

An evolutionarily conserved mechanism for presynaptic trapping

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Abstract Presynaptic differentiation takes place over three interrelated acts involving the biogenesis and trafficking of molecular complexes of active zone material, the “trapping” or stabilization of active zone sites, and the subsequent development of mature synapses. Although the identities of proteins involved with establishing presynaptic specializations have been increasingly delineated, the exact functional mechanisms by which the active zone is assembled remain poorly understood. Here, we discuss a theoretical model for how the *trapping* stage of presynaptic differentiation might occur in developing neurons. We suggest that subsets of active zone proteins containing polyglutamine domains undergo concentration-dependent prion-like conversions as they accumulate at the plasma membrane. This conversion might serve to aggregate the proteins into a singular structure, which is then able to recruit scaffolding agents necessary for regulated synaptic transmission. A brief informatics analysis in support of this ‘Q’ assembly hypothesis—across commonly used models of synaptogenesis—is presented.

Keywords Active zone · Presynaptic assembly · Presynaptic trapping · Prion · Neurobiology · Bassoon · Bruchpilot · Syd-2

Non-pathogenic prion proteins are able to assume two different functionally relevant conformations, one of which is transmissible to other proteins of the same type and causes self-aggregation. Nearly 6 years ago, Si et al. [1] reported that a brain-specific isoform of the *Aplysia californica* cytoplasmic polyadenylation element binding protein (CPEB) has prion-like features that are likely to contribute to how it normally maintains spatially restricted changes in long-term synaptic strength (i.e., a universally accepted mechanism for how animals learn and remember). They proposed a model wherein CPEB accumulation in response to synaptic activity triggers a semi-permanent, wholesale conversion of all of the local CPEB molecules to the ‘prion’ state. To our knowledge, no other brain-specific proteins with an ability to undergo prion modification as part of their *normal function* have been suggested since, although it is becoming clear that prion-like transitions in protein folding might serve as a post-translational modification in general biology [2].

Aggregate forming domains in prion proteins very often exhibit amino acid (AA) sequence compositions that are biased towards glutamine (Q) or asparagine (N) residues. A search of the UniProt Protein Knowledgebase reveals that mammalian presynaptic active zone (AZ) and postsynaptic density (PSD) proteins are defined by a narrow Q content range of only ~5–6% (average \pm SD, AZ, $5.8 \pm 0.02\%$, $n = 20$; PSD, $4.9 \pm 0.02\%$, $n = 17$). Against this backdrop, however, we find that there are three structurally important AZ proteins in humans (h) and mice (m) with

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putative prion-determinant regions: Bassoon, Piccolo, and MALS1. These specific ~60–100 AA regions are highly Q enriched (25–50%), are nestled in general areas of the protein where the majority of the protein's total Q residues are concentrated, and appear to border coiled-coil and PDZ protein interaction motifs (Fig. 1, top 3 panel sets). Such regions are not evident in any other AZ or PSD proteins including RIM1 α , CAST/ERC2, MUNC13-1, liprin- α 1, CASK, Mint1, Shank1-3, Homer1-3, SAPAP1-4, the MAGUKS PSD-93, PSD-95, SAP97 or SAP102, BEGAIN, MAGI-2, or GKAP (Swiss-Prot and TrEMBL). Bassoon and Piccolo are known to be homo- and heterodimerizing binding partners [3–10], and regions of the proteins that harbor Q domains have been shown, inexplicably, to form dense aggregates upon over-expression in neurons and heterologous cells [6–8]. In the case of Bassoon—where this phenomenon has been best documented—painstaking analysis of various deletion constructs reveals that AAs ~2000–2600 (i.e., Bassoon's first potential prionic area, Fig. 1) are crucial for clustering behavior [7, 8]. While full-length Bassoon and Bassoon (2,088–2,564) exhibit aggregation, Bassoon (Δ 2,088–2,564) displays only diffuse intracellular labeling [7]. Intriguingly, fragments of Bassoon containing the first prionic area form agglomerates that remain functional in so far as they are still able to recruit other AZ constituents [8]. Conversely, fragments not containing this area are unable to be anchored to the cytomatrix associated with the AZ [9] and render a significant fraction of synapses inactive [10].

Regarding MALS1, little is known about its presynaptic function beyond participation in a tripartite complex with CASK and Mint1 via each member's L27 domain [11]. It has not been determined, for instance, if Piccolo and MALS1 bind one another via their consensus type I PDZ domains; ligands for these modules have yet to be identified in the AZ proper. But, given the proteins' proximity to one another and the large stretch of AAs dedicated to the MALS1 PDZ motif, this would not be an unreasonable expectation. PDZ-PDZ interactions have already been found to occur between PSD-95 and nNOS that mediate nNOS's synaptic localization and generally have been hypothesized to organize macromolecular signaling complexes at synaptic membranes [12].

Drosophila melanogaster (dm) does not have orthologs of Bassoon or Piccolo, despite the fact that the majority of proteins that contribute to human presynaptic architecture are well conserved in insects [13]. Another coiled-coil protein—Bruchpilot—has been suggested to take on some of Bassoon's functions at the fly AZ along with the fly ortholog of RIM1, dmRIM (comparatively, a *much* larger protein showing regions of homology with mammalian Piccolo [14, 15]). Bruchpilot, in some ways reminiscent of the role that Bassoon plays at photoreceptor ribbons [16], is

absolutely essential for the structural integrity of the AZ as evidenced by the lack of T bars in *brp* mutants [17, 18]. dmRIM, in contrast to mammalian RIM1 α , does not function as a Rab3 effector, hinting at a different biological role for the protein in invertebrates versus vertebrates [19]. Both Bruchpilot and dmRIM exhibit several putative prion-determinant regions that are unusually rich in Q or Q/N (33–52%). As was the case for Bassoon-Piccolo-MALS1, these specific domains are found in larger areas of the protein where the majority of the protein's Q's are clustered (Fig. 1, fourth set of panels). Q domains are not evident in the *Drosophila* MALS1 ortholog, Veli (TrEMBL). The N-terminal domain of Bruchpilot displays significant sequence homology with ERC [17]. Although not experimentally demonstrated, Bruchpilot and dmRIM are thought to be potential binding partners in flies as ERC and RIM1 α are in mammals (D. Wagh, personal communication). Of note, Bruchpilot has also been recently shown to form ordered, clear vesicle dotted agglomerates upon over-expression. These free-floating AZs appear to be unconnected to plasma membrane [20].

Another invertebrate model organism *C. elegans* does not have direct homologs to Bassoon and Piccolo, but does have a RIM homolog named UNC-10. Like dmRIM, worm UNC-10 does not function as a Rab3 effector [19], again suggesting that the role of invertebrate RIM is fundamentally different from that of vertebrate RIM. SYD-2 (liprin- α), as demonstrated by electron microscopy, is centered within the very base of the worm AZ and is thought to be the main scaffolding organizer in worm presynaptic assembly [21, 22]. Its *Drosophila* counterpart, too, appears to surround the core of Bruchpilot-defined AZs [23]. Interestingly, UNC-10 and SYD-2 show a number of potential prion-determinant regions enriched for Q or Q/N (20–58%) that are located in larger areas with biased Q/N expression (Fig. 1, last set of panels). Similar to mammalian and insect Q domain containing proteins, UNC-10 and SYD-2 have been found to associate [24]. Q domains are not evident in the *C. elegans* MALS1 homolog, LIN-7 (TrEMBL, NCBI).

The presence of putative prion-like 'Q' or 'Q/N' domains in subsets of interacting AZ proteins in humans, mice, flies, and worms implies a conserved mechanism of presynaptic trapping, whereby intra- and inter-protein Q aggregates might lock and define the location of formal presynaptic macromolecular assemblies. In this model, the steady membrane accumulation of the aforementioned AZ molecules would eventually trigger a protein folding switch that—in a regulated manner—serves to establish the core of the AZ and to recruit a critical mass of peripheral scaffolding agents needed for neurotransmission. The signals that would initiate this synaptogenic event would vary, but possibly include Ca²⁺ considering its role in

and other insects that have been sequenced (i.e., *Anopheles gambiae*, *Nasonia vitripennis*) (NCBI), we believe that it is a hypothesis worth carefully exploring.

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