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Microexons: at the nexus of nervous system development, behaviour and autism spectrum disorder

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The discovery and characterization of a network of highly conserved neuronal microexons has provided fundamental new insight into mechanisms underlying nervous system development and function, as well as an important basis for pathway convergence in autism spectrum disorder. In the past few years, considerable progress has been made in comprehensively determining the repertoires of factors that control neuronal microexons. These results have illuminated molecular mechanisms that activate the splicing of microexons, including those that control gene expression programs critical for neurogenesis, as well as synaptic protein translation and neuronal activity. Remarkably, individual disruption of specific microexons in these pathways results in autism-like phenotypes and cognitive impairment in mice. This review discusses these findings and their implications for delivering new therapeutic strategies for neurological disorders.

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A neuronal microexon network frequently disrupted in autism spectrum disorder

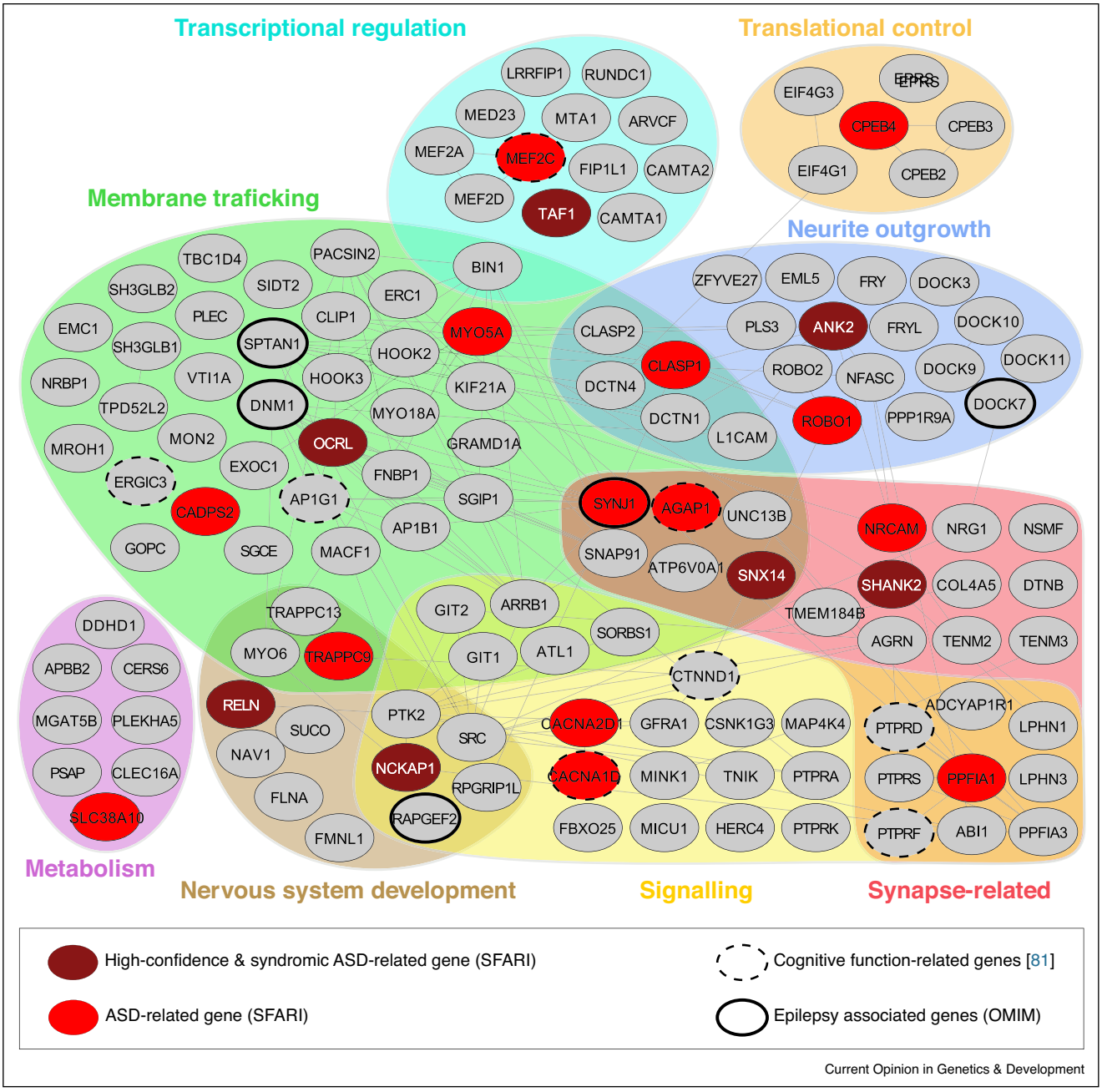
High-throughput RNA sequencing (RNA-Seq) studies during the past 12 years have revealed that alternative splicing is widespread and contributes greatly to the transcriptomic complexity of the vertebrate nervous system [1–4]. Among several thousand detected neural differential splice variants is a program of ~250 predominately neuronal-included, 3–27 nucleotide long microexons. These microexons represent the most highly conserved program of alternative splicing detected to date in vertebrates and typically display pronounced switch-like regulation during neuronal differentiation [5,6]. Moreover, they are strongly enriched for frame preservation and frequently insert one to nine amino acids in or proximal to interaction domains of proteins associated with neurogenesis, axonogenesis and synaptic functions [5,6] (Figure 1). Consistent with these findings, accumulating evidence indicates that neuronal microexons have important roles in shaping protein–protein interactions required for brain-specific functions [5,7••,8].

Another striking feature of the neuronal microexon program is that it is frequently disrupted in the brains of autism spectrum disorder (ASD) subjects. RNA-Seq analysis of post-mortem brain samples indicated that approximately 40% of detected neuronal microexons displayed increased skipping in 30% of analyzed individuals with idiopathic autism, relative to matched controls, whereas a significantly lower percentage of longer cassette exons (~5%) displayed misregulation [5]. These observations were subsequently confirmed in analyses of larger cohorts of ASD patient and control samples [9,10••]. Importantly, supporting a relationship between this dysregulation and neurological disorders, neuronal microexons are often found in genes with genetic links to ASD [5] (Figure 1). Moreover, a mouse model that recapitulates disruption of neuronal microexons was shown to have multiple hallmark ASD-like features, including social behaviour deficits, sensitivity to environmental stimuli, aberrant cortical layering and altered synaptic transmission [11,12]. Interestingly, the disruption of neuronal microexons has recently been linked to additional neurodevelopmental disorders including schizophrenia [10••]. Collectively, these studies have provided evidence that a dynamic neuronal microexon network is critical for nervous system development and function, and that when disrupted contributes to neurological disorders. These initial studies raised several important questions, namely: how are microexons activated in neurons despite their short length; which of the many microexons contribute to ASD-associated or possibly other neurological phenotypes; and what are the mechanisms by which microexons function? The following sections of this review describe recent advances in addressing these questions. Earlier studies focusing on the discovery and characterization of microexons are reviewed in Refs. [13–15]. Microexons that function in non-neural cells are also not discussed in this review.

(Mis-)regulation of neuronal microexons

Microexons are generally too short to be amenable to previously described exon recognition mechanisms, which

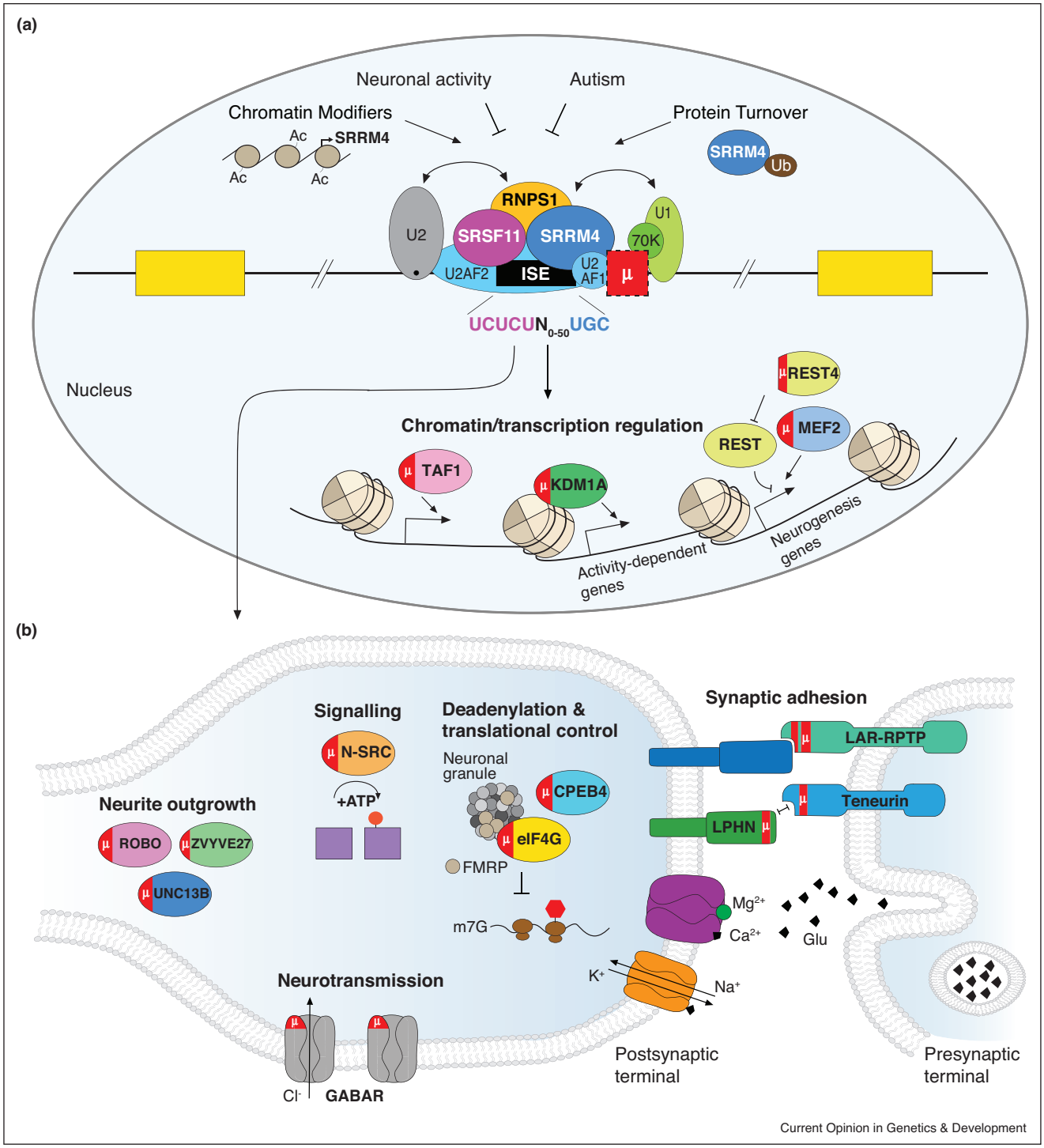
Figure 1



A neuronal microexon network associated with nervous system development, function and autism spectrum disorder.
The diagram models a protein–protein interaction network (based on STRING annotations [80]) representing 136 genes that encode a neuronal-specific microexon conserved between human and mouse and disrupted in neurological disorders. Genes genetically linked with autism (SFARI; https://gene.sfari.org/database/human-gene/), epilepsy (OMIM; https://omim.org/) and cognitive functioning [81] are indicated. The functional groups were manually curated.

involve networks of 'exon definition' interactions that bridge between 5′ and 3′ splice sites across exons. Analyses of the intronic sequences surrounding microexons revealed that they are on average significantly more conserved than intron sequences flanking longer alternative exons, and also that they are typically demarcated by relatively weak 3′ splice sites and strong 5′ splice sites [5,6]. These observations suggested that intronic elements proximal to microexon 3′ splice sites may facilitate their regulated activation. Knockdown or overexpression of several alternative splicing regulators that display differential expression between neural and other tissues, including the neuronal-specific

Figure 2



Regulation and function of neuronal microexons.
(a) Model for the recognition of neuronal microexons involving a network of protein–protein and protein–RNA interactions that span a specialized intronic enhancer and core splicing signals. Upstream regulatory factors and processes that impact microexons are indicated, as are examples of downstream target microexons that control core gene regulatory processes. **(b)** Illustration of how different neuronal microexons impact processes critical for nervous system development and function, including translational control, neurite outgrowth, neurotransmission and signalling. μ, Microexon; MEF2, Myocyte-specific enhancer factor 2; TAF1, Transcription initiation factor TFIID subunit 1; Ub, Ubiquitin; ISE, intronic splicing enhancer element; U1, U1 small nuclear ribonucleoprotein (snRNP); 70K, U1 snRNP 70 kDa; U2, U2 snRNP; GABAR, gamma-aminobutyric acid receptors; Glu, glutamate; m7G, 7-methylguanosine cap; ZFYVE27, Protrudin; LAR-RPTP, Leukocyte common antigen-related receptor tyrosine phosphatase; LPHN, Latrophilin.

Serine/Arginine Repetitive Matrix 4 protein (SRRM4; also known as nSR100), RBFOX and PTBP1, followed by RNA-Seq analysis, revealed that these factors extensively regulate microexon splicing, with SRRM4 and RBFOX predominantly activating – and PTBP1 repressing – inclusion, respectively [5,6]. These and subsequent studies revealed that SRRM4 has the greatest impact on microexons; it activates more than half of all neural microexons, through binding UGC-containing motifs located between the 3′ splice site and polypyrimidine tract [5,16,17]. However, how SRRM4 functions with other factors to define microexons, and how it and other directly acting regulators themselves are controlled was unclear.

To systematically define the repertoires of factors and pathways that regulate neuronal microexon splicing, we performed CRISPR-Cas9 loss of function screens targeting ~20 000 protein coding genes in neural cells expressing dual-fluorescent microexon splicing reporters from the Mef2d and Shank2 genes [18•]. These screens revealed that extensively overlapping sets of ~200 genes impact splicing of these microexons and act at multiple regulatory levels, including RNA processing, chromatin regulation and proteolysis. Remarkably, these factors are significantly enriched in genetic links to autism. In addition to SRRM4 and its closely related paralog, SRRM3, screen hits with among the largest positive impact on microexon inclusion included the widely expressed SR-related proteins, SRSF11 and RNPS1. Further analysis revealed that these proteins interact with SRRM4 and a bi-partite intronic splicing enhancer element (UCUCUCN$_{1\text{-}50}$UGC) that is enriched upstream of neuronal microexons [18•] (Figure 2a). It was also shown that SRRM4, through its C-terminal intrinsically disordered domain (dubbed the 'eMIC domain'), which is necessary and sufficient for promoting microexon splicing, forms interactions with the branchpoint binding protein SF1, and the U2 snRNP auxiliary factor (U2AF) [19••]. Collectively, these interactions are likely at the core of early steps in the recognition and activation of microexon splicing [18•,19••]. Moreover, these and additional results reveal how exons that are too short to harbour splicing enhancer sequences nevertheless depend on exon definition-like interactions that instead span an upstream intronic enhancer and the microexon 5′ splice site [13,18•] (Figure 2a).

To further investigate the pathways that impact neuronal microexon splicing, the reporter screens described above were complemented by genome-wide CRISPR-Cas9 screens designed to detect factors that affect SRRM4 protein levels [18•]. Genes identified in both screens included chromatin regulators, such as the histone acetyltransferases EP300 and CREBBP, which impact microexon splicing by promoting *SRRM4* gene expression. The screens also identified FBXW11, a component of the Skp-Cullin-F-box complex, which interacts with and reduces SRRM4 expression via ubiquitin-dependent proteolysis. The screens described above thus defined direct and indirect mechanisms and pathways that control microexon splicing (Figure 2a). These approaches further enabled linking genetics with underlying molecular mechanisms; in addition to other factors defined in the CRISPR screens, SRSF11, RNPS1, EP300, and CREBBP have all been genetically linked to ASD and intellectual disability [20–23].

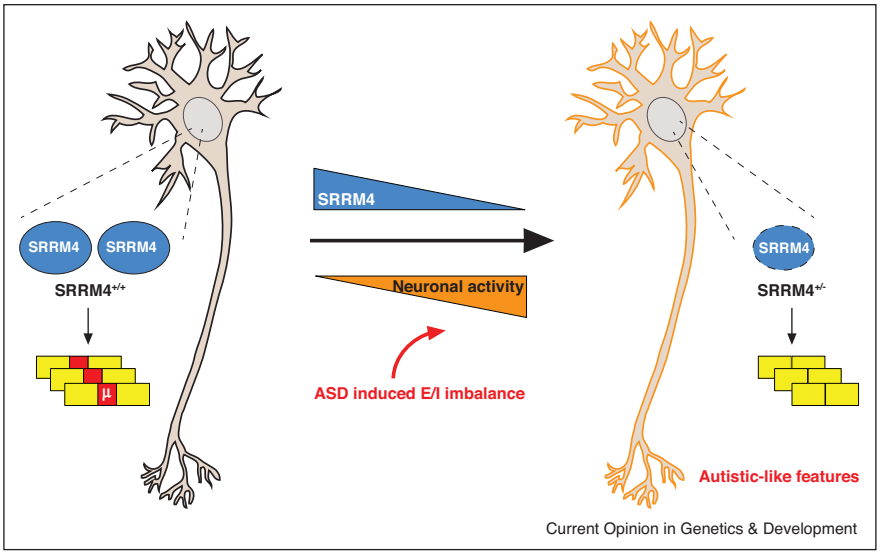
Another important aspect of the regulation of microexons is that they are disproportionately impacted by changes in neuronal activity. Following KCl-induced depolarization of neurons, 22% of all microexons show increased skipping, in contrast to 7% of longer cassette exons [11]. The reduced splicing levels of microexons is consistent with a rapid reduction in SRRM4 protein detected in response to induced neuronal activity [11]. These findings suggested a possible mechanism for phenotypic convergence arising from the diverse genetic alterations impacting ASDs, namely, that increased neuronal activity, as a comorbid feature associated with a subset of the genetic insults contributing to ASD, results in a loss of SRRM4 and other factors that in turn leads to decreased splicing of microexons and consequent disruption of neuronal development and function [24] (Figure 3). Interestingly, microexons not only respond to – but also elicit – activity-dependent pathways that impact neuronal plasticity and memory formation [7••,25•,26•]. As such, the microexon regulatory network likely functions as a homeostatic sensor to ensure proper brain function [7••,25•,26•,27]. A key challenge presented by this model is to establish functions of individual neuronal microexons regulated by SRRM4 and other splicing factors, and to link these with neurodevelopmental and ASD-associated phenotypes.

Functions of neuronal microexons

As mentioned above, the vast majority of neuronal microexon coding sequences overlap with and preserve open reading frames [5,6,28]. Microexons are significantly enriched for overlap with predicted disordered regions of proteins, although to a lesser extent than observed for longer-neural differential exons. They are also significantly enriched for overlap with – or in close proximity to – modular-type interaction domains of proteins, relative to longer neural differential exons. Consistent with these findings, the host proteins are enriched for membership in protein complexes compared to proteins lacking neuronal microexons, and in several tested cases deletion of a microexon affects interactions with partner proteins. Taken together, these data support a major role for microexons in activity-dependent and activity-independent regulation of protein-interaction networks with critical functions in neurons (Figure 2b) [5,6,11].

Specific examples of important roles for microexons in the control of protein–protein interactions are described later. A notable exception is a frame-disrupting neuronal

Figure 3



Model for convergence in ASD through activity-dependent disruption of the neuronal microexon network.
SRRM4 activates neuronal microexons and its haploinsufficiency results in microexon skipping and autistic-like behaviours. Reduced expression of SRRM4 is induced by increased neuronal activity, resulting in microexon skipping. These observations suggest a mechanism of convergence in ASD, whereby excitation to inhibition (E/I) imbalances, as a consequence of diverse genetic alterations, disrupt microexon splicing and contribute to autistic-like phenotypes [11].

microexon in the RE1-silencing transcription factor (REST/NRSF). REST is a C2H2 zinc finger transcriptional repressor that silences genes required for neurogenesis in non-neural cells. Inclusion of the frame-disrupting exon (16-nt in mouse and 50-nt in human) introduces a downstream termination codon, which results in a truncated isoform ('REST4') lacking several zinc finger domains required for efficient DNA binding and gene silencing [29]. Disruption of this microexon in mice results in inner ear hair cell degeneration and deafness [30••]. Furthermore, a C > G mutation upstream of the orthologous human exon, which activates a cryptic 3′ splice site and renders the exon in-frame, is associated with deafness in humans [30••]. The REST frame-disrupting exon is activated in neurons by SRRM4 and SRRM3 [29,30••,31], and a mutation in the Srrm4 locus, which deletes part of the eMIC domain, causes deafness and balance defects in the Bronx waltzer mouse strain [17]. Taken together, these data suggest that hair cell development and associated deafness and balance deficits are particularly sensitive to the effects of altered REST4 splicing levels controlled by Srrm4 and Srrm3 [17,30••]. However, more extensive disruption of Srrm4 function results in additional neurodevelopment defects, whereas mice haploinsufficient for Srrm4 exhibit multiple ASD-like deficits [11].

Interestingly, aberrant activation of REST has been linked to autistic-like behavioural phenotypes in a mouse model of the high confidence ASD-risk gene *CHD8*, which encodes a chromatin remodeller [32]. Mice haploinsufficient for CHD8 show reduced levels of numerous REST target genes and, consistently, exhibit neurodevelopmental delay. Given that SRRM4 promotes REST inactivation, it is conceivable that loss of its expression may in part contribute to phenotypes through reduced splicing of the REST4 microexon [17,29]. However, it is becoming increasingly clear that SRRM4, together with additional splicing regulators, is required for nervous system development and animal behaviour through its role in regulating a broad program of frame-preserving neuronal microexons [11,12,29,31,33] (Figures 1, 2 and Table 1), examples of which are highlighted below.

Neurogenesis and neurite outgrowth

An increasing number of recent studies have linked microexons to the regulation of neurite outgrowth (Figure 2b). For example, the neural-enriched NOVA splicing factors regulate microexons in the ROBO1 and ROBO2 membrane receptors, which are critical for the control of axonal repulsion during development [34••]. Increased expression of NOVA1/2 during development results in Robo1/2 microexon skipping, which is characterized by greater repulsive activity. In NOVA deficient embryos, inclusion of the microexons is aberrantly elevated and the commissural axons fail to enter the brain hemisphere midline [34••,35]. Furthermore, SRRM4-dependent microexons in Unc13b, Zfyve27/Protrudin, Kdm1a and L1cam have been individually shown to impact neurite outgrowth and morphogenesis [12,36–38]. Importantly, overexpression of

Table 1

List of microexons characterized at the functional level

Gene name (Protein name)	Microexon length	Regulation	Molecular function	Associated phenotype upon perturbation
APBB1	6-nt	SRRM4/RNPS1 [18•]	Promotes interaction with KAT5 [5]	–
AP1S2	9-nt	SRRM4/RNPS1 [18•]	Promotes interaction with AP1B1 [5]	–
CPEB4	24-nt	SRRM4/SRSF11/ RNPS1 [7••]; RBFOX [82]	Controls deadenylation and protein expression of ASD-risk genes [51••]	Decrease spine density; Synaptic transmission alterations; Social behaviour deficits [51••]
EIF4G1 *EIF4G3*	21-nt	SRRM4/SRSF11/ RNPS1 [7••]; RBFOX [7••]	Overlaps prion-like domains. Promotes multivalent interactions with granule components including FRMP and stalls translation [7••]	Learning and memory deficits; Social behavior deficits; Synaptic plasticity and transmission alterations [7••]
GABRG2	24-nt	NOVA [83,84]; RBFOX [84,85]	Phosphorylated by PKC [86,87] to modulate GABAergic transmission [88]	–
ITSN1	15-nt	SRRM4 [12]	Overlaps SH3 domain and promotes interactions with Dynamin-1, Synaptojanin-1, and Rho GTPase-activating protein and supress interactions with SOS-1 and CBL [89,90]	–
KDM1A	12-nt	NOVA [91]; SRRM4 [12,91]	Upon phosphorylation prevents interactions with CoREST and HDAC1/2 [52]; Promotes interaction with SVIL [36]; Underlies activity-dependent gene expression [25•,26•]	Learning and memory deficits [25•]; Decreased seizure susceptibility [91]; Reduced anxiety-like phenotypes [26•]
L1CAM	12-nt	SRRM4/RNPS1/ SRSF11 [18•]	Targets L1CAM to axonal growth cones [92]; Is required for L1CAM endocytosis by promoting an interaction with AP-2 [93]	–
L1CAM	15-nt	SRRM4/RNPS1/ SRSF11 [18•]	Promotes homophilic and heterophylic ligand interactions [37,94]	Reduced neurite outgrowth [38]
LPHN1 *LPHN2* *LPHN3* *(Latrophilins)*	12-/15-nt	SRRM4/RNPS1/ SRSF11 [18•]; RBFOX [18] (evidence for the 12-nt LPHN2 microexon)	Supresses interactions with Teneurins [48]	–
MEF2A *MEF2C* *MEF2D*	24-/24-/ 21-nt	SRRM4/RNPS1/ SRSF11 [16,18•]; RBFOX [95]	Promotes transcriptional activity on reporter constructs [96]	–
PTPRD *PTPRF* *PTPRS* (LAR-RPTPs)	27-nt	SRRM4/RNPS1 [18•] (evidence for the PTRF microexon)	Promotes interaction with SALM3 [46]; Required for interaction of PTPδ with IL1RAPL1 [44]; Promotes interaction of PTPδ with IL-1RAcP [45]; Represses interaction of PTPσ with TrkC [43]	–
PTPRD *PTPRF* *PTPRS* (LAR-RPTPs)	12-nt	SRRM4 [12] (evidence for the PTRD microexon)	Required for interaction with SALM3 [46]; Required for interaction with Slitrk1 [47]; Promotes interaction of PTPδ with IL1RAPL1 [44] and IL-1RAcP [45] Represses interaction of PTPσ with TrkC [43]	–
REST	16-nt	SRRM4, SRRM3 [17,29,30••,31]	Frame-disruptive exon, inactivates REST transcriptional repressive activity [29]	Neurogenesis defects [29]; Deafness [30••]; Controls GABAergic neurotransmission [31]
ROBO1 *ROBO2*	9-/12-nt	NOVA [34••]	Promotes SLIT/ROBO signalling [34••]	Commissural axon midline crossing defects [34••]
SRC	18-nt	SRRM4/RNPS1/ SRSF11 [18•]; PTBP1 [39,97,98]	Overlaps an SH3 domain and supresses interaction with Dynamin-1 [89]; Alters kinase substrate specificity [40]	Reduced neurite outgrowth [41,99,100]; Neurogenesis defects [41]
TAF1	6-nt	SRRM4/RNPS1/ SRSF11 [18•,101]	Controls TAF1 distribution [101]	–
TENM2 *(Teneurin)*	27-nt	–	Inhibits interactions with Latrophilins [49]	Controls post-synaptic specialization [49,50]
UNC13B	6-nt	SRRM4 [12]	–	Reduced neurite outgrowth [12]
ZFYVE27 (Protrudin)	21-nt	SRRM4/RNPS1 [18,102]	Promotes interaction with VAP [37]	Reduced neurite outgrowth [37,102]

Characteristics of microexons, including their length, known regulators, and their molecular and phenotypic impact are summarized.

the microexon-containing isoform of Unc13b, but not the microexon-lacking isoform, rescues neurite extension defects in Srrm4-deficient hippocampal neurons [12]. In addition to neurite outgrowth, microexons have been associated with neurogenesis [12,29]. An interesting example is represented by an neuronal-specific, SRRM4-activated and PTBP1-repressed microexon in the tyrosine-protein kinase SRC [18•,39]. The SRC microexon has been proposed to modulate kinase substrate specificity and its disruption results in neurodevelopmental defects in Xenopus [40,41]. Collectively, these studies demonstrate important roles for neuronal microexons in neurogenesis, axonogenesis and neurite formation (Figure 2b).

Neurotransmission, cognitive functioning and animal behaviour

Multiple RNA binding proteins that regulate neuronal transcript localization, translation and/or splicing have been shown to play critical roles in synaptic maturation, transmission and plasticity, and as a consequence altering their levels impacts neuronal wiring, behaviour and cognitive functioning (reviewed in Refs. [14,42]). For example, disruption of the neuronal microexon network in SRRM4 heterozygous mice has been implicated in alterations in synaptic transmission associated with abnormalities in pre-synaptic neurotransmitter release [11]. Consistent with these observations, microexons are highly enriched in genes that function in vesicle trafficking and membrane synaptic organization [5] (Figure 1). Moreover, a set of microexons have been identified in genes encoding pre-synaptic adhesion molecules comprising the Leukocyte common antigen-related receptor tyrosine phosphatases (LAR-RPTPs) family. The peptides encoded by these microexons are located in the extracellular region and play a critical role in regulating cross-synaptic interactions between LAR-RPTPs with various post-synaptic ligands, therby controlling synapse development [43–47]. Furthermore, recently described microexons in Teneurins and Latrophilins regulate interactions between these proteins that are important for formation of excitatory or inhibitory synapses [48–50] (Figure 2b and Table 1). Finally, as described below, microexons in chromatin, transcription and translation factors further affect synaptic transmission and plasticity, likely by controlling the expression of numerous important synaptic proteins [7••,31,51••]. For example, REST4 alternative splicing controls the transition from excitatory to inhibitory neurotransmission during nervous system development by regulating the expression of the KCl cotransporter 2 gene, *Kcc2* [31]. Collectively, these studies demonstrate that a major role of neuronal microexons is the control of synaptic development and transmission.

Several recent studies have linked individual neuronal microexons to animal behaviour and cognitive functioning (Figure 2b). One of the first mammalian microexons characterized *in vivo* is an Srrm4 and Nova-regulated, 12-nt exon in the *Kdm1a* (also known as *Lsd1*) gene, which encodes the Lysine-specific histone demethylase 1A. KDM1A is a chromatin regulator that specifically demethylates monomethyl and dimethyl Lysine-4 of histone H3 (H3K4) [8]. Inclusion of this microexon generates a dominant-negative KDM1A isoform that is unable to bind the co-repressors CoREST and HDAC1/2 upon phosphorylation of a threonine residue encoded by the microexon, and instead acquires substrate specificity for H4K20 demethylation [25•,42]. Disruption of the Kdm1a microexon impairs activity-dependent gene expression, which is critical for linking sensory experiences to long-lasting changes in brain function and behaviour through the modulation of synaptic strength [25•,26•]. Mice deficient of the Kdm1a microexon display learning and memory deficits and reduced anxiety-like behaviour [25•,26•].

More recently, individual microexons in translation-associated factors have been shown to impact animal behaviour and cognitive functioning [7••,51••]. Altered neuronal translational outputs contribute to neurodevelopmental disorders, including autism and intellectual disability, by impacting synaptic function and plasticity [53–57]. Eukaryotic translation is primarily initiated by eIF4F, a complex comprising the scaffolding protein eIF4G, which interacts with the mRNA 5′ cap binding protein eIF4E and DEAD-box helicase eIF4A. eIF4F facilitates recruitment of the 43S pre-initiation complex for 5′ UTR AUG scanning [58]. Interestingly, genetic alterations that result in increased cap-dependent translation due to enhanced association of eIF4E with eIF4G lead to autistic-like phenotypes, as well as hippocampal-dependent learning and memory deficits in mice [53,54,58,59]. Pharmacological inhibition of the eIF4E–eIF4G interaction is sufficient to rescue molecular, electrophysiological and behavioural deficits associated with autism [53,54,57,60]. A critical role for translational control in cognitive functioning is also exemplified by the Fragile X Mental Retardation Protein, or FMRP, which is inactivated in the Fragile X syndrome (FXS), the most common inherited form of intellectual disability and leading monogenic cause of autism (reviewed in Ref. [56]). FMRP is a multifunctional protein that controls both translation initiation and elongation, as well as mRNA expression levels [61–65]. FMRP contains a C-terminal intrinsically disordered region which, upon phosphorylation, can phase separate and repress translation *in vitro* [64,66–68]. A CGG tri-nucleotide repeat expansion in the *FMR1* gene, which encodes FMRP, leads to loss of FMRP expression and subsequent increased expression of synaptic protein expression, which is linked to FXS phenotypes.

We recently demonstrated that genetic ablation of conserved neuronal microexons in the eIF4G1 and eIF4G3 paralogs, which are regulated by SRRM4 and neuronal activity, and misregulated in autistic brains, results in the increased expression of numerous proteins that impact

synaptic transmission and plasticity [7••]. Mice deficient of the Eif4g1 microexon display altered social behavior and memory deficits, accompanied by an aberrant form of protein synthesis-dependent synaptic plasticity [7••]. Interestingly, the eIF4G microexons appear to primarily regulate neuronal translation by promoting ribosome stalling, through a mechanism associated with the increased coalescence of numerous neuronal granule components, including FMRP. Consistently, *in vitro* experiments revealed that the eIF4G microexon promotes phase separation with a phosphorylated form of FMRP linked to translation repression [7••,69], and splicing of the microexons in neurons elicits translation repression and ribosome stalling on transcripts that significantly overlap with known FMRP targets [7••,70]. The mechanistic similarities by which the eIF4G microexons and FMRP control translation of synaptic protein expression provides an intriguing link between idiopathic cases of autism, in which pronounced eIF4G1 microexon skipping is often detected, and FXS.

CPEB4 contains another SRRM4-dependent microexon [7••,12] that has also recently been implicated in the control of the neuronal proteome and autistic-like phenotypes [51••]. CPEB4 binds cytoplasmic polyadenylation element motifs within the 3′ UTR of transcripts and induces shortening or elongation of poly(A)-tails, which in turn affects mRNA translation. Post-mortem brain samples exhibiting microexon skipping display altered deadenylation of transcripts encoded by genes with high-confidence ASD-risk variants [51••]. A mouse model conditionally overexpressing Cpeb4 lacking the microexon recapitulates altered deadenylation with associated suppression of protein expression of important synaptic proteins. These mice also show reduced dendritic spine density and frequency of neurotransmitter release, as well as social interaction deficits [51••]. Although these effects could in part be due to Cpeb4 overexpression, the results nevertheless implicate critical roles for the microexon and are consistent with earlier work demonstrating overlapping phenotypes in *Srrm4* haploinsufficient mice [11]. The mechanism by which the CPEB4 microexon impacts translation is not currently known. However, similar to eIF4G and other CPEB family proteins, CPEB4 possesses a prion-like domain that may control learning and memory through coalescence-based mechanisms [71]. It is thus interesting to speculate that additional regulated neuronal microexons may promote coalescence of low complexity regions of multiple proteins in ways that impact higher-order cognitive functioning (Figure 2b).

Collectively, the mouse models described above demonstrate that critical regulatory processes acting at the levels of chromatin, transcription, and mRNA translation, are coordinated by a microexon network that shapes the neuronal proteome to control plasticity, circuit formation and animal behaviour.

Conclusions and future perspectives

Advances in transcriptome profiling technologies, including the development of increasingly sophisticated RNA-Seq analysis pipelines, has revealed a large network of vertebrate-conserved neuronal microexons that is often disrupted in autism spectrum disorder [5,6,9,10••,15,28,72]. The advent of CRISPR-based genome-wide screens coupled with molecular characterization has delineated factors and mechanisms that control this program [18•,19••]. It can be anticipated that as the depth and resolution of RNA-Seq profiling and computational approaches increases, the network(s) of biologically important microexons will continue to expand. For example, it will be important to define microexons associated with neuronal subtype specificity [72], as well as the factors determining this specificity. Recent advances in single cell RNA-Seq profiling coupled with methods such as neuronal subtype conditional tagging of RNA binding proteins will increasingly afford the generation of RNA binding maps that impact microexon splicing across different neuronal subtypes [65,72,73].

Another critical advance will be the development of methods that enable the more efficient functional characterization of microexons (and other forms of transcript isoform diversity). The recent development of CRISPR-based exon-resolution functional screening strategies holds promise in this regard [74,75]. Similarly, the development of methods capable of linking neurodevelopmental disorder-associated genetic variants detected in genome sequencing studies with microexon disruption and phenotypic outcomes will also be important, Collectively, these approaches are expected to pave the way for the systematic identification and characterization of the microexons and other alternative splicing events that impact nervous system development and function, and in turn may facilitate precision medicine strategies directed at correcting splicing defects to treat and cure human diseases and disorders. This outcome is supported by rapid advances in the development of splicing directed therapies based on antisense oligonucleotide-based, small molecule-based, and CRISPR-based modulators of splicing [76–78], some of which have already proved to be highly effective in the clinic [79]. The discovery that disruption of a microexon regulatory network represents a convergence 'hub' in autism spectrum disorder [5,7••,10••,11,51••] points to targeting key regulators of this network as a widely applicable potential future therapeutic strategy for autism.

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Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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