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Brain Translation: A Feather Tips the Scale

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In this issue of *Molecular Cell*, Gonatopoulos-Pournatzis et al. (2020) report a neuron-specific microexon in eIF4G translation initiation factors that dampens synaptic protein translation. Autism-associated disruption of this exon results in increased protein production, likely through reduced coalescence with cytoplasmic ribonucleoprotein granule components, including FMRP.

The pathogenesis of autism spectrum disorders (ASDs), due to their highly heterogeneous genetic landscape, remains a serious challenge for neuroscience. Several convergent insights have emerged in recent years, including the now well-established association of protein translation dysregulation with ASD phenotypes observed in human patients and animal models. In addition, multiple studies have shown the importance of regulated alternative splicing in the nervous system and identified disrupted splicing as one of the main causes or contributors to neurodevelopmental disorders, including ASDs (Mullins et al., 2016). In this issue of *Molecular Cell*, Gonatopoulos-Pournatzis et al. (2020) propose a novel link between these two processes. The authors discovered a highly conserved, neuron-specific microexon in eukaryotic translation initiation factors 4G1 and 4G3 (eIF4G1 and eIF4G3), critical members of the cap-dependent translation initiation complex. Skipping of these neuronal activity-regulated exons has specific effects on synaptic protein abundance and is associated with ASD-like cellular and behavioral phenotypes (Figure 1).

Microexons are a unique class of alternative exons, only 3–27 nucleotides long, with a coding capacity of up to 9 amino acids. Development of deep RNA sequencing technologies and computational tools have led to the widespread discovery of these alternative exons, which are especially prevalent in the nervous system, highly conserved in mammals, and frequently altered in ASDs (Ustianenko et al., 2017). However, characterizing the precise function of these alternative exons and their effects on cellular, circuit, and behavioral phenotypes remains a major challenge for the field. Previous work published by the Blencowe group reported SRRM4 (nSR100) to be one primary RNA-binding protein factor regulating microexons in the brain (Irimia et al., 2014). SRRM4 is neuron-activity dependent, and its haploinsufficiency in mouse models can lead to alteration of an activity-dependent splicing program, as well as neural morphological, electrophysiological, and ASD-like social behavior changes. Which individual microexons are underlying these functional changes? Is there a mechanistic connection between misregulation of alternative splicing and the imbalance of translation observed in ASDs? Gonatopoulos-Pournatzis et al. (2020) describe two highly homologous and conserved microexons encoding seven amino acids in the protein paralogs of translation initiation complex factors eIF4G1 and eIF4G3. While these translational regulators are ubiquitously expressed, inclusion of the microexons is developmentally and activity regulated, highly neuron specific, and controlled by SRRM4 and several other neuronal splicing factors. Interestingly, the eIF4G1 microexon ranked third highest among differentially spliced microexons in ASD patient samples, suggesting a possible link between dysregulated splicing and translation in autistic brains.

There is growing evidence that fine-tuned protein translation is an important hub in the gene regulatory networks disrupted in ASDs, as exemplified by causative mutations in FMRP, TSC1/2, PTEN, eIF4E, and others (see references in Gonatopoulos-Pournatzis et al., 2020). Fragile X syndrome, caused by FMRP protein deficiency, is the most common inherited form of intellectual disability and ASD. FMRP is an RNA-binding protein shown to prevent excessive translation of synaptic proteins by stalling ribosomes (Darnell et al., 2011; Das Sharma et al., 2019). Given this association, the authors explored the possibility that eIF4G microexon inclusion might impinge on similar cellular processes. Despite being part of a generically important translational complex, inclusion of the eIF4G microexons indeed has an outsized effect on the abundance of hundreds of proteins enriched in synaptic functions, and, furthermore, their splicing is dynamically regulated in response to neuronal depolarization. The link between neuronal activity and the regulation of new protein synthesis is an area of particular interest, as neuronal plasticity and synaptic proteins are also lynchpins in autism pathology. Consistent with a proposed mechanism of the eIF4G microexon acting as a "translational brake," Gonatopoulos-Pournatzis and colleagues show that exclusion of the eIF4G microexon results in an increase of synaptic components, such as GluN1 and gephyrin (Gonatopoulos-Pournatzis et al., 2020). This finding is remarkably reminiscent of fragile X phenotypes in which depletion of FMRP leads to an increased overall protein production, particularly those present in synapses, including the mGlu5 receptor.

Taking a mechanistic approach, Gonatopoulos-Pournatzis et al. (2020) detected a reduction in stalled polyribosomal puncta in cultured microexon knockout neurons, a feature also associated with the primary function of the FMRP protein. Detailed analysis of ribosomal footprints at the stalled ribosome sites further revealed an enrichment of FMRP binding sites, suggesting that a role for the microexons in dampening translation is likely, at least in part, through FMRP. The peptides encoded by the microexon lie in an

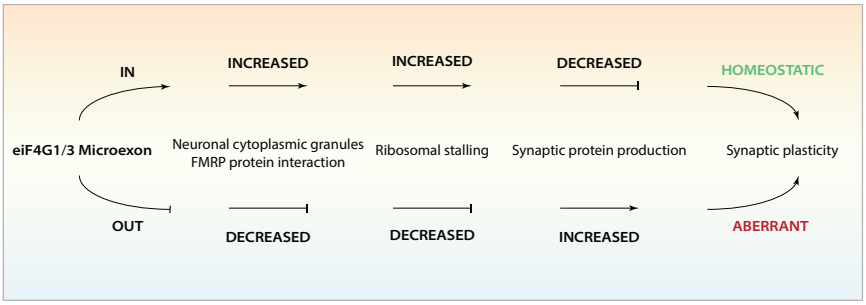

Figure 1. Proposed Model of the Neuron-Specific eIF4G Microexon in Affecting Translation of Synaptic Proteins and ASD-Related Phenotypes

Skipping of the exon releases stalled ribosomes resulting in increased protein translation. This is associated with altered interaction of eIF4G with many components of cytoplasmic neuronal granules, including FMRP.

unstructured and flexible part of the eIF4G protein with a predicted prion-like domain. Similar protein domains were recently reported to contribute to a liquid-liquid phase separation, resulting in droplet-like, membraneless assemblies of interacting proteins. Strikingly, the microexon-deleted eIF4G1 proteins have reduced interaction with many proteins associated with cytoplasmic neuronal granules, as revealed by proximity-based labeling with BioID. The authors also found that microexon inclusion facilitated the interaction between eIF4G and FMRP and the ability of phosphorylated FMRP to form liquid droplets *in vitro*. These data collectively suggest that the microexon-promoted formation of neuronal granules may be an important aspect of stalling translation along polyribosomes.

This work highlights several outstanding issues and questions. First, why does the eIF4G microexon specifically affect production of synaptic proteins? FMRP is also known to selectively target mRNAs encoding synaptic components, though its binding footprints are widespread across the coding region, and thus its sequence specificity is still somewhat under debate (Darnell et al., 2011). Alternatively, the selectivity can be potentially derived from synaptic/local translation regulated by microexon-mediated interactions between eIF4G, FMRP, and possibly other granule components. This is consistent with an enrichment of dendritically localized transcripts among those regulated by the eIF4G microexon. Along this line, FMRP is actively transported by kinesin motors to the sites of localized translation that occurs at the basal parts of dendritic spines as part of dynamic cytoplasmic neuronal granules containing translationally silent mRNA (Telias, 2019). To address the question, it will be interesting to test whether the eIF4G isoforms are differentially localized to neuronal soma versus processes. Second, to what extent does the translational repression activity of the microexon and that of FMRP depend on each other? The authors observed significant overlap between genes translationally affected by the eIF4G microexons and FRMP. Importantly, mice deficient of the eIF4G1 microexon reported in the current study recapitulate many FMRP knockout cellular and behavioral phenotypes, such as impaired synaptic plasticity, contextual fear memory, and social novelty testing. However, FMRP has mainly been implicated in regulation of translational elongation, although its interaction with translational initiation complex components has also been proposed previously (Napoli et al., 2008). The potential interplay of initiation and elongation steps have certainly made the interpretation of ribosome footprinting data more complicated, as ribosome occupancy reflects translational efficiency only when one can assume a constant ribosome elongation rate. FMRP-bound target transcripts show reduced ribosome occupancy upon FMRP depletion, which has been interpreted as the result of stalled ribosome release and thus an increased ribosome elongation rate (Das Sharma et al., 2019). On the other hand, depletion of the eIF4G microexons did not result in a global change in ribosome occupancy but rather reduced ribosome stalling, which is most compatible with an increase in both translational initiation and elongation. Taken together, these data suggest both overlapping and unique aspects of downstream pathways affected by the microexons and FMRP.

To conclude, this and other recent studies on microexons (e.g., Parras et al., 2018) suggest that the concept of a "specialized ribosome" (Shi and Barna, 2015) can be extended to many regulatory factors affecting translation in time and space, and inclusion of tiny bits of coding sequence by alternative splicing can exert a global impact on development and disease.

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