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Scaffold remodeling in space and time controls synaptic transmission

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Scaffolding proteins that are associated with glutamate receptors in dendritic spines govern the location and function of receptors to control synaptic transmission. Unraveling the spatio-temporal dynamics of protein-protein interactions within components of the scaffolding complex will bring to light the function of these interactions. Combining bioluminescence resonance energy transfer (BRET) imaging to electrophysiological recordings, we have recently shown that GKAP, a core protein of the scaffolding complex, interacts with DLC2, a protein associated with molecular motors. Synaptic activity-induced GKAP-DLC2 interaction in spines stabilizes the scaffolding complex and enhances the NMDA currents. Interestingly, this work placed emphasis on the bioarchitectural dependence of protein-protein interaction dynamics. Depending on physiological conditions, the modulation in space and time of protein-protein interaction is acutely regulated, engendering a subtle control of synaptic transmission in the state of the individual synapse.

Keywords: protein-protein interaction, bioluminescence resonance energy transfer (BRET), scaffolding proteins, glutamate receptors, guanylate kinase-associated protein (GKAP), dynein light chain 2 (DLC2), synaptic transmission, dendritic spine

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The communication between neurons is mainly achieved at the level of chemical synapses. In the presynaptic terminal, synaptic vesicles containing neurotransmitters (NT) are recruited to specialized release sites termed active zones. Once released, the NT diffuses within the synaptic cleft and binds to postsynaptic receptors that change the membrane potential and initiate signal transduction cascades. Glutamate is the main excitatory NT in the mammalian brain. Most excitatory inputs find postsynaptic connections at dendritic spines. Spines are postsynaptic specialized protrusions, dynamic structures that ensure the compartmentalization of biochemical and electrical signals.[1,2] Spines contain NT receptors, organelles, and signaling systems essential for synaptic function and plasticity. Right beneath the postsynaptic membrane resides an electron-dense organelle named the postsynaptic density (PSD). In the PSD, multiprotein complexes mediate the organization and clustering of receptors and orchestrate their specific coupling to various signaling pathways.[3] A loss or dysregulation of these scaffolding proteins can generate a variety of neurological diseases.[4] However, although the importance of receptor scaffolding proteins in the control of receptor functions is well established, the molecular detail of the endogenous interactions and the roles that they play in the regulation of synaptic transmission is poorly defined because of the dearth of methods for acutely and specifically monitoring the binding interactions and their dynamics. Recently, a chemically based approach for acutely disrupting endogenous interactions within the postsynaptic scaffolding complex has proved the importance of scaffolds in the stabilization of glutamate receptors at the synapse.[5] Scaffolding complexes trigger the specific anchoring of glutamate receptors within synapses.[6] Furthermore, genetic perturbations that reduce the stability of these scaffolding protein complexes would sustain synaptic and behavioral dysfunction in a mouse model of autism.[7] These recent publications highlight the need to study such dynamic interactions in native conditions.

Macromolecular complexes that are held together by protein-protein interactions govern the functioning of excitatory

synapses in the mammalian brain. Scaffolding proteins function not only as anchors, but also as signaling proteins for neurotransmitter receptors. As synapses are dynamic structures, studying the dynamics of such synaptic receptor scaffolds and their role in neurotransmission is an essential query. In particular, the molecular mechanisms regulating the postsynaptic targeting and assembly of neurotransmitter receptors and associated scaffolding proteins in the PSD are still poorly understood. Guanylate kinase-associated protein (GKAP) is a core protein of the PSD95-GKAP-Shank-Homer scaffolding complex linking glutamate receptors (NMDA and group I mGlu receptors, **Fig. 1**). This complex governs glutamate receptor location and function in dendritic spines.[8-12] Interestingly, GKAP also interacts with DLC2,[13] a light chain shared by cytoplasmic dynein and myosin-V.[14] This adaptor protein functions as a molecular motor that drives the trafficking of cargoes along microtubules and actin filaments.[15-17] The association of GKAP with molecular motors raised the question of its functional interaction in the organization and activity of the glutamate receptors. An original combination of bioluminescence resonance energy transfer (BRET) imaging[18,19] with immunofluorescence staining and electrophysiological recordings recently allowed us to better understand the physiological role of GKAP-DLC2 complex in postsynaptic glutamate receptor assembly and function.[20] Studying the spatio-temporal dynamics of GKAP-DLC2 interaction in living hippocampal neurons, we found that GKAP-DLC2 interaction was prominent in dendritic spines and could be exacerbated by sustained synaptic activity. Indeed DLC2 specifically interacts with GKAP but not other PSD-associated scaffolding elements. We showed that GKAP-DLC2 interaction has two complementary functions, both solicited by neuronal activity (**Fig. 1**): First, GKAP-DLC2 interaction within dendritic spines stabilizes the postsynaptic complex at the PSD, highlighting the role of DLC2 as a hub protein that interacts with partially disordered proteins to promote their adequate organization. Second, GKAP-DLC2 increases the spine preferential expression of GKAP and its cognate scaffolding partners in dendritic spines. As a light chain of myosin V, DLC2 seems to be an adaptor protein that functions as a molecular motor to drive the specific trafficking of GKAP toward dendritic spines along actin filaments. The overall functional consequence of GKAP-DLC2 interaction is the enhancement of NMDA synaptic currents. This extended role of GKAP-DLC2 complex is in agreement with the existence of non-synaptic clusters of synaptic proteins[21] and would explain the essential role of the actin cytoskeleton in both maintenance and reorganization of the PSD.[22]

In their physiological environment, membrane receptors form complexes with scaffolding proteins that link them to the cytoskeleton as well as receptor's intracellular signal transduction pathways. Despite the relatively stable structure of such receptor-associated scaffolds, the exchange of individual adaptor proteins within complexes can occur within a short period of time and in a highly regulated manner, which provides fine-tuning, speed and specificity to the receptor's signaling. Depending on physiological conditions, protein-protein interactions will be modulated in space and time. Therefore, an essential biological concern is to understand how proteins are activated as free molecules or part of complexes. Our study throws new light on the spatio-temporal dynamic of GKAP-DLC2 interaction and its function. Thus, modulation of protein-protein interactions within scaffold complexes governs synaptic transmission. Interestingly, BRET signals (reporting the interaction between GKAP and DLC2) differed between spines. These differences placed emphasis on physiological disparities between spines. The amount of proteins per spine may be different, thus small differences in DLC2 and GKAP protein expression ratio will influence the effectiveness of the interaction. But more importantly, spines are dendritic protrusions allowing the isolation of the biochemical and electrical signals generated by receptors. Neuronal activity is not homogenous and will vary from one spine to another, controlling the efficiency of GKAP-DLC2 interaction. DLC2 is a light chain of myosin V, this adaptor protein links GKAP to the molecular motor and drives the specific trafficking of GKAP along actin filaments up to the PSD in spines. This confirms the critical role of filamentous actin in determining the extent of dynamic reorganization in PSD molecular composition.[22] Spines have an elaborate mechanical nature that is regulated by actin fibers.[23] This morphology-dependent modulation of protein-protein interactions introduces a supplemental level of complexity in the control of cell signaling. These subtle and confined variations of interactions accurately control the cell signaling. Regarding neuron morphology in particular, spines are especially advantageous to neurons due to their role in compartmentalizing biochemical and electrical signals. This can help to encode changes in the state of an individual synapse without necessarily affecting the state of other synapses of the same neuron. This undoubtedly affects the efficiency of protein-protein interactions between spines.

We evidenced for the first time the spatio-temporal occurrence and dynamics of protein-protein interactions in living neurons to control receptor function and synaptic transmission.

The development of sensitive tools (rather than general approaches that miss subtle variations of protein interactions in space and time) is a major advance in the field of cell signaling. Thus, activity-induced modulation can be directly compared with the basal interaction in the same neuron, same spine, pixel by pixel. The present findings provide a novel regulatory pathway of synaptic transmission that depends on activity-induced remodeling of the postsynaptic scaffold protein complex. Other regulatory roles for scaffolding proteins will soon be revealed. For example, scaffold remodeling also represents a form of homeostatic control of synaptic excitability.[24] Indeed, the PSD95-GKAP-Shank-Homer scaffolding complex disassembly (triggered by sustained activation of synaptic NMDA receptors) induces a physical and functional interaction between NMDA and group I mGlu receptors. Interestingly, this protein scaffold remodeling is spine-confined and results in a negative feedback loop on NMDA receptor activity. It

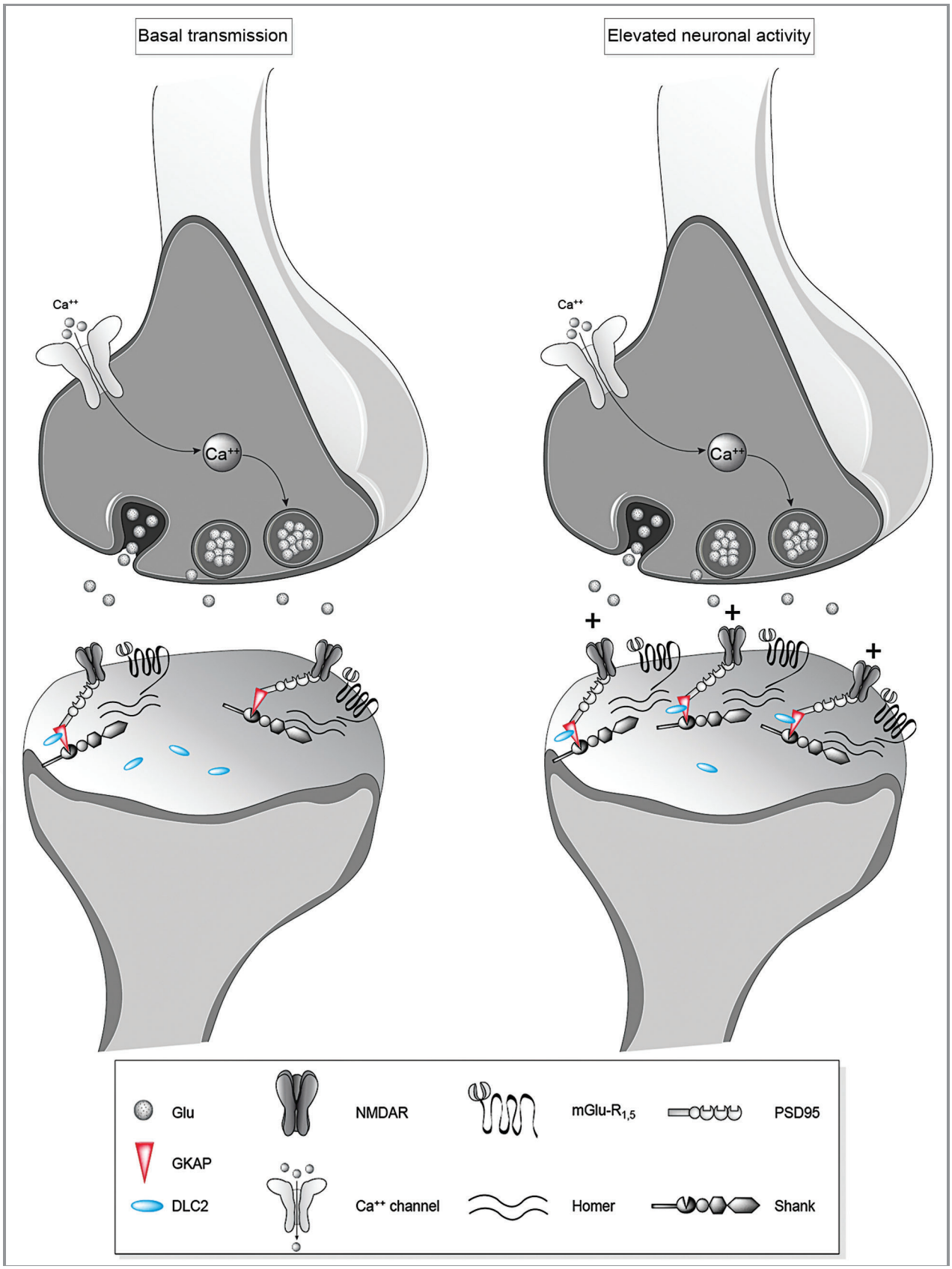

Figure 1. GKAP-DLC2 interaction, favored by sustained synaptic activity in the dendritic spine, stabilizes scaffolding protein expression at the PSD and enhances synaptic glutamate receptor activity.

has also become clear that scaffold proteins have a crucial role in regulating various signaling cascades in many other cell types (such as immune-cell signaling,[25] or infection[26,27]). Therefore, this ubiquitous functional importance of protein-protein interactions within scaffolding complexes stresses the need to study the spatio-temporal dynamic of complexes and to understand their functions.

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