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Presynaptic protein homeostasis and neuronal function

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Proteome integrity is maintained by a coordinated network of molecular chaperones, by protein degradation machineries and by their regulators. Numerous human pathologies are considered as diseases of compromised protein homeostasis (proteostasis), including neurodegeneration. These are characterized by the accumulation of neuronal protein aggregates and by synaptic defects followed by loss of connectivity and cell death. While this suggests that synaptic terminals are particularly sensitive to proteostasis imbalance, our understanding of protein turnover mechanisms and regulation at the synapse remains limited. Recent reports show that different proteolytic pathways act at synapses, including several forms of autophagy. The role of chaperones in controlling the balance between synaptic protein refolding and degradation and how this complex network regulates neuronal function also begins to be unraveled.

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Introduction

Synapses, and in particular the contacts sites between presynaptic and postsynaptic neurons, contain an extremely dense networks of proteins [1•]. At presynaptic terminals these proteins function in a highly coordinated and dynamic manner to support neuronal communication, allowing neurotransmitters to be released within 1 ms upon arrival of an action potential and with a frequency that sometimes exceeds 100 Hz. This intense activity places presynaptic proteins at a high risk of misfolding and molecular damage [2]. Moreover the postmitotic nature of neurons and their long lifetimes, potentially for the entire life of the organism, imply that the presynaptic proteome accumulates stress over long periods of time. Given the polarized morphology of neurons, in many respect synapses function as semi-autonomous entities and this is also true in terms of protein quality control. In this manuscript we review the current knowledge on mechanisms that act at the presynapse to prevent protein damage and that have the goal to repair or degrade dysfunctional protein assemblies. In this review we focus on how these processes affect presynaptic activity in healthy neurons, but it is clear that when deregulated, these mechanisms are central to neurodegenerative disease as well [3,4].

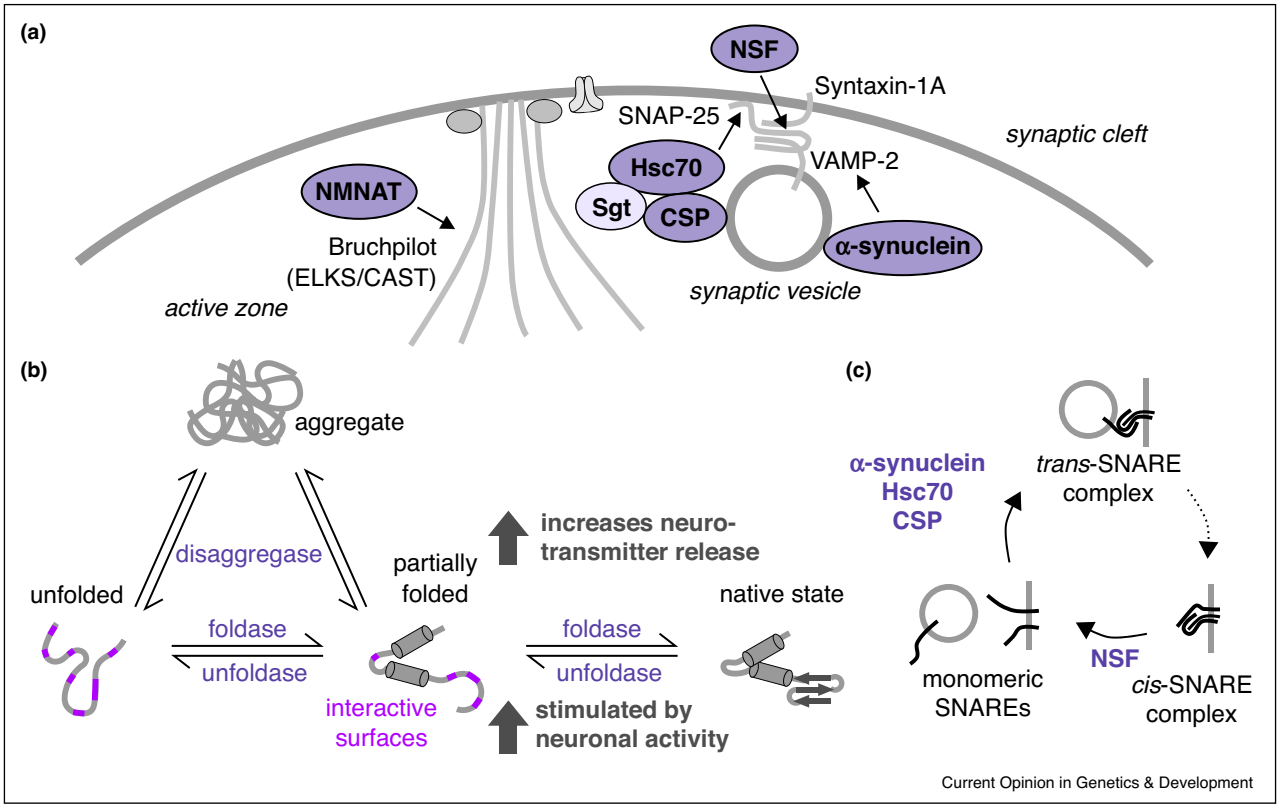
Chaperones watch over the presynaptic proteome

Neurotransmission relies on a complex cascade of temporally and spatially controlled protein–protein interactions [5,6] (Figure 1a). During biological activity-induced conformational changes, interactive surfaces of proteins become exposed to the intracellular environment. Molecular chaperones act to prevent or correct unwanted interactions that may occur between these surfaces [7] (Figure 1b). By doing so, chaperones can regulate protein function, avoid or reverse the formation of non-functional protein assemblies and influence the decision to target client proteins for degradation.

Maintenance of the synaptic vesicle (SV) exocytic machinery requires the action of the Cystein string protein – heat shock cognate 70 – Small glutamine-rich tetratricopeptide repeat-containing protein (CSP-Hsc70-SGT) chaperone complex. This complex binds the plasma membrane R-SNARE protein SNAP-25 in its monomeric form through the Hsc70 chaperone and refolds this client into a SNARE-complex assembly competent state [8] (Figure 1a and c). This activity is indispensable to maintain neurotransmitter release at high frequency during the entire life of the organism, and lack of the CSP co-chaperone leads to presynaptic defects and ultimately neurodegeneration both in *Drosophila* and in mouse [9–12].

Trans-SNARE complex assembly is also promoted by Alpha-synuclein, and expression of this abundant presynaptic protein can reverse the lethal neurodegeneration observed in *Dnajc5* (encoding CSP) knockout mice [13,14]. Alpha-synuclein acts as a SNARE chaperone by simultaneously binding to the vesicular Q-SNARE VAMP-2 and to phospholipids, and this function gains importance with aging and upon increased neuronal

Figure 1



Presynaptic chaperones promote neurotransmission by keeping active zone proteins properly folded.
(a) Key active zone proteins known to rely on chaperones (dark purple) and co-chaperones (light purple) to maintain their normal function. See the text for details. **(b)** Chaperones are best known for their ability to act as foldases that help proteins reach their native folding state. Some chaperones also prevent protein aggregation by 'holding' unfolded client proteins and in some cases even reverse aggregation. Importantly chaperones can also act as unfoldases, an activity that is critical, for example during cycles of protein–protein binding such as seen during the SNARE assembly/disassembly cycle depicted in **(c)**. All three chaperones activities promote SV exocytosis and counteract use-dependent protein damage. **(c)** Neurotransmitter release involves the formation of membrane-bridging *trans*-SNARE complexes between the SV Q-SNARE VAMP2 and the plasma membrane R-SNAREs Syntaxin-1 and SNAP-25. *trans*-SNARE complexes formation is promoted by the overlapping chaperone activities of the CSP-Hsc70-Sgt complex and of Alpha-synuclein. After membrane fusion and collapse of the SV into the plasma membrane, *cis*-SNARE complexes must be disassembled to allow endocytic recycling of VAMP-2 and another round of neurotransmitter release. This disassembly is catalyzed by the NSF AAA+ ATPase in an ATP-dependent manner.

activity [15]. After fusion of SVs with the plasma membrane, highly stable *cis*-SNARE complexes are disassembled by the AAA+ ATPase *N*-ethylmaleimide-sensitive fusion factor (NSF) [16] (Figure 1c).

Other presynaptic proteins beside SNAREs also require chaperones to protect them from activity-dependent damage. Lack of the neuronal maintenance factor NMNAT (nicotinamide mononucleotide adenylyltransferase) [17] leads to reduced levels of several presynaptic markers including CSP itself, Synaptotagmin 1 and the active zone protein Bruchpilot [18]. NMNAT, which was identified in a genetic screen for *Drosophila* factors involved in synaptic function [19], functions as a *bona fide* chaperone independently of its enzymatic function in nicotinamide adenine dinucleotide (NAD) synthesis [20,21]. NMNAT directly binds to Bruchpilot in an activity-dependent manner and protects it against ubiquitylation and proteasomal degradation [18] (Figure 1a).

To date we only know of a few examples where chaperones directly control the activity of presynaptic proteins. Yet the classical dogma that proteins must adopt and retain a particular tridimensional structure to be functional no longer holds true, and protein folding is now instead recognized as an extremely dynamic process [22]. In this context chaperones undoubtedly play a central role in protecting the presynaptic proteome from aggregation and in facilitating changes in folding status during biological activity (Figure 1).