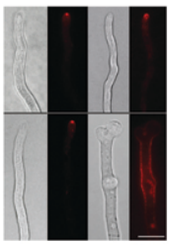
Communicative & Integrative Biology



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ARTICLE ADDENDUM

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Novel mRNA-silencing bodies at the synapse: A never-ending story

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ABSTRACT

Several cellular responses depend on translational regulation and in most cases, this involves the formation of cytoplasmic granules that contain repressed mRNAs. In neurons, numerous mRNAs travel along dendrites to be locally regulated upon synapse activity and we have recently shown that the exoribonuclease XRN1 forms dynamic aggregates at the post synapse that respond to specific stimuli.[1] These foci were termed SX-bodies and are distinct from stress granules (SGs), processing bodies (PBs) and other RNA granules previously described. Together with Smaug1-foci and FMRP-granules, the SX-bodies contribute to dynamically shape the transcriptome available for translation at the post-synapse.

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It is a growing concept that translational repression is linked to the formation of large supramolecular complexes that contain silenced mRNAs in association with RNA-binding proteins and additional repressor factors. Processing bodies (PBs) and Stress granules (SGs) are the founding members of this novel family of cytosolic assemblages, that we collectively termed mRNA-silencing bodies. Both PBs and SGs are highly dynamic and it is believed that their remodeling or dissolution releases transcripts thus allowing their translation.[2-6] A large number of cellular responses are regulated at the translational level and synapse plasticity greatly depends on the regulation of mRNAs localized at the post-synaptic compartment. The importance of local translation in memory consolidation and learning was shown in a wide range of organisms including *Aplysia*, *Drosophila* and mammals.[7-9] Paralleling the formation of PBs or SGs in other cellular contexts, specific mRNA-silencing bodies were shown to dynamically form at the post-synapse. Among others, the RNA regulator Smaug1/Samd4a and Fragil X Mental Retardation Protein (FMRP) form related bodies collectively termed SyAS-foci, as they are dependent on synapse activity.[4,10] The Smaug1 bodies, named S-foci, respond to NMDAR stimulation dissolving and releasing *CamKII* mRNA and likely several other transcripts. Interestingly, NMDA induces a global translational silencing and the formation of specific bodies upon NMDAR stimulation was hypothesized.[10] In following up these studies, we have recently described a new type of SyAS-foci apparently linked to NMDAR activation, that we termed SX-bodies, as they contain the 5′-3′ exoribonuclease XRN1. Remarkably, although in most cell types XRN1 is present in PBs –which include several molecular complexes involved in decapping, repression and decay– we found that the SX-bodies present in hippocampal synapses lack several PB components, including DCP1a, an obligate cofactor for decapping. These recent findings open new questions that remain to be solved.

First, which is the mechanism of mRNA repression that operates at the SX-bodies? XRN1 recognize monophosphate 5′ ends, as those produced by decapping of mRNAs. The apparent absence of DCP1/2 decapping activity at the SX-bodies is puzzling and suggests that the SX-bodies are not linked to mRNA decay. However, other decapping activities may be involved and recently, the decapping molecule DCPS1 and XRN1 were shown to work together in the degradation of specific long noncoding RNAs. DCPS1 activity is blocked by RG3039—a drug with therapeutic potential against spinal muscular atrophy—and a few non-coding RNAs affected by this inhibitor are targeted by XRN1.[11] Regulation by non-coding RNAs is an important

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mechanism for the modulation of local protein synthesis upon synapse activity and the possibility that the SX-bodies are involved in this pathway should be considered. However, decapping and decay might not be connected to SX-bodies and these might operate as RNA storage centers. Which specific RNAs are repressed and protected in these silencing bodies remains a mystery.

Alternatively, the accumulation of XRN1 at the post-synaptic density could be linked to the storage or regulation of XRN1 activity. Recent work in yeast cells shows the unexpected presence of XRN1 at unusual locations. As in most mammalian cells, the yeast homolog XRN1p localizes at PBs and SGs when these are induced upon acute stress insults. Recent findings show that after glucose exhaustion, yeast XRN1p reversibly accumulates in cortical clusters, at the cytosolic side of specific membrane compartments termed eisosomes.[12] Eisosomes are multiprotein complexes *a priori* not involved in mRNA metabolism, and similar to the SX-bodies in neurons, the yeast eisosome contains XRN1 but no other PB component, opening the question on the functional relevance of XRN1 recruitment to these specific subcellular sites. The work in *S. cerevisiae* is inspiring as eisosomes are involved in signal transduction and sensing of environmental stresses. A tempting hypothesis is that both, the eisosome and the post-synaptic density are signaling knobs that regulates XRN1 activity upon external stimuli.

Another important finding related to our observations is that XRN1p is imported to the yeast nucleus to directly regulate gene expression.[13] A complex including XRN1p and other decay factors interacts with the chromatin to couple decay with transcription, accounting for a robust mechanism to control mRNA levels in a narrow range, where more decay is followed by more transcription and more transcription is followed by more decay. Whether XRN1 has a role in transcription in neurons is unknown. Interestingly, several transcription factors are present at dendrites and post-synapses, opening the unexplored possibility of an interaction between transcription factors and XRN1, which would link synapse function, mRNA decay and gene expression in an hypothetical regulatory network.

Nevertheless, our recent work suggests that the synaptic XRN1 clusters help the translational silencing triggered by NMDAR stimulation. NMDA induces the accumulation of SX-bodies and XRN1 KD impairs the translational silencing.[1] In contrast to the effect elicited by NMDA, the SX-bodies slowly dissolve upon stimulation of metabotropic receptors, which induces a concomitant stimulation of dendritic protein synthesis. Altogether these observations support a role for SX-bodies as mRNA-silencing centers that respond to synaptic activity.

SX-bodies are different from canonical PBs and from S-foci and moreover, both SX-bodies and S-foci are different from granules containing FMRP.[1,14] Along with additional RNA granules, the 3 types of bodies are present at hippocampal synapses and display different responses to different stimuli. Whereas the S-foci rapidly dissolve upon activation of NMDA or metabotropic receptors, the FMRP granules do not respond to NMDA but they rapidly dissolve upon metabotropic receptor stimulation. More recently, we found that a Smaug1 splicing variant, termed ΔEIII, which has a shorter RNA-binding domain and however display a normal repressor activity, is expressed in neurons together with the full length Smaug1 and the highly homologous Smaug2, and moreover, the 3 Smaug isoforms form cytosolic bodies (Fernandez-Alvarez et al., this issue). Whether Smaug variants are present in distinct dendritic mRNA-silencing bodies that control specific subsets of transcripts and/or respond to different stimuli remains to be investigated. In a recent work, Amadei et al reported that Smaug2 is expressed in cortical neuron precursors, whereas Smaug1 levels increase later during development. Thus, developmental regulation adds another layer of complexity to the universe of RNA-silencing bodies.[15]

We speculate that the aggregation and dissolution of these diverse mRNA-silencing bodies would affect the local repertoire of transcripts that are available to enter translation. In addition, granules containing stalled ribosomes are present in dendrites and furthermore, the stimulation of specific receptors has consequences on the activity of a number of translation factors (reviewed in refs. 16, 17). As a result of this complex regulatory networks, specific translational changes are expected to be achieved for each stimulation pattern (Fig. 1).[17,18]

In addition to the open questions on the biological significance of the different mRNA-silencing bodies present at synapses, another point that remains to be unraveled is which are the molecular determinants and pathways that govern their aggregation and dissolution. Similarly to the formation of SGs, which requires the action of microtubule and microfilament-dependent molecular motors,[19] we anticipate that the assembly of silencing bodies at the synapse surroundings involves the active transport of molecules and/or particles. Substantial advances have been made in identifying the molecular motors involved in the transport of RNA granules along dendrites as well as in their entrance to dendritic spines.[20] All this knowledge will inspire future studies on the subcellular transport involved in the aggregation and dispersion of synaptic mRNA-silencing bodies.

Work from several labs in several organisms point to the common observation that several PB and SG components contain prion-related domain or low complexity regions (LCR) that mediate homotypic protein-protein

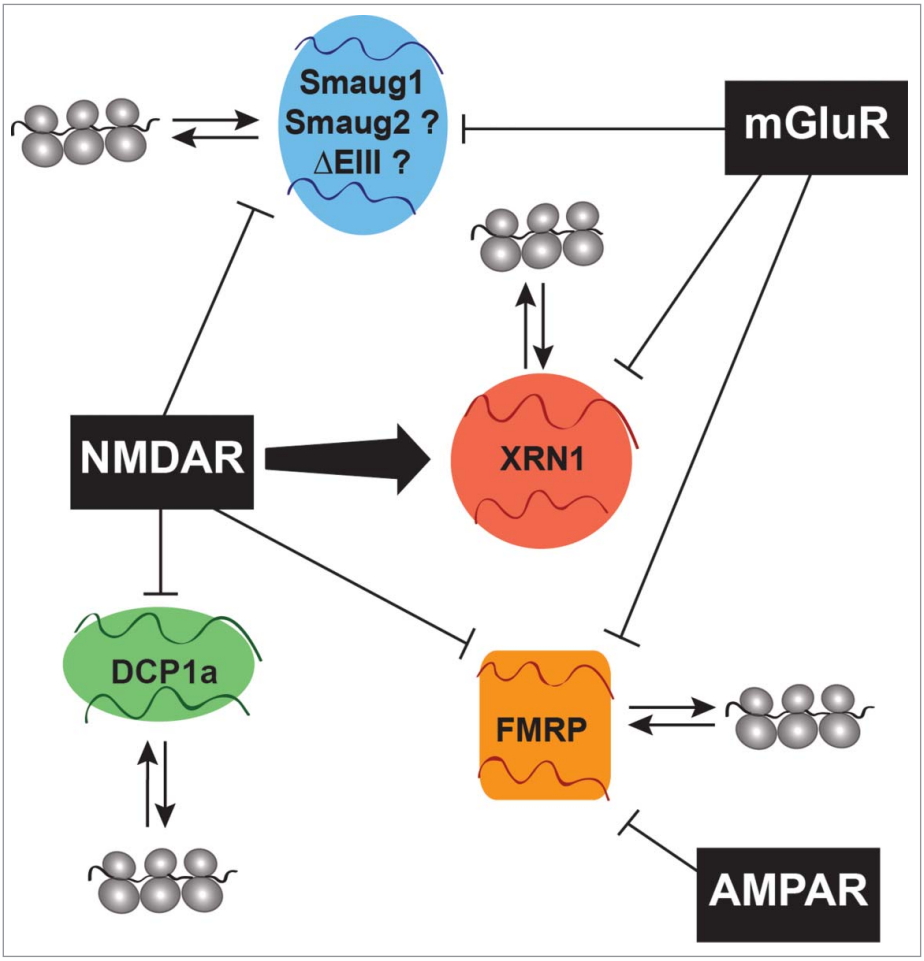

Figure 1. Multiple mRNA-silencing bodies regulate the transcriptome at the post-synapse. The exoribonuclease XRN1, together with Smaug, FMRP and additional RNA-binding proteins including decapping factors; Pumilio; RNG105; ZBP1; TDP43 and FUS/TLS (not depicted) among other molecules involved in post-transcriptional regulation form specific bodies at dendrites and post-synapses. These bodies may respond to synaptic stimulation by dissolving and releasing transcripts to allow their translation, or with an increased assembly linked to translation repression (see text). Remarkably, the SX-bodies (in red) are the only ones described to date to increase in size and number upon NMDAR stimulation, which triggers a global translational silencing. In contrast, NMDAR stimulation triggers the dissolution of Smaug1-foci (in blue) and similarly affects specific bodies containing DCP1a and termed dendritic P-body-like structures (dlPbodies) (in green), with no effect on FMRP granules (orange). The activation of metabotropic receptors provokes the rapid dissolution of the S-foci and FMRP granules, and a much slower dissolution of the SX-bodies. At least 3 Smaug isoforms exist in mature neurons, namely Smaug1, Smaug1 ΔEIII –a splicing variant with a shorter RNA-binding domain– and the highly homologous Smaug2, product of a different gene. Whether these major Smaug isoforms have redundant or specific functions, and whether they form different dendritic bodies remain unknown. The SX-bodies exclude decapping molecules, which are present in dlPBs. Conversely, dlPBs exclude XRN1, suggesting that both types of bodies are connected to mRNA storage rather than decay.[1,30]

interactions thus helping the formation of RNP aggregates that behaves as liquid droplets.[4-6,17,21-23] The conserved presence of aggregation modules in RBPs linked to neuron physiology is somehow surprising given that protein aggregation is connected to neurodegeneration. The current view is that RNA-silencing bodies and granules are reversible and their transformation into irreversible hydrogels or amyloid-like fibers drives toxicity. For example, mutant hnRNPA1 or mutant FUS (Fused in Sarcoma) form anomalous RNP aggregates that disturb the normal dynamics of RNA granules thus affecting local translation.[23,24] The biophysics of these phase-separation processes driven by normal and mutant prion-like domains and related aggregation modules has been discussed elsewhere and open important questions on how aggregation and dispersion are controlled downstream of synaptic activity. Phosphorylation of multiple residues in the oligomerization domain is emerging as an important mechanism to control aggregation in several cell systems.[21,25-27]

In addition to mRNA repression, controlled oligomerization of RNA-binding proteins may also be linked to mRNA activation. A recent report in *Drosophila* indicates that the oligomerization of Orb2—which is driven

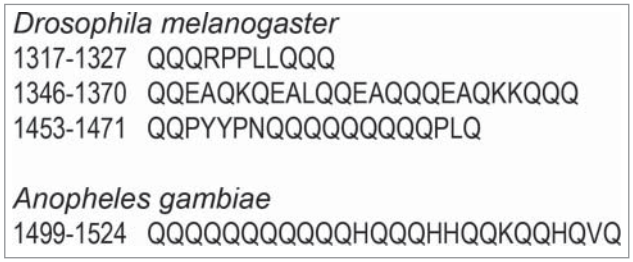

Figure 2. Insect XRN1/Pacman contain Q-rich regions that are absent from vertebrate XRN1. Both in vertebrates and invertebrates, the XRN1 catalytic domain is located at the N-terminus and additional conserved regions in the first 1100 aa help enzyme activity. The C-terminus is less conserved and includes several low complexity regions (LCRs) rich in K, E and P (described in ref. 1). LCRs frequently help protein aggregation and are common in RNA-binding proteins.[21,31] The relevance of these LCRs in XRN1 aggregation remains to be investigated. In addition to LCRs of variable composition, the C-terminus of *Drosophila* (NP_001162796) and *Anopheles* (XP_313715) Pacman/XRN1—which show overall low homology—include Q-rich stretches that are absent from human XRN1 (NP_061874). The percentage of Q in the C-terminus of *Drosophila* (aa 1137–1613), *Anopheles* (aa 1161–1705) and *homo sapiens* (aa 1178–1706) is 13%; 11%; and 7% respectively.

by a polyQ region—helps translation of target mRNAs, whereas the Orb2 monomeric form mediates mRNA repression. It is believed that Orb2 behaves as a prion and oligomeric Orb2 drives the oligomerization of more Orb2 molecules, thereby perpetuating a local response and helping synapse consolidation.[28] In the case of XRN1, aggregation into SX-bodies correlates with translational silencing. Vertebrate XRN1 contains several LCRs at the C-terminal end, and their contribution to SX-body aggregation is likely (discussed in ref. 1). Interestingly, the XRN1 C-terminus is not fully conserved along evolution and the insect proteins contain multiple short Q-rich regions, which are absent from vertebrate XRN1 molecules, including human, zebrafish and xenopus (Fig. 2). The presence of polyQ regions seems to be a common feature in several other yeast and insect PB components and it is less frequent in vertebrates.[28] It has been suggested that mammalian cells are particularly sensitive to the presence of polyQ aggregates, all these suggesting that polyQ-mediated aggregation has evolutionary constrains likely connected to the toxicity of these aggregates.[29] We hypothesized that the LCRs at the C-terminal domain can facilitate the self-aggregation of XRN1. In addition, the interaction with other yet-unknown SX-body components is expected to help their assembly. Likewise, the molecular determinants of Smaug aggregation remain unknown. We recently found that Smaug1 splicing variants and the highly homologous Smaug2 also aggregate when overexpressed in cell lines, and moreover, all these Smaug molecules colocalize in specific cytosolic assemblages that exclude XRN1, suggesting that the molecular determinants of aggregation are distinct for Smaug and XRN1 (Fernandez Alvarez et al., this issue). Future work will help understanding common and specific pathways that control the formation and dissolution of an increasing number of synaptic RNA bodies involved in local translation and ultimately, synapse plasticity and memory formation.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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