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Structure–function analysis of SAP97, a modular scaffolding protein that drives dendrite growth

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ABSTRACT

Activation of AMPA receptors assembled with the GluA1 subunit can promote dendrite growth in a manner that depends on its direct binding partner, SAP97. SAP97 is a modular scaffolding protein that has at least seven recognizable protein–protein interaction domains. Several complementary approaches were employed to show that the dendrite branching promoting action of full length SAP97 depends on ligand(s) that bind to the PDZ3 domain. Ligand(s) to PDZ1, PDZ2 and I3 domains also contribute to dendrite growth. The ability of PDZ3 ligand(s) to promote dendrite growth depends on localization at the plasma membrane along with GluA1 and SAP97. These results suggest that the assembly of a multi-protein complex at or near synapses is vital for the translation of AMPA-R activity into dendrite growth.

1. Introduction

The interaction of a young animal with its environment evokes patterns of synaptic activity within the nervous system that influences the form and function of neurons (Hubel and Wiesel, 1970; Hubel et al., 1977; Wiesel, 1982). The molecular mechanisms by which synaptic activity operates have been well studied when activation of N-methyl-D-aspartate-type glutamate receptors (NMDA-Rs) is the initiating event. This has crystallized into a version of the "synaptotrophic hypothesis" in which transient pre- and post-synaptic contacts (mediated by adhesive molecules (Chen et al., 2010)) are stabilized if they lead to activation of NMDA-Rs (Constantine-Paton, 1990; Cline and Haas, 2008; Chia et al., 2014). Stabilized synapses lead to stabilized neurites while preliminary contacts (and the neurites that bear them) that fail to lead to active NMDA-Rs decay (Ruthazer et al., 2003; Hua and Smith, 2004; Niell et al., 2004; Haas et al., 2006). This form of activity-dependent development has been extensively studied in the sensory systems (most notably the visual system).

An additional form of activity-dependent development is initiated by activation of (2-amino-3-(5-methyl-3-oxo-1,2- oxazol-4-yl)) propanoic acid-type glutamate receptors (AMPA-Rs) assembled with the GluA1 subunit. The developing locomotor system has provided a useful platform for mechanistic insight into this form of activity-dependent plasticity. Neonatal motor neurons display calcium-permeable AMPA receptors (Carriedo et al., 1996; Vandenberghe et al., 2000) — consistent with the observation that they express particularly high levels of GluA1 during this period (Jakowec et al., 1995a,b). Elimination of GluA1 leads to stunted motor neuron dendrite growth, deranged segmental spinal cord circuitry and sub-normal motor behavior (Zhang et al., 2008). Overexpression of GluA1 in mature motor neurons leads to remodeling of the dendritic tree with an increase in branch abundance (Inglis et al., 2002). These events occur in an NMDA-R independent manner (Inglis et al., 2002). These observations provide the first clues that GluA1 containing receptors can control activity-dependent plasticity in parallel with the better understood NMDA-R mediated pathway.

The capacity of AMPA-Rs assembled with GluA1 to control the various aspects of motor system development is entirely dependent on its ability to form a native complex with synapse associated protein of 97 kDa molecular weight (SAP97). This has been most convincingly shown by studies of mice with a knock-in allele of GluRA1 that lack the C-terminal 7 amino acids (originally designated GluR1Δ7, now called GluA1Δ7) (Zhou et al., 2008). AMPA-Rs are processed normally in the GluA1Δ7 mice, show normal synaptic currents and normal synaptic plasticity (such as NMDA-R-dependent hippocampal plasticity) (Kim et al., 2005; Zhou et al., 2008). Yet motor neuron dendrite growth is aberrant both in vitro and in vivo. These affects can be ascribed to the inability of GluA1 to chaperone SAP97 to synapses because the GluA1Δ7 phenotype can be

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completely rescued by membrane targeting SAP97. As would be expected, conditional deletion of SAP97 from motor neurons phenocopies the loss of GluA1 (Zhou et al., 2008). These observations are most parsimoniously explained by a GluA1-mediated form of neuronal plasticity that is driven by a GluA1/SAP97 synaptic complex that does not involve activation of NMDA-Rs.

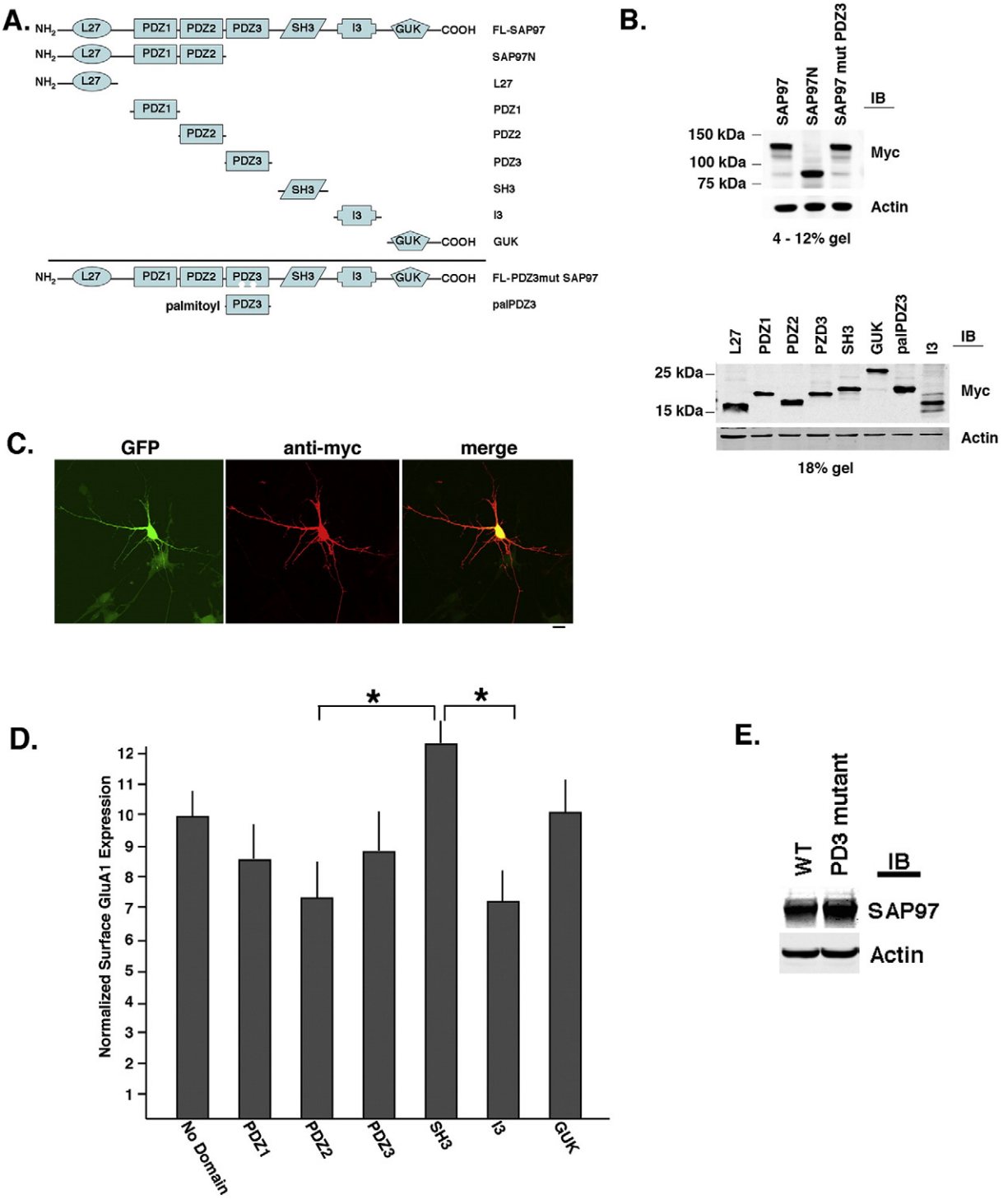

Fig. 1. Domain structure of SAP97 constructs, expression levels, cellular distribution and effects on neuronal cell surface GluA1 abundance. Panel A. A cartoon depiction of recognizable protein–protein interaction domains of full length SAP97 (FL-SAP97) and the various truncation and point mutants employed in this study. Panel B. Western blots of various constructs expressed in HEK 293 cells. There are minor variations in the expression levels of individual constructs but no correlation between expression level and biological effects on dendrite growth. Panel C. Immunocytochemical localization of myc tagged PDZ3 shows that the protein is widely distributed throughout neuronal processes. GFP was used to delineate neuronal architecture and the myc-PDZ3 was imaged in the red channel. The merge image shows perfect co-localization. Calibration bar = 25 μm. Panel D. Quantification of cell surface GluA1 immunoreactivity in neurons expressing individual domains. The reported values are a ratio of GluA1 fluorescence to GFP (the co-transfection marker). In comparison with no domain controls, there were few differences in surface GluA1 abundance. Neurons expressing the SH3 domain had significantly more surface GluA1 in comparison with neurons expressing the PDZ2 or the I3 domain. It is noteworthy that expression of the SH3 domain was associated with more GluA1 expression but had no effect on dendrite growth (see Table 5). Panel E. Expression levels of FL WT SAP97 and FL PDZ3 mutant SAP97 are very similar as are actin levels.