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Commentary

RPS23RG1 May Prevent Ubiquitin-Proteosomal Degradation of Postsynaptic Densities-93 and -95 to Protect Synaptic Function: Implications for Alzheimer's Disease

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Alzheimer's disease (AD) is characterized by the hallmark pathologies of amyloid-β (Aβ) plaques and tau tangles. Recently, many drug trials and much research have focused on preventing or removing these pathologies, especially Aβ. While some trials have been able to effect change in amyloid levels, none have succeeded in improving cognition. Although the trials may have involved patients that were too advanced for benefit with antiamyloid therapies, an inability of these drugs to protect synaptic function may have contributed to their failure. It was reported as early as 1990 that synaptic loss correlated strongly with dementia (1,2), suggesting that protecting synapses could slow cognitive decline. Given the recent challenges in developing effective therapies targeting amyloid and tau, there has been a renewed interest in understanding synapse degeneration and loss in AD [reviewed in (3,4)] and the investigation of synaptic function as a potential biomarker (5) and therapeutic target. In this issue of *Biological Psychiatry*, Zhao *et al.* (6) investigated a novel role for a little-known protein, RPS23RG1, in preserving postsynaptic structure and function in an amyloid pathology mouse model.

In 2009, Zhang *et al.* (7) published the results of a genetic screen that was designed to find genes that reduced Aβ production by looking for the accumulation of amyloid precursor protein (APP) β C-terminal fragments, which uncovered *Rps23rg1*. They demonstrated that *Rps23rg1* exerts its effects on Aβ generation and tau phosphorylation by downregulating the activity of glycogen synthase kinase 3. Importantly, Zhang *et al.* (7) showed that RPS23RG1 interacts with adenylate cyclase to upregulate cyclic adenosine monophosphate levels and activate protein kinase A phosphorylation of glycogen synthase kinase 3, reducing its activity. Ultimately, this mechanism leads to reduced Aβ generation and decreased tau phosphorylation.

Evolutionarily, *Rps23rg1* arose from the retroposition of the ribosomal protein 23 gene and is transcribed in the opposite direction as its parent gene. To determine the biological role of this novel protein, Zhao *et al.* (6) generated a *Rps23rg1* knockout (KO) mouse line using a transcription activator–like effector nucleases–based gene targeting strategy. *Rps23rg1* messenger RNA is expressed throughout the body, suggesting an important physiological function. *Rps23rg1* null mice were born at the expected Mendelian ratio and were viable, with grossly normal brain structure at 1 month of age. Despite this, the mice showed striking cognitive deficits in learning and memory at an early age, as measured by T-maze, novel object recognition, fear conditioning, and the Morris water maze tasks. A physiological correlate of learning and memory, long-term potentiation (LTP), was reduced in the *Rps23rg1* KO mice. Both mini excitatory postsynaptic currents and evoked excitatory postsynaptic currents mediated by alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) receptors were lower in KO mice neurons compared with control mice neurons, indicating impairment of glutamatergic neurotransmission and synaptic function.

To find potential causes for the electrophysiological postsynaptic changes in *Rps23rg1* KO mice, Zhao *et al.* (6) examined spine and synapse structure, observing a reduction in mature spine number in cultured neurons and in the CA1 region of the brain of the *Rps23rg1* null animals. Electron microscopy revealed a reduction in the size of *Rps23rg1* KO postsynaptic densities (PSDs), prompting an investigation of two important postsynaptic structural proteins, PSD-95 and PSD-93. Both PSD-95 and PSD-93 proteins were reduced in KO mice owing to increased turnover, which led to reduced surface expression of AMPA and NMDA receptor subunits. A specific interaction between RPS23RG1 and PSD-95/PSD-93 was found to be dependent on five amino acids, TTLAH, conserved between mouse and human proteins, in the 19 amino acid intracellular domain of RPS23RG1 (Figure 1). The association between RPS23RG1 and PSD-95/PSD-93 attenuated the interaction of PSD-95/PSD-93 with murine double minute 2, an E3 ubiquitin ligase, in a dose-dependent manner. By blocking the interaction with murine double minute 2, which ubiquitinates PSD-95/PSD-93 to target them for degradation by the proteasome, RPS23RG1 elevated the levels of PSD-95/PSD-93, which could in turn increase surface levels of AMPA and NMDA receptors.

To determine if loss of PSD-95/PSD-93 is the cause of cognitive impairment in the *Rps23rg1* KO mice, Zhao *et al.* (6) injected viruses expressing these proteins into the ventricles of postnatal day 0 mice to boost PSD levels. At 2 months of age, *Rps23rg1* KO mice that coexpressed PSD-95 and PSD-93 performed like wild-type mice on novel object recognition and almost like wild-type mice on the T-maze task. Elevation of either protein alone did not have an effect on behavior, while expression of either or both in the *Rps23rg1* KO mice improved LTP in the hippocampus, indicating that loss of PSD-95/PSD-93 plays a key role in synaptic dysfunction in *Rps23rg1* null mice.

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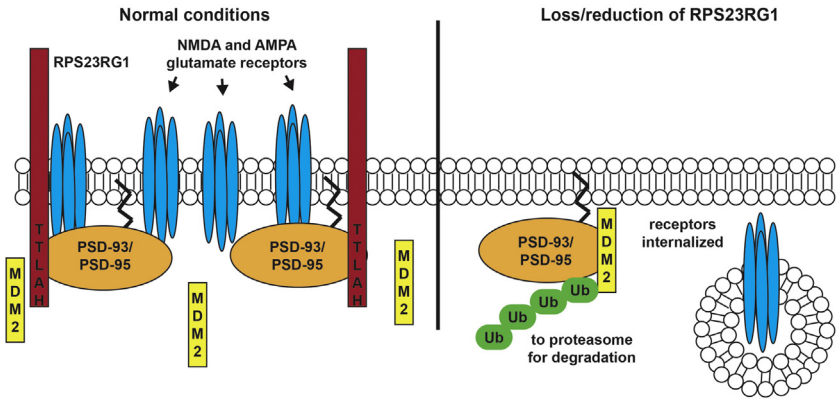

Figure 1. RPS23RG1 stabilizes postsynaptic structure through an interaction with postsynaptic densities-95 and -93 (PSD-95/PSD-93). (Left panel) Under normal physiological conditions, RPS23RG1 interacts with the postsynaptic structural proteins PSD-95/PSD-93 through the five amino acid sequence TTLAH found in the small intracellular domain. RPS23RG1 binding blocks the interaction of the E3 ubiquitin ligase murine double minute 2 (MDM2) with PSD-95/PSD-93, preventing their degradation by the proteasome and increasing steady state levels of PSD-95/PSD-93. PSD-95/PSD-93 prevents internalization of the alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) receptors to help maintain consistent postsynaptic activity. (Right panel) When RPS23RG1 is absent, such as in knockout mice, or reduced, as is observed in patients with Alzheimer's disease and aged APP/PS1 amyloid model mice, MDM2 is free to interact with PSD-95/PSD-93, resulting in its ubiquitination (Ub) and degradation by the proteasome. Decreased levels of PSD-95/PSD-93 lead to more internalization of NMDA and AMPA receptors and reduced postsynaptic activity, which is associated with cognitive impairments in learning and memory tasks.

Next, Zhao *et al.* (6) investigated whether intraperitoneal injection of synthetic RPS23RG1 19 amino acid C-terminal domain harboring the TTLAH PSD-95/PSD-93 binding motif could rescue PSD-95/PSD-93 levels and behavioral and synaptic deficits. To improve cellular uptake of the peptide, an 11 amino acid human immunodeficiency virus trans-activator of transcription (TAT) transduction sequence was conjugated to the biotin-TTLAH peptide or a scrambled peptide control. Though only a small portion of TAT-conjugated protein entered the brain 24 hours after intraperitoneal injection, PSD-95/PSD-93 could be coimmunoprecipitated with biotin-TTLAH-TAT from brain lysates, indicating target engagement. *Rps23rg1* KO mice injected for 3 days with biotin-TTLAH-TAT peptide showed increased levels of PSD-95/PSD-93 and enhanced LTP compared with mice injected with scrambled peptide-TAT and performed like wild-type mice on novel object recognition and T-maze tasks. In addition, the increased association between PSD-95/PSD-93 and murine double minute 2 observed in *Rps23rg1* KO mice was eliminated in mice injected with TTLAH-TAT peptide, supporting the hypothesis that increased degradation of PSD-95/PSD-93 leads to synaptic dysfunction.

Rps23rg1 overexpression had already been shown to decrease the AD pathologies of amyloid plaques and abnormal tau phosphorylation, and in this article was shown to protect synaptic integrity, so Zhao *et al.* (6) further investigated the role of *Rps23rg1* in AD. In human AD and an amyloid mouse model (APP/PS1), messenger RNA and levels of RPS23RG1 were reduced, correlating with a decrease in PSD-95 and PSD-93. Zhao *et al.* (6) also assessed whether the synapse-preserving effect of the TTLAH motif could have a beneficial effect in the APP/PS1 transgenic mouse model of amyloid pathology. After 3 days of biotin-TTLAH-TAT injection, 7-month-old APP/PS1 mice had a restoration of PSD-95/PSD-93 levels, showed normal behavior in T-maze and novel object recognition, and exhibited partially restored LTP.

Zhao *et al.* (6) demonstrates that RPS23RG1 is an important protein in maintaining PSD-93 and PSD-95 levels, and thus that glutamatergic synaptic function is necessary for normal learning and memory functions. This pathway may be compromised in AD, as *RPS23RG1* mRNA levels are reduced in human AD patients, and within this group, low levels of RPS23RG1 protein correlate with low levels of PSD-95/PSD-93. In an AD mouse model, blocking PSD-93/PSD-95 degeneration through injections of the RPS23RG1 TTLAH domain improved synaptic function and memory, suggesting that this could be a useful approach in AD. Along these lines, future work should focus on finding a brain-penetrant small molecule that could mimic the interaction between the RPS23RG1 TTLAH domain and PSD-95/PSD-93 and investigating its efficacy in maintaining synaptic function in preclinical models and in human patients. The mechanism of the persistence of the therapeutic effect also requires further investigation, as it seems that only 3 days of treatment provides efficacy that lasts as long or longer, despite the relatively rapid turnover of PSD-93/PSD-95.

In conclusion, Zhao *et al.* (6) present a new pathway through which synaptic integrity may be compromised in AD and that may provide novel opportunities for intervention.

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Article Information

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