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Review Article

The presynaptic active zone: A dynamic scaffold that regulates synaptic efficacy

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ABSTRACT

Before fusing with the presynaptic plasma membrane to release neurotransmitter into the synaptic cleft synaptic vesicles have to be recruited to and docked at a specialized area of the presynaptic nerve terminal, the active zone. Exocytosis of synaptic vesicles is restricted to the presynaptic active zone, which is characterized by a unique and highly interconnected set of proteins. The protein network at the active zone is integrally involved in this process and also mediates changes in release properties, for example in response to alterations in the level of neuronal network activity. In recent years the development of novel techniques has greatly advanced our understanding of the molecular identity of respective active zone components as well as of the ultrastructure of this membranous subcompartment and of the SV release machinery. Furthermore, active zones are now viewed as dynamic structures whose composition and size are correlated with synaptic efficacy. Therefore, the dynamic remodeling of the protein network at the active zone has emerged as one potential mechanism underlying acute and long-term synaptic plasticity. Here, we will discuss this recent progress and its implications for our view of the role of the AZ in synaptic function.

Contents

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Introduction

Information transfer in the brain, from acute initiation of movement to higher cognitive functions like memory and emotions, takes place at contact sites between neurons and their target cells. At this cell to cell connection, the synapse, information arrives in form of an electrical signal and is transmitted to the target cell via neurotransmitters, chemical signaling molecules. This information transfer is mediated by the fusion of neurotransmitter filled synaptic vesicles (SVs) at a highly specialized area of the presynaptic plasma membrane, the active zone (AZ). The AZ is characterized by an electron-dense protein network, the cytomatrix of the active zone (CAZ), and a precise alignment with the postsynaptic reception apparatus, the postsynaptic density (PSD). To achieve the high spatial and temporal precision of synaptic transmission the process of SV fusion is tightly regulated. Before SVs can fuse with the plasma membrane in response to Ca^{2+}-influx through voltage-gated calcium channels (VGCCs) they have to be recruited to and docked at the AZ followed by a maturation process ("priming") that renders them fusion-competent (reviewed in [1]). Presynaptic active zones are integrally involved in multiple processes that control and enable the high speed, temporal and spatial control as well as plasticity of synaptic vesicle fusion: (1) Through specific trans-synaptic cell adhesion molecules AZs coordinate the precise opposition of the pre- and postsynaptic specializations; (2) AZs contribute to the spatial restraint of the fusion process as SVs dock and fuse at the AZ; (3) AZ components play a role in the recruitment of VGCCs to the presynaptic plasma membrane and in the efficient coupling of SVs to VGCCs, which in turn is crucial for the high speed of synchronous release; (4) the AZ contributes to the sorting of SV proteins to the endocytic machinery and (5) AZ proteins play important roles in the processes mediating activity-induced presynaptic short- and long-term plasticity. However, there are still many open questions regarding the functional contribution of individual AZ components to the individual steps of the SV cycle. In this review we will focus on recent progress in our understanding of the functioning of the molecular machinery at the AZ, in particular on novel insights into the correlation between ultrastructure, molecular composition and function.

The active zone proteome

In contrast to the proteomes of synaptosomes, synaptic vesicles and the postsynaptic density (PSD), which have been characterized in multiple studies (reviewed in [2]), the composition of the cytomatrix at the presynaptic active zone has remained less well defined, mainly due to difficulties in obtaining reasonably pure preparations. However, analyses of genetic mutations, mainly in the worm *Caenorhabditis elegans*, the fruit fly *Drosophila* and rodents, as well as of protein–protein interactions have revealed five evolutionarily conserved proteins, RIM (Rab3-interacting molecule), Munc13, ELKS/CAST/Bruchpilot (glutamic acid (*E*), leucine (*L*), lysine (*K*), and serine (*S*)-rich protein; *CAZ-a*ssociated *st*ructural proteins), α-Liprin/syd-2 (synapse-defective 2), and RIM-BP (RIM-binding protein), that are highly enriched at and form the core of the *c*ytomatrix at the *a*ctive *z*one (CAZ) (reviewed in [2–4]). In vertebrates, two additional large homologous proteins, Bassoon and Piccolo/Aczonin, are associated with active zones. Moreover, in mammals the five core active zone proteins are encoded by multiple genes, which are further diversified by alternative splicing. Via their conserved domains, like PDZ, SH3, coiled-coil, SAM, Zn^{2+}-finger, and C2, the core CAZ components can interact with each other, form oligomers and a tight protein network that regulates synaptic vesicle fusion. Furthermore, these multiple connections are thought to contribute to the synapse's ability to both alter its efficacy in response to activity (plasticity) and to maintain properties over long periods of time (tenacity). It is important to note that the core active zone proteins are not exclusively localized to this specialized area of the presynaptic plasma membrane but are only strongly enriched at the AZ. They are partially expressed also in neuroendocrine and neurosecretory or even non-neuronal cells. In recent years different strategies have been employed to isolate presynaptic fractions of high purity and yield in order to identify the complete AZ proteome using an unbiased approach. Here by, synaptosomes were treated by hypo-osmotic shock to release two populations of synaptic vesicles, one free and a second one that remained docked to the presynaptic plasma membrane. Both fractions were further purified by immunoisolation using antibodies against SV proteins [5–7]. In particular two methodological improvements introduced by Boyken et al., separation of the pre- from the postsynaptic compartment by a mild proteolytic digestion of the synaptosomes before the hypo-osmotic lysis and the quantitative analysis of the composition of the "free" versus the "docked" SV fraction, provided for the first time quantitative information about the protein complement of the SV docking complex and the AZ [6]. In addition to the core AZ proteins, ion channels, transporters and cell adhesion molecules with known presynaptic functions 30 so far uncharacterized proteins were identified, of which around 50% were strongly enriched in the "docked" SV fraction. Future studies will be required to determine if these proteins play a role in the regulation of AZ structure and function. An important finding of this study was the observation that glutamatergic and GABAergic docking complexes exhibit only few quantitative differences indicating that the release machineries of these two synapse types are very similar or even identical. This is in contrast to the molecular composition of the postsynaptic scaffolding and signaling complexes, which differ significantly between excitatory and inhibitory synapses [8,9]. However, synapse-type specific proteins might have been missed in this global proteomics approach due to their low abundance. Furthermore, synapses throughout the brain exhibit considerable morphological and functional heterogeneity [10]. This heterogeneity might be mediated by a diversity in their molecular composition, for example by the exact complement of alternatively spliced variants or family members of AZ proteins present in a specific synapse type or even in individual synapses. The recently reported purification of synaptosomes from distinct synaptic subtypes using the Fluorescence Activated Synaptosome Sorting (FASS) method together with high-resolution imaging techniques for validation and fine mapping of the distribution of distinct proteins will allow to address these open questions [10,11].

Recently, Wilhelm et al. have reported a first quantitative molecular-scale model of an "average" synapse. They have used an integrative approach combining quantitative immunoblotting and mass spectrometry to determine the abundance and super-resolution-fluorescence microscopy to define the localization of