 CrossRef PubMed

39 Darbelli, L. and Richard, S. (2016) Emerging functions of the Quaking RNA-binding proteins and link to human diseases. Wiley Interdiscip. Rev.: RNA **7**, 399–412 CrossRef PubMed

40 Zearfoss, N.R., Farley, B.M. and Ryder, S.P. (2008) Post-transcriptional regulation of myelin formation. Biochim. Biophys. Acta **1779**, 486–494 CrossRef PubMed

©2016 The Author(s). Published by Portland Press Limited on behalf of the Biochemical Society.

41 Fujita, N., Sato, S., Kurihara, T., Inuzuka, T., Takahashi, Y. and Miyatake, T. (1988) Developmentally regulated alternative splicing of brain myelin-associated glycoprotein mRNA is lacking in the quaking mouse. FEBS Lett. **232**, 323–327 CrossRef PubMed

42 Wu, J.I., Reed, R.B., Grabowski, P.J. and Artzt, K. (2002) Function of quaking in myelination: regulation of alternative splicing. Proc. Natl. Acad. Sci. U.S.A. **99**, 4233–4238 CrossRef PubMed

43 Zhao, L., Mandler, M.D., Yi, H. and Feng, Y. (2010) Quaking I controls a unique cytoplasmic pathway that regulates alternative splicing of myelin-associated glycoprotein. Proc. Natl. Acad. Sci. U.S.A. **107**, 19061–19066 CrossRef PubMed

44 Zhao, L., Tian, D., Xia, M., Macklin, W.B. and Feng, Y. (2006) Rescuing qkV dysmyelination by a single isoform of the selective RNA-binding protein QKI. J. Neurosci. **26**, 11278–11286 CrossRef PubMed

45 Mandler, M.D., Ku, L. and Feng, Y. (2014) A cytoplasmic quaking I isoform regulates the hnRNP F/H-dependent alternative splicing pathway in myelinating glia. Nucleic Acids Res. **42**, 7319–7329 CrossRef PubMed

46 Arning, S., Gruter, P., Bilbe, G. and Kramer, A. (1996) Mammalian splicing factor SF1 is encoded by variant cDNAs and binds to RNA. RNA **2**, 794–810 PubMed

47 Wahl, M.C., Will, C.L. and Luhrmann, R. (2009) The spliceosome: design principles of a dynamic RNP machine. Cell **136**, 701–718 CrossRef PubMed

48 Rutz, B. and Seraphin, B. (1999) Transient interaction of BBP/ScSF1 and Mud2 with the splicing machinery affects the kinetics of spliceosome assembly. RNA **5**, 819–831 CrossRef PubMed

49 Guth, S. and Valcarcel, J. (2000) Kinetic role for mammalian SF1/BBP in spliceosome assembly and function after polypyrimidine tract recognition by U2AF. J. Biol. Chem. **275**, 38059–38066 CrossRef PubMed

50 Shitashige, M., Naishiro, Y., Idogawa, M., Honda, K., Ono, M., Hirohashi, S. and Yamada, T. (2007) Involvement of splicing factor-1 in beta-catenin/T-cell factor-4-mediated gene transactivation and pre-mRNA splicing. Gastroenterology **132**, 1039–1054 CrossRef PubMed

51 Haraguchi, N., Andoh, T., Frendewey, D. and Tani, T. (2007) Mutations in the SF1-U2AF59-U2AF23 complex cause exon skipping in Schizosaccharomyces pombe. J. Biol. Chem. **282**, 2221–2228 CrossRef PubMed

52 Jang, Y.H., Park, H.Y., Lee, K.C., Thu, M.P., Kim, S.K., Suh, M.C., Kang, H. and Kim, J.K. (2014) A homolog of splicing factor SF1 is essential for development and is involved in the alternative splicing of pre-mRNA in *Arabidopsis thaliana*. Plant J. **78**, 591–603 CrossRef PubMed

53 Corioni, M., Antih, N., Tanackovic, G., Zavolan, M. and Kramer, A. (2011) Analysis of *in situ* pre-mRNA targets of human splicing factor SF1 reveals a function in alternative splicing. Nucleic Acids Res. **39**, 1868–1879 CrossRef PubMed

54 Frisone, P., Pradella, D., Di Matteo, A., Belloni, E., Ghigna, C. and Paronetto, M.P. (2015) SAM68: signal transduction and RNA metabolism in human cancer. Biomed. Res. Int. **2015**, 528954 CrossRef PubMed

55 Zong, F.Y., Fu, X., Wei, W.J., Luo, Y.G., Heiner, M., Cao, L.J., Fang, Z., Fang, R., Lu, D., Ji, H. and Hui, J. (2014) The RNA-binding protein QKI suppresses cancer-associated aberrant splicing. PLoS Genet. **10**, e1004289 CrossRef PubMed

56 Lu, Y., Ryan, S.L., Elliott, D.J., Bignell, G.R., Futreal, P.A., Ellison, D.W., Bailey, S. and Clifford, S.C. (2009) Amplification and overexpression of Hsa-miR-30b, Hsa-miR-30d and KHDRBS3 at 8q24.22-q24.23 in medulloblastoma. PLoS One **4**, e6159 CrossRef PubMed

57 Reichelt, A.C., Rodgers, R.J. and Clapcote, S.J. (2012) The role of neurexins in schizophrenia and autistic spectrum disorder. Neuropharmacology **62**, 1519–1526 CrossRef PubMed

58 Haroutunian, V., Katsel, P., Dracheva, S. and Davis, K.L. (2006) The human homolog of the QKI gene affected in the severe dysmyelination "quaking" mouse phenotype: downregulated in multiple brain regions in schizophrenia. Am. J. Psychiatry **163**, 1834–1837 CrossRef PubMed

59 Kumar, R., Corbett, M.A., Smith, N.J., Jolly, L.A., Tan, C., Keating, D.J., Duffield, M.D., Utsumi, T., Moriya, K., Smith, K.R. et al. (2015) Homozygous mutation of STXBP5L explains an autosomal recessive infantile-onset neurodegenerative disorder. Hum. Mol. Genet. **24**, 2000–2010 CrossRef PubMed

Received 31 March 2016
doi:10.1042/BST20160084

©2016 The Author(s). Published by Portland Press Limited on behalf of the Biochemical Society.