REVIEW

Altered localization of Staufen1 in Amyotrophic Lateral Sclerosis

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Localized protein expression is crucial for the health and survival of axons and dendrites in a rapidly changing environment. This process, however, cannot take place without the precise spatiotemporal localization of the cellular translational machinery and of mRNA. mRNA transport and localization requires a variety of RNA-binding proteins. Here, we highlight a recent publication which presents evidence for the altered localization of the dsRNA-binding protein Staufen1 as a result of Amyotrophic Lateral Sclerosis (ALS) - linked mutations, supporting the perception of ALS as a RNA spatiotemporal mislocalization disease.

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Neurons maintain broad inter-cellular communication networks by extending dendrites and axons over long distances. However, this highly polarized morphology poses a challenge for intra-cellular communication. Thus, the neuron requires tightly regulated mechanisms of cellular transport, signal transduction and localized protein synthesis. A recent publication revealed a possible role for the RNA-binding protein Staufen1 in Amyotrophic Lateral Sclerosis (ALS), demonstrating both *in vitro* and *in vivo* alterations in its localization to the synapse [1].

The correct spatiotemporal localization of local protein synthesis is essential for a neuron's health and facilitates the neuron's ability to respond to external stimuli in a precise spatial and temporal manner. It is a highly regulated event that requires the coordination of different processes including the transport, targeting, anchoring, and on-site translation of mRNA [2].

Alterations in RNA localization and local synthesis have been associated with neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS), Spinal Muscular Atrophy (SMA), Huntington's disease (HD) and others [3, 4]. ALS is a progressive neurodegenerative disease that affects both upper and lower motor neurons, resulting in a phenotype of muscle atrophy that eventually leads to death. Research over the past several years has pointed to a growing role for RNA metabolism and intracellular transport in ALS, with the association of RNA binding proteins TDP-43 and FUS/TLS, whose cellular pathology include aggregation into cytoplasmic inclusions [5] with the disease. C9ORF72, the most prevalent genetic cause for ALS, is not an RNA-binding protein, but its pathology includes RNA foci, aggregates that include both RNA and RNA-binding proteins and hinder RNA splicing, and disrupted nucleocytoplasmic transport [6-8].

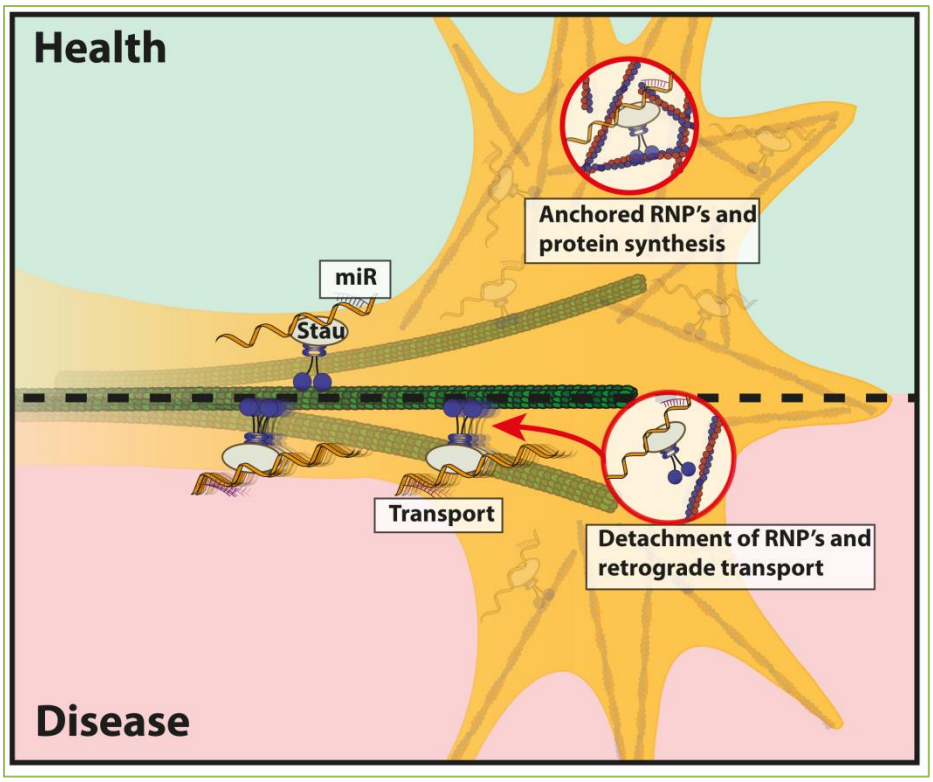

Figure 1. Suggested model: Dynein switches between a trafficking role and an anchoring one as a result of ALS-linked mutations. In the healthy neuron, dynein serves to anchor the Staufen1-mRNA complex to a precise synaptic location, possibly enabling local synthesis of the tethered mRNA. In the diseased neuron, the Staufen1-RNA complex detaches from the synapse and is actively transported retrogradely by dynein away from the synapse. The switch between anchor and motor may be a result of the phosphorylative state of dynein. This depletion of Staufen1 and mRNA is suggested to increase the neurite's susceptibility to environmental stressors, and to lead to neurodegeneration.

The subcellular localization of mRNAs in eukaryotic cells requires the coordination of different processes including the transport, targeting, anchoring, and on-site translation of mRNA[2]; these are facilitated in great part by RNA-binding proteins (RBPs) that transport and anchor mRNAs as part of a RNA-protein (RNP) complex. The RBPs that bind to the mRNA have an additional role in maintaining the translationally repressed state, until the arrival of a triggering signal – such as a guidance cue, or injury [9-11]. This provides both spatial and temporal control [2, 3, 9, 12, 13]. RBPs recognize and bind mRNAs based on primary sequences, i.e. a consensus "zip code" sequence, or secondary sequences in the mRNA that target them for transport into subcellular compartments [14].

The cytoskeleton "highways" and motor protein "vehicles" are fundamental in maintaining axonal transport, whose dysfunction has been linked to various neurodegenerative diseases. Indeed, perturbations of the retrograde transporting dynein complex are sufficient in causing mild, late-onset neurodegeneration [15]. However, beyond general slowing and disturbance in both anterograde and retrograde transport, the nature of transport can hold valuable information as to how transport can contribute to the development and progression of neurodegeneration. For example, the retrograde transport of pro-survival neurotrophic factors to the cell body is significantly inhibited in ALS model mice, and replaced with stress-related factors [16]. Altogether, this mislocalization renders the axon far more vulnerable to environmental stress [16]. Moreover, the same perturbations which cause mild neurodegeneration in healthy mice have an attenuating effect on disease progression in mSOD1 mice [17], further stressing the role of the transported cargo in axonal health.

With this in mind, Gershoni-Emek *et al*. sought to identify proteins whose interaction with the retrograde transport motor protein dynein in synaptic fractions may be altered as a result of the ALS-linked mutation mSOD1^{G93A} [1]. Mass spectrometry (MS) of dynein-immunoprecipitated complexes was complemented with bioinformatics network reconstruction using the Advanced Network Analysis Tool (ANAT) [18], and this highlighted a predicted role for dsRNA-binding protein Staufen1 as a central node in dynein

interactions with the predicted proteins EIF2AK2 (eukaryotic translation initiation factor kinase), PABPC1 (poly (A)-binding protein C) and PPP1CA (protein phosphatase A, catalytic subunit C), all of which play a part in the regulation of gene expression, suggesting a role for dynein-bound Staufen1 complexes in translational regulation of local expression. One of the proteins identified by MS uniquely in the mSOD1 sample was Protein Phosphatase 1, catalytic subunit β (PP1B), which was predicted to interact with Staufen1 via PP1A. Dynein motility can be modulated by its phosphorylation state [19], and PP1B has been shown to dephosphorylate cytoplasmic dynein, suggesting it to switch dynein between a trafficking and an anchoring mode [20,21].

The predicted interaction of Staufen1 and PP1B with dynein was subsequently demonstrated *in vivo* and *in vitro*, and the effect of ALS-linked mutations on their synaptic localization and interaction with dynein was studied. A decreased synaptic localization of both Staufen1 and PP1B as a result of the ALS-linked mutations $mSOD1^{G93A}$ and $TDP43^{A315T}$ in vitro was shown, together with a depletion of Staufen1 from the neuromuscular junction (NMJ) in vivo in the $mSOD1^{G93A}$ mouse model.

Taken together, Gershoni-Emek *et al*. suggest a model in which under physiological conditions dynein anchors Staufen1 together with its bound mRNAs to a precise synaptic location, consistent with a previously described tethering role for dynein in maintaining synaptic stability [22]. This anchored RNA complex at a distinct place provides the neuron the ability to respond to alterations in its microenvironment. As a result of ALS-linked mutations, dynein switches to a trafficking mode, clearing Staufen1 and its bound mRNAs from the NMJ, resulting in an increased vulnerability of the neuron to stress.

Staufen1 has a well-described role in spatiotemporal localization of mRNA in the D. melanogaster oocyte [23, 24], as well as in the formation, transport and anchoring of RNA granules in neurons [24, 25]. Staufen1 has been linked to numerous neuronal functions, among them neuronal plasticity during memory formation [26, 27], as the dendritically localized mRNA CamKIIa whose translation is activity-dependent, can be found in Staufen1 mRNPs [28]. Within neuronal Staufen1 mRNP granules, translational repression has recently been suggested to be maintained by the inclusion of inhibitory microRNAs together with their mRNA targets [29]. In this context, anchoring may be an essential step preceding the release from translational repression.

Although Staufen localization in muscles and at the NMJ has been previously described [30, 31], Gershoni-Emek and colleagues are the first to show alterations in Staufen1 as a result of ALS. Previous works have reported an association between TDP-43 and Staufen1, suggesting a role for TDP-43 in shuttling of mRNA between mRNP granules [32]. Moreover, an activation-regulated, functionally coordinated complex of TDP-43, Staufen1 and FMRP (Fragile X-related Mental Retardation Protein) has been shown to play a role in neuroprotection [33, 34].

In order to further examine the role of Staufen1 in axonal health and ALS-related neurodegeneration, it would be beneficial to characterize the milieu of Staufen1-bound mRNAs in axons, and to probe how the spatiotemporal localization of these mRNAs is altered as a result of ALS. Additionally, it would be interesting to understand how Staufen1 is trafficked and localized, and whether it interacts directly with dynein.

In summary, recent work supports the concept of spatiotemporal mislocalization in neurodegeneration [3], and provides an exciting new avenue to explore in understanding ALS pathology.

Conflicting interests

The authors have declared that no competing interests exist.

Abbreviations

ALS: Amyotrophic Lateral Sclerosis; ANAT:Advanced Network Analysis Tool; CamKIIa: calcium calmodulin dependent kinase II, subunit a; dsRNA: double stranded RNA; EIF2AK2: eukaryotic initiation factor 2 kinase; FMRP: Fragile-X related mental retardation protein; FUS/TLS: Fused in sarcoma/ translocated in sarcoma; HD: Huntington's disease; MS: Mass Spectrometry; NMJ: neuromuscular junction;PABPC1: poly A binding protein, cytoplasmic; PP1A: protein phosphatase 1, subunit A; PP1B: Protein phosphatase 1, subunit B; RBP: RNA binding protein; RNP: RNA-protein; SMA: Spinal Muscular Atrophy; SOD1: superoxide dismutase 1; TDP-43: TAR DNA binding protein.

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