

of the C2A-C2B interaction and may provide insight into the membrane fusion activity of this protein.

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A Novel Target of Proteasomal Degradation Induces Homeostatic Plasticity

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¹Neuroscience, University of Michigan, Ann Arbor, MI, USA, ²Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI, USA. Chronic manipulation of synaptic activity drives bidirectional alterations in synaptic efficacy. Compensatory tuning of presynaptic neurotransmitter release maintains levels of synaptic activity within an optimal range for efficient information processing. This homeostatic form of plasticity is apparent in cultured hippocampal neurons and appears to involve the ubiquitin-proteasome system (UPS). Examination of presynaptic mechanisms underlying homeostatic plasticity has identified multiple positive regulators of exocytosis subject to proteasomal degradation. However, homeostatic compensation can involve up- or down-regulation of exocytosis and substantially less is known of the negative regulation of vesicle release. Tomosyn, a presynaptically active SNARE protein, is unique in that it is cytosolic and serves to potently inhibit vesicle release at central synapses. Proteomic analysis of tomosyn revealed an interaction with the E3 ubiquitin-ligase HRD1. Here we aim to test the hypothesis that the UPS serves as an activity-dependent mechanism to precisely regulate tomosyn proteostasis, and in turn manipulates exocytosis. Consistent with this hypothesis, endogenous tomosyn levels in cultured rat hippocampal neurons (18-25 DIV) increase following application of the proteasome inhibitors MG132 (50 μ M, 4h) and lactacystin (10 μ M, 4h). Moreover, our data indicate that the tomosyn-HRD1 interaction is activity-dependent, as chronic AMPAR blockade (CNQX, 24h) results in an increase in HRD1 co-immunoprecipitation with tomosyn and consequentially a decrease in overall tomosyn protein level. To assess tomosyn's role homeostatic plasticity we use an optical reporter of exocytosis, vGlut1-pHluorin. Results show that chronic activity blockade via CNQX (40 μ M, 24h) enhances vesicle release in response to a stimulus train (10Hz, 10s) as compared to non-stimulated controls. This effect is dependent upon tomosyn, as shRNA-mediated tomosyn knock-down mitigates the compensatory enhancement of exocytosis. These data strongly implicate tomosyn as a key presynaptic molecular target which is subject to regulation by the UPS and facilitates activity-dependent homeostatic plasticity.

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A Novel Inhibitory Pathway Modulates the Fraction of Release-Competent Synaptic Vesicles

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¹Neuroscience, University of Michigan, Ann Arbor, MI, USA, ²Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI, USA. Homeostatic synaptic plasticity (HSP) maintains circuits within a dynamic range suitable for encoding information. HSP-induced changes in neurotransmission result from chronic increases in activity and lead to compensatory decreases in vesicle release (and vice versa). One mechanism for modulating release in response to a homeostatic challenge is to shift the fractional size of resting vs. recycling vesicle pools. In addition, strong evidence indicates that cyclin-dependent kinase 5 (CDK5) is critical for determining the fraction of vesicles in the resting, non-releasable state. Although CDK5 activity strongly affects synaptic release, the specific mechanism(s) exerting this function remains unknown. Current investigations aim to identify and characterize the CDK5 effector pathway that is required for its maintenance of the resting pool of vesicles. Here we test the hypothesis that tomosyn, a soluble R-SNARE protein is this CDK5 effector. Indeed our data indicate that tomosyn can be phosphorylated by CDK5 *in vitro*. We next determined the functional reliance of CDK5 on the presence of tomosyn by knocking-down (KD) or overexpressing (OE) tomosyn. These experiments utilized cultured rat hippocampal neurons (15-21 DIV) expressing vGLUT1-pHluorin, an optical reporter of vesicle release. Tomosyn KD increased mobilization of vesicles into recycling (active) pools (68% vs. 51% of totally releasable pool (TRP) in control); and, conversely, tomosyn OE resulted in a decrease in the recycling pool (36% of TRP). Pharmacological inhibition of CDK5 via roscovitine (100 μ M, 30m) mirrored the tomosyn KD results wherein a significantly greater fraction of vesicles appeared in a releasable state (70% of TRP). Importantly, combination of the two perturbations (tomosyn KD + roscovitine) did not result in an additive increase in the releasable pool (72% of TRP) further demonstrating that CDK5 and tomosyn likely exert control over vesicle release through a common signaling pathway.

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The Role of Doc2B in Depolarization-Evoked and G Protein-Coupled Receptor Modulated Exocytosis in Mouse Chromaffin Cells

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Previously we demonstrated that activation of Gq protein-coupled H1 histamine receptors potentiates stimulus-coupled exocytosis in bovine chromaffin cells, despite inhibiting Ca²⁺ influx through voltage-gated calcium channels (VGCCs). This potentiation is restricted to the immediately releasable pool (IRP) of vesicles coupled to VGCCs, and requires the priming protein Munc13-1. Doc2B is a calcium-binding protein, known to interact with Munc13 to promote exocytosis; whether it plays a role in agonist-regulated exocytosis is unknown. To address this question, we isolated chromaffin cells from Doc2B (+/+), (+/-) and (-/-) mice and examined the effects of PLC-coupled GPCR activation on depolarization-evoked exocytosis.

We found that under control stimulus conditions, depolarization-evoked exocytosis, measured as changes in membrane capacitance, was not significantly different between Doc2B (+/+), (+/-) and (-/-) cells; similarly no differences were found between the sizes of the releasable vesicle pools (IRP, RRP, SRP). Equimolar replacement of extracellular Ca²⁺ by Ba²⁺ also revealed no significant differences in the synchronous and asynchronous phases of Ba²⁺-evoked exocytosis in Doc2B (+/+), (+/-) or (-/-) cells. Finally, although more than 80% of cells, independent of their genotype, responded to histamine with a reduction of Ca²⁺ influx, potentiation of exocytosis was surprisingly modest (2-fold) and, even in Doc2 (+/+) cells rarely observed (5/17 cells, (+/-): 5/24 cells, (-/-): 2/18). This is in stark contrast to bovine cells, where 80% of the cells show a 5-fold potentiation of exocytosis. Importantly, Doc2B (+/+), (+/-) and (-/-) cells responded to the DAG analog PMA with potentiation. Taken together, the results suggest that there are profound species differences in the coupling between GqPCRs and the exocytotic machinery in chromaffin cells and that Doc2B is not required for agonist-dependent potentiation of exocytosis in mice.

Nucleo-Cytoplasm Transport

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A Ran-Dependent Importin-Beta/Nup153 Barrier in the Nuclear Pore Complex

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The nuclear pore complex (NPC) is a large multi-protein assembly that mediates the selective transport of molecules between the cytoplasm and nucleus in eukaryotic cells. While the factors involved in various transport pathways are largely known, the physical mechanism by which the NPC supports both efficient and selective molecular translocation remains unclear. We have identified a component of the NPC selectivity barrier consisting of the transport receptor importin- β and the nucleoporin Nup153 which can be modulated by the GTPase Ran. We have investigated importin- β and Nup153's effect on the kinetic rates of both passive and active nuclear import and its regulation by Ran. Furthermore, using photobleach step counting and super-resolution imaging, we have characterized the numbers and spatial distributions of importin- β within the pore suggesting that this transport receptor is a functional and surprisingly stable component of the NPC. Finally, we demonstrate *in vitro* with fluorescence fluctuation spectroscopy that importin- β and Nup153 bind to form large cross-linked complexes which can be dispersed by RanGTP. Our results suggest that importin- β in conjunction with Nup153 can constitute a selective 'gate' in the NPC whose permeability towards both inert and transport-competent cargos is sensitive to RanGTP.

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Conformational Behavior of the Confined Fg-Repeat Domains in the Nuclear Pore

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The nuclear pore complex, NPC, is responsible for selectively conducting transport to/from the nucleus in eukaryotes. While we are entering the sixth