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Mechanisms controlling assembly and plasticity of presynaptic active zone scaffolds

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Cognitive processes including memory formation and learning rely on a precise, local and dynamic control of synapse functionality executed by molecular changes within both presynaptic and postsynaptic compartments. Recently, the size of the presynaptic active zone scaffold, a cluster of large multi-domain proteins decorating the presynaptic plasma membrane, was found to directly scale with the action potential evoked release of synaptic vesicles. The challenge now is to constitute an integrated picture of how long-range axonal transport, local exchange and localization mechanisms at the scaffold and degradation processes are integrated to allow for dynamic and controlled scaffold rearrangements. Here we discuss findings from multiple model systems emphasizing both short-term and long-term regulations of active zone composition and function.

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Introduction

Functionality of the nervous system is based on a rapid communication between neurons and their target cells through specialized cell–cell contacts generically termed synapses. Appropriate synaptic function is essential for all types of cognitive processes, including memory formation and learning. Chemical synapses are asymmetrically organized with a presynaptic 'active zone' (AZ) capable of neurotransmitter release upon action potential arrival and a postsynaptic compartment able to receive and further process this signal. The presynaptic compartment usually accumulates large numbers of synaptic vesicles (SVs). The cytoplasm of the presynaptic bouton moreover is populated with several hundred protein species in copy numbers ranging over several orders of magnitude [1•]. However, the AZ scaffold, an electron dense structure essential for synapse tenacity, localization of SV fusion and positioning of voltage-dependent calcium channels, involves only a few canonical protein families: ELKS/CAST family, RIM-superfamily, including the mammalian Piccolo and Bassoon, RIM-BP, (M)UNC-13, Liprin-a and SYD-1 (Table 1) [2–5]. The use of electron tomography and super-resolution light microscopy revealed underlying macromolecular 'architectures' within presynaptic scaffolds [6–8]. Scaffold assembly is based on defined and dynamically regulated protein–protein interactions using a conserved set of interaction surfaces including both intramolecular and intermolecular coiled-coil interactions, SAM and PDZ domain interactions [2]. The possibility of multiple potentially parallel interactions results in at least partial functional 'redundancy' between AZ scaffold components, thus complicating stringent functional analysis and necessitating the simultaneous manipulations of several genetic loci. This biochemical and genetic complexity is likely a direct reflection of the tailoring of these crucial neuronal compartments towards robustness, combining the high stability of a lifelong structure with the demand for dynamic changes adapting to plasticity requirements. In fact, recent data show that at individual AZs, scaffold size scales with the probability of SV release on the time scale of several minutes only. The difficulty now is to elucidate the detailed cell-biological mechanisms integrating assembly and maintenance with dynamic plasticity processes in different biological contexts concerning neuron type, developmental state and age of the organism. Here we review and try to conceptualize recent findings to create an integrated picture of the regulatory processes determining AZ scaffold architecture, size and function.

Dynamic control of active zone scaffold size and release function

Work at both mammalian and *Drosophila* synapses provides evidence for a tight link between AZ size and complexity and the resulting functional synaptic output. A recently developed assay monitoring the Ca^{2+} influx through postsynaptic glutamate receptors of neuromuscular synapses of *Drosophila* larvae allows for the detection of single AZ release events [9]. This assay provides the possibility to relate the microscopic organization of an individual AZ to its functional properties concerning spontaneous and evoked release. Interestingly it could

Table 1

Presynaptic active zone components and their metabolic and residency turnover rates

Component	Name	Function	Metabolic turnover rate $t_{1/2}$ (days)	Residency turnover rate (FRAP)	Comment
ELKS/CAST family	Glutamic acid (E), leucine (L), lysine (K), and serine (S)-rich protein (ELKS)/ cytomatrix at the active zone (CAZ)-associated structural proteins (CAST)	Scaffolding protein	3.30	Unknown	*Drosophila melanogaster* homologue: Bruchpilot (BRP)
RIM	Rab3-interacting molecule	Scaffolding protein	Unknown	RIM1: 78 ± 5% after 5 min mean recovery $t_{1/2}$: 17 ± 5 s [31]	
RIM-BP	RIM-binding protein	Scaffolding protein	Bzrap1: 59.62	Unknown	*Rattus norvegicus* homologue: Bzrap1
RIM superfamily: Piccolo, Bassoon,		Scaffolding protein	Piccolo: 2.54 Bassoon: 2.57	Bassoon: 100% after 16–18 hours [30]	*Drosophila melanogaster* homologue of Piccolo: Fife
(M)UNC-13 a	*Mus musculus* uncoordinated-13	Scaffolding protein and essential release factor	1.32	MUNC-13-1: exchange time constant for fast pool 3 min; for slow pool 80 min [29]	
Liprin-α/SYD-2	Synapse-defective 2	Assembly and scaffolding protein	Liprin-α2: 3.18	Liprin-α2: 67 ± 4% after 8 min mean recovery $t_{1/2}$: 13 ± 3 s [31]	Additional function in transport
SYD-1	Synapse-defective 1	Assembly and scaffolding protein	Unknown	Unknown	
SPN	Spinophillin	Assembly and scaffolding protein	Neurabin1: 2.08 Neurabin2: 3.04	Unknown	*Homo sapiens* homologue: neurabin1/2
NRX	Neurexin	Assembly and synaptic cleft spanning protein	Neurexin1: 2.89	~90% after 60 s for αNrxn ~70% after 60 s for βNrxn [52]	Transsynaptic complex with NLG
NLG	Neuroligin	Assembly and synaptic cleft spanning protein	Neuroligin2: 2.56	Unknown	Transsynaptic complex with NRX

be shown that neither spontaneous nor evoked release probability is uniformly distributed between the individual AZs of a synaptic terminal [10]. Instead, they segregate in subsets with either high or low probability for evoked release events. Notably, the size of the scaffold at individual AZs was found to scale with their overall probability to display evoked release in response to an action potential. Thereby, increasing levels of the AZ component Bruchpilot (BRP, a member of the ELKS family, Table 1) favors evoked over spontaneous transmission [11••]. A recent study at the *Drosophila* neuromuscular junction describes a spatial gradient of AZ size with larger and BRP enriched presynapses at the distal, compared to the proximal end of the terminal, correlating with larger and faster distal postsynaptic responses [12]. Similarly, at rat hippocampal neurons, evoked release per AZ scales with the ultrastructural AZ area and the local amounts of the scaffold proteins RIM1/2, Bassoon and Ca^{2+} channels [13,14]. What might be the mechanistic rationale behind this close relation between scaffold size and function? Potentially the AZ scaffold might provide 'SV fusion slots' where close proximity between Ca^{2+} channels and the SVs fusion machinery is established [8,15,16]. Additionally, because AZ scaffold components BRP, RIM-BP and RIM promote Ca^{2+} channel clustering at AZs [17–20], the correlation between AZ scaffold sizes and their microscopic probability of evoked release might also result from increased Ca^{2+} channel density and thus increased Ca^{2+} influx at larger AZs. While additional studies to deeper understand the interplay between AZ architecture and AZ function are needed, several reports, using different synaptic preparations, describe dynamic changes of AZ scaffolds operating on the minute timescale. These changes are either spontaneous, activity