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RNA biology: Alternative splicing hits synaptic function and behavior

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A new study finds the spliceosome protein SNRNP70 in cytoplasmic RNA granules in zebrafish motoneurons. Intriguingly, cytoplasmic SNRNP70 is essential for functional neuromuscular junctions, possibly due to a role in alternative splicing of *z+agrin* mRNA.

Once generated, young neurons and muscles still lack an essential feature — the ability to communicate in a fast, controllable, and meaningful manner. The establishment of this communication is not only one of the most fascinating developmental processes, but also the basis for movement in general and essential for the survival of the animal. Synapses, the specialized zones of communication between excitable cells, have been in the minds of scientists since their discovery by Sir Charles Sherrington in 1897[1]. It has become apparent that the establishment and modification of synaptic connections contribute to many important nervous system functions, such as learning, memory and consciousness, among other cognitive traits. Consequently, synapse dysfunction causes many prominent neurological diseases, for example, schizophrenia, epilepsy and bipolar disorders to name a few. Different types of synapses exist: between nerve cells, between neurons and oligodendrocyte progenitor cells (specialized types of glial cells), and between the motor neuron and the muscle fiber (the neuromuscular junction).

Especially neuromuscular junctions are essential for muscle contraction and motor control. It is by now textbook knowledge that during neuromuscular junction assembly signals from both sides, the terminal end of the motoneuron and the muscle cell, are required. Here, the Sanes laboratory has provided major molecular and functional insight into neuromuscular synaptogenesis by identifying a key player, the proteoglycan agrin[2,3]. The name refers to its potential to aggregate acetylcholine receptors (AChRs) on the surface of muscle cells facing muscle-innervating axons[4]. Interestingly, there is a specific neuronal agrin isoform termed 'Z+ agrin' that mediates this clustering and which has been shown to be essential for neuromuscular junction function[5]. In a new study published in this issue of *Current Biology*, Nikolaou *et al.* provide significant new insight into how AChR clustering and neuromuscular synaptogenesis work[6] (see also Figure 1). The authors show that a cytoplasmic pool of a primarily nuclear RNA-binding protein, SNRNP70, regulates splice variants of transcripts such as *agrin*, with important implications for neuromuscular junction assembly and function.

Nikolaou *et al.* had previously shown that SNRNP70 is present in the cytoplasm of zebrafish motor axons[7]. This is surprising as SNRNP70 is a splicing factor, and mRNA splicing normally occurs in the nucleus. This raised the question: what is the role of SNRNP70 in the cytoplasm of highly compartmentalized cells like nerve cells consisting of a soma containing the nucleus, as well as axons and dendrites. DNA is transcribed into mRNA in the nucleus, where it is normally spliced and then exported into the cytoplasm to be translated into proteins. Up to now, SNRNP70 has known roles in the nucleus, as it has a canonical function in spliceosome assembly[8], and in chromatin retention of non-coding RNAs[9]. To assess the role of this cytoplasmic pool of SNRNP70, the authors came up with a very clever approach.

First, by using state-of-the-art CRISPR/Cas9 technology, they generated a loss-of-function line by producing a deletion (referred to as 'mutant' hereafter) inside the first coding exon of the zebrafish snrnp70 gene. Due to nonsense-mediated mRNA decay, the homozygous mutants have no SNRNP70 left and display various defects including abnormal tail extension, heart edema and cell death, as well as defects in the growth of motor nerves and in AChR clustering. Importantly, the mutant fish also show a clear change in behavior as their startle response is impaired (Figure 1B). This is an evolutionarily preserved defense response, manifesting as a quick 'body-bend' in reaction to a sudden sensory stimulus (Video: https://www.youtube.com/watch?v=fTHFKA_eFkU). Second, the authors generated an altered SNRNP70 protein that is excluded from the nucleus. To achieve this, they deleted two obvious nuclear localization signals within SNRNP70 and simultaneously added three nuclear export signals to the carboxyl terminus. This allowed the authors to reintroduce this cytoplasmic variant of the engineered protein into the mutant zebrafish background lacking the snrnp70 gene. Although this clever manipulation did not fully rescue the observed morphological defects of the homozygous mutants, they observed a partial recovery of AChR clustering, growth of motor nerves and interestingly, touch-evoked startle response (Figure 1). These elegant functional experiments were complemented by cell transplantation assays to demonstrate that SNRNP70 is necessary for neuromuscular junction formation in motoneurons.

In order to identify the RNA targets of cytoplasmic SNRNP70, the authors

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performed RNA-seq on the mutant embryos that either expressed or lacked the cytoplasmic SNRNP70-eGFP protein and identified a total of 347 differentially expressed transcripts. They also discovered 93 alternatively spliced events that were rescued by non-nuclear SNRNP70, indicating that the cytoplasmic protein can indeed regulate the abundance and/or localization of transcripts. Next, they took a candidate approach to identify SNRNP70 targets that could explain the AChR clustering phenotype, choosing to focus on the above-mentioned neuronal Z+ agrin isoform. Rewardingly, they showed by RT-PCR that *z+agrin* expression was reduced in mutant fish, but expression of cytoplasmic SNRNP70-eGFP protein brought expression levels back to normal. The neural z+ isoforms containing additional micro-exons encoding 8, 11 or 19 amino acids are most likely generated by alternative splicing in the nucleus[10]. The authors themselves are very careful in their article by stating that their study "provides (…) evidence that the cytoplasmic pool of a spliceosome component [SNRNP70] is functionally active independently from its nuclear translocation"[6], suggesting a possible role of SNRNP70 in cytoplasmic splicing. Furthermore, nuclear on-demand splicing, regulated by synaptic activity, might affect the exported transcripts subsequently in the cytosol.

Indeed, there is recent ground-breaking work by the Scheiffele lab that showed substantial on-demand splicing in response to cellular signals. Many transcripts harboring introns are stably maintained in the nucleus. Remarkably, their splicing is completed in response to neuronal network activity, followed by their cytoplasmic export and translation[11]. These findings suggest that intron retention in the nucleus serves as a widespread mechanism to control storage and on-demand release of mRNAs[12]. It is by now accepted that intron retention globally impacts gene expression by negatively regulating cytoplasmic transcript levels.

Of course, as with any other break-through study, the interesting findings from Nikolaou *et al.* raise many questions. First, what could be the function of alternatively spliced transcripts in the neuronal cytoplasm or in specific neuronal compartments? The Holt lab recently identified axon-specific sequence motifs linking alternative splicing to axonal localization and translation[13]. They speculated about a potential role of the RNA-binding protein FMRP1 in translational control. Indeed, it will be exciting to learn whether RNA-binding proteins in general, for example, Staufen2, FMRP1, Pumilio2 and Nova, among others[14], contribute to localization and subsequent translation at synapses. Second, are all introns removed in the nucleus? Notably, not all transcripts that will eventually be exported from the nucleus are devoid of introns. Recently, transcripts have been identified in dendrites of mature neurons that contain introns, either in their coding region[15] or in their 3'-untranslated region (3'-UTR)[16,17]. This fits well with the authors' identification of intron-containing transcripts that are regulated by cytosolic SNRNP70. The presence of SNRNP70 together with components of the spliceosome[18] or splicing factors, e.g. Nova[19] or SFPQ[7], in axons and dendrites[14] suggests that those transcripts might be spliced in the cytoplasm to (further) increase the diversity of the proteome. However, it has been far from trivial to convincingly demonstrate that splicing really occurs in the cytoplasm of either axons or dendrites, especially locally in the proximity of synapses. Third, although these intron-containing transcripts, e.g. *Arc*, Ca^{2+}/CaM-dependent protein kinase 2 alpha (*Camk2α*), calmodulin 3 (*Calm3*) or the newly identified alternatively spliced transcripts that are regulated by cytosolic SNRNP70, are found in dendrites of mature neurons, they might not necessarily be translated into proteins. Notably, both *Camk2α* and *Calm3* mRNAs are transported to dendrites via the RNA-binding protein Staufen2 in an activity-dependent manner. So, what could be the function of such dendritically localized transcripts? Ortiz *et al.* offer an interesting hypothesis that they might act as sponges for either miRNAs and/or

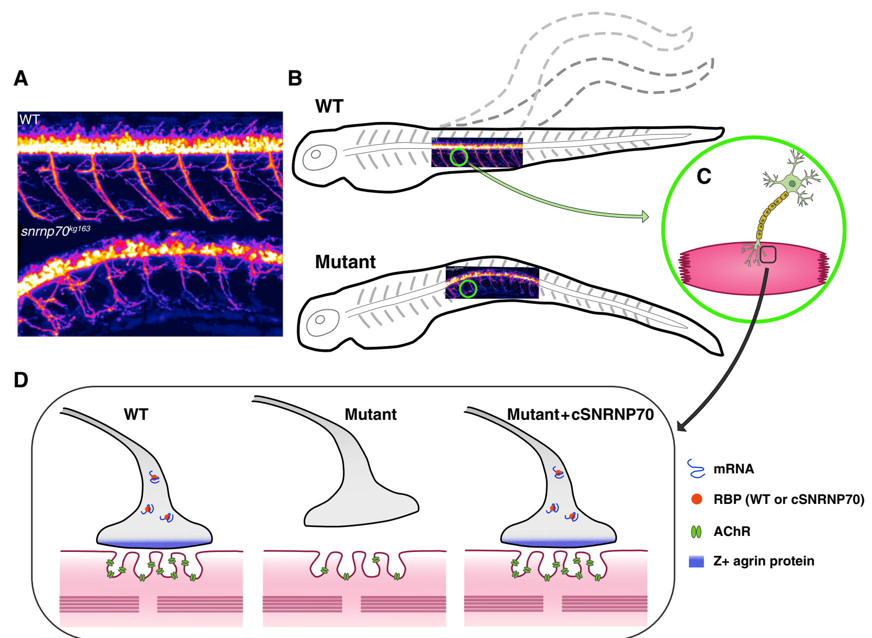

Figure 1. From a protein (RNA-binding protein) to behavior (startle response).
(A) Fluorescently labelled motor nerves in wild-type (WT, top) and snrnp70 null mutant (bottom) zebrafish larvae at two days post-fertilization. Note that in mutant animals deficient for SNRNP70 ($snrnp70^{kg163}$), motor nerves are significantly shorter, and their terminals are largely reduced in complexity, size, and myotome coverage. Pictures from WT and mutant fish: courtesy of N. Nikolaou and C. Houart. (B) Motor nerve defects in zebrafish snrnp70 null mutants (bottom) prevent the startle response (indicated by tail movement in grey) observed in WT larvae (top). (C,D) Cytosolic SNRNP70 (cSNRNP70) mediated modulation of alternative splicing of neuronal *z+agrin* affects synaptogenesis, thereby controlling clustering of acetylcholine (Ach) receptors on the myotome and eventually the startle response (shown in WT larvae in (B)). RBP, RNA-binding protein. Illustration by Dr. Barbara Nitz.

RNA-binding proteins[15]. Clearly, sophisticated, systematic work is necessary to understand the function of such introns in the 3'-UTR of transcripts[17], whether alternatively spliced transcripts are generated in the cytoplasm near synapses and to determine their role in local translation in either axons or dendrites.

Finally, this brings us to the overall biological role of cytoplasmic SNRNP70 at synapses. As the authors indicate, it is very likely that specific alternatively spliced transcripts that contain selective exons contribute to synaptogenesis and eventually to neuronal connectivity. It will be interesting to learn whether the presence of cytoplasmic RNA-binding proteins with a role in splicing will turn out to be of general biological importance. Undoubtedly, the alternative splicing of transcripts would not only influence the formation of synapses but also the regulation of synaptic strength, which is considered to be the cellular basis for plasticity in the nervous system[20].

DECLARATION OF INTERESTS

The authors declare no competing interests.

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Programmed DNA elimination: New metazoan models

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Programmed DNA elimination (PDE) occurs in various metazoans. Parasitic nematodes have long been the major experimental model for PDE investigation. New studies have reported that some genetically tractable free-living nematodes also undergo PDE, paving the way for understanding the molecular mechanisms of PDE in metazoans.

Most of the cells in the body have identical genomes. Therefore, we can produce induced pluripotent stem cells from fibroblasts and establish a clone by transferring a nucleus from a skin cell to an enucleated oocyte. However, in some organisms, the genomes of somatic cells differ from those of germline cells due to the removal of some determined DNA segments during the development of somatic lineages. The process is called programmed DNA elimination (PDE, also called programmed genome rearrangement or chromatin diminution).

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