Lecture 7:
Roles for MAGUKS in Activity-dependent Synaptogenesis
From: Kennedy (2000) Science
MEMBRANE ASSOCIATED GUANYLATE KINASES
MAGUKS are the protein scaffolds of the post-synaptic density

From: Kennedy (2000) Science
### Direct Protein Interactions of NMDA Receptor Binding MAGUKs

<table>
<thead>
<tr>
<th>Protein</th>
<th>Bonding Domain on MAGUK</th>
<th>Proposed Function</th>
<th>Source</th>
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</thead>
<tbody>
<tr>
<td>* NR2A</td>
<td>PDZ&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Scaffolds NRS to MAGUKs</td>
<td>Korneau et al.'95; Lau et al.'96; Miller et al.'96; Neithammer et al. '96</td>
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<tr>
<td>* NR2B</td>
<td>PDZ&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Scaffolds NRS to MAGUKs</td>
<td></td>
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<tr>
<td>* GKAP95/130</td>
<td>GK</td>
<td>Couples MAGUKS to SHANK and dynein light chain</td>
<td>Kim et al.'97; Naisbitt et al.'97; Tuo et al.'97</td>
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<tr>
<td>* Stargazan</td>
<td>PDZ</td>
<td>Stabilizes AMPARs in synaptic membrane</td>
<td>Chen et al. '00</td>
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<tr>
<td>* KA&lt;sub&gt;1&lt;/sub&gt;</td>
<td>SH&lt;sub&gt;3&lt;/sub&gt; + GK</td>
<td>Scaffolds KARs to MAGUKS, block KAR desensitization</td>
<td>Garcia et al. '98; Mehta et al.'01</td>
</tr>
<tr>
<td>* GluR&lt;sub&gt;B&lt;/sub&gt;</td>
<td>PDZ</td>
<td>Scaffolds KARs to MAGUKS, block KAR desensitization</td>
<td>Garcia et al. '98</td>
</tr>
<tr>
<td>AKAP</td>
<td>SH&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Scaffolds CaN, PKA, PKC; regulation actin dynamics</td>
<td>College et al. '00; Gomez et al. '00</td>
</tr>
<tr>
<td>SynGAP</td>
<td>PDZ</td>
<td>ras GAP</td>
<td>Chen et al. '98; Kimetal. '98</td>
</tr>
<tr>
<td>Neuroligin</td>
<td>PDZ</td>
<td>Adhesion, binding partner of Neurexin</td>
<td>Irie et al. '97; Bolliger et al. '01, Song et al. '01</td>
</tr>
<tr>
<td>ErbB&lt;sub&gt;4&lt;/sub&gt;</td>
<td>PDZ&lt;sub&gt;1,2&lt;/sub&gt;</td>
<td>Neuregulin receptor signals to the nucleus</td>
<td>Huang et al. '01</td>
</tr>
<tr>
<td>Erbin</td>
<td>PDZ</td>
<td>Increases Erb expression</td>
<td>Huang et al. '01</td>
</tr>
<tr>
<td>K&lt;sup&gt;+&lt;/sup&gt; channel</td>
<td>PDZ</td>
<td>Membrane polarization</td>
<td>Imamura et al. '02</td>
</tr>
<tr>
<td>NOS</td>
<td>PDZ</td>
<td>Synthesizes NO, a putative retrograde signal</td>
<td>Craven &amp; Bredt. '02</td>
</tr>
<tr>
<td>Fyn</td>
<td>PDZ</td>
<td>Tyrosine kinase, phosphorylates NR2A</td>
<td>Tezuka et al. '99</td>
</tr>
<tr>
<td>Cript</td>
<td>PDZ</td>
<td>Assoc. w/tubulin; relieves GK domain from inhibition of binding</td>
<td>Neithammer et al.'98; Passafaro et al. '99</td>
</tr>
<tr>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt;-ATPases 2a, 4b</td>
<td>PDZ</td>
<td>Maintaining Ca&lt;sup&gt;2+&lt;/sup&gt; homeostasis PSD-95 binds both, SAP102</td>
<td>Demarco &amp; Strehler, '01</td>
</tr>
<tr>
<td>Kalirin-7</td>
<td>PDZ</td>
<td>Rho family GEP that affects dendrite actin dynamics; inte w/all NR-binding MAGUKs</td>
<td>Penzes et al. '01</td>
</tr>
</tbody>
</table>

**TABLE I: Proteins that Bind Domains of NR-binding MAGUKs**

- * not known whether all NR binding MAGUKs will interact with most of these proteins
- **fetal and postnatal period: SAP 102**
- **juvenile-adult: PSD-95; in some regions PSD-93**
The NMDAR is a tetramer consisting of 2 NR1 subunits and 2 NR2 subunits

![Diagram of NMDAR subunits](image)

<table>
<thead>
<tr>
<th></th>
<th>NR2A</th>
<th>NR2B</th>
<th>NR2C</th>
<th>NR2D</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR2A</td>
<td>AA</td>
<td>AB</td>
<td>AC</td>
<td>AD</td>
</tr>
<tr>
<td>NR2B</td>
<td>BA</td>
<td>BB</td>
<td>BC</td>
<td>BD</td>
</tr>
<tr>
<td>NR2C</td>
<td>CA</td>
<td>CB</td>
<td>CC</td>
<td>CD</td>
</tr>
<tr>
<td>NR2D (embryo)</td>
<td>DA</td>
<td>DB</td>
<td>DC</td>
<td>DD</td>
</tr>
</tbody>
</table>

Postnatal
Midbrain and forebrain express NR2A & NR2B
Developmental decreases with age in NMDAR synaptic current decay times are due to the loss of NMDARs enriched in the NR2B subunit.

Studies on hippocampal autaptic synapses in isolated island cultures

Experiments testing the efficacy of ifenprodil against 2NR1: 2NR2B receptors Transfected into HEK293 cells

Extrasynaptic NMDARs out-number synaptic NMDARs by ~ 3 to 1 in ≤ 7 DIV neurons. Use of autapses allows synaptic currents and whole cell currents of the same cell to be compared.

EPSCs are less sensitive than whole cell currents to ifenprodil and are smaller than whole cell currents.

MK801 is a non-competitive NMDAR channel blocker. Therefore, it can be used to specifically block synaptic NMDAR currents.

This difference represents the contribution of NMDAR synaptic current to NMDAR whole-cell currents.
Many antibodies against MAGUKS are not specific.
In hippocampus, as in many other brain regions as development proceeds, the number of glutamate receptors increase but the type of NMDA receptor changes.

Sequence
2NR1:2NR2B to 2NR1:1NR2B:1NR2A to 2NR1:2NR2B
The NMDAR scaffolding molecules also change with age.
SAP-102 is synaptic (as well as extrasynaptic) in neonates and becomes progressively perisynaptic (extrasynaptic) with age.

PSD-95 is expressed at low levels in neonates and increases at synapses with age.
NR2B always immunoprecipitates more SAP-102 than PSD-95.

NR2A immunoprecipitates more PSD-95 than SAP-102.

PSD-95 and NR2A are not at immunoblot detectable levels in the neonate.
Synaptic Activity Controls:

1. The levels of NR2B transcription (e.g. increased activity, decreased NR2B transcript).

2. The transport of PSD-95 to the synapse.

3. Possibly the local synaptic translation of NR2A.
In Wildtype Mice Both NR2A and PSD-95 Become Enriched In the Synaptoneurososome (Dendritic) Fraction In the P8-P11 Interval

Tissue from the superficial visual layers of the superior colliculus (sSC)

This increase is eliminated when the photoreceptor to bipolar to ganglion cell pathway is blocked

PSD-95 increases in NR2A KO synaptoneurosome fractions.

However, this PSD-95 does not bind NMDA receptors effectively.

Townsend, M et al., (2003) PNAS.
The normal development of the retina provides natural predictable changes in the amount and patterning of activity to central visual neurons. Post-synaptic responses of visual neurons can therefore be studied after known in vivo stimulation.

Western blots of dendritic fractions (synaptoneurosomes) from the visual layers of the superior colliculus & visual cortex reveal changes in PSD proteins with age.

Yoshii et al., 2003
PSD-95 Increases In Dendritic Fractions Within Hours of Controlled Eye-Opening

PSD-95 is concentrated in cell bodies before eye-opening.

(Yoshii et al. 2003, PNAS)
PSD-95 is Redistributed to Synapses after Eye-Opening

Green: PSD-95
Red: MAP2

BEO  6hrs AEO

(Yoshii et al. 2003, PNAS)
Eye-opening produces a switch in the ratios of the NR2B and the NR2A subunits associated with PSD-95 in dendritic fractions.

Yoshii et al., 2003
Over-expression of tagged PSD-95 and either tagged NR2A or NR2B shows co-localization of NR2A and PSD-95 at synapses


Figure 6. Distinct colocalization of PSD-95gfp with NR2A-flag and NR2B-flag clusters
GKAP95 increases at the synapse with PSD-95, while GKAP130 levels remain constant.

Yoshii et al, 2003

Data suggest:

Naisbitt et al, 2000
The Flailer mouse has an additional truncated myosin Va expressed only in brain. It is ataxic and has seizures. The truncated myosin Va appears to operate as a dominant negative (Jones et al., 2000)
Localizing Synaptic Distribution of PSD-95 (1)

Wild type

Flailer

DIV 14

Red: Phalloidin
Green: PSD-95
Blue: Synaptophysin
Localizing Synaptic Distribution of PSD-95 (2)

**Wild Type**
- synaptophysin
- PSD-95
- Phalloidin
- Overlay

**Flailier**
- synaptophysin
- PSD-95
- Phalloidin
- Overlay
Hypothesis: The entire ionotropic glutamate receptor scaffolding, trafficking, and signaling complex changes with developmental increases in activity.

Culture hippocampal slices. After ~ 4 days transfect with tagged PSD-95.

Several days later record from an infected cell and a non-infected neighbor.

Figure 1. Expression of PSD-95 enhances the amplitude and frequency of mEPSCs. A, Five superimposed sample traces showing mEPSCs from a control cell (left) and a PSD-95-expressing cell (right). Note that there are many more events in the PSD-95-expressing cell, and that some of the events are larger than events recorded in a control cell. B, Cumulative frequency distributions of the amplitudes of mEPSCs recorded from control cells (closed circles) and PSD-95-expressing cells (open circles) \((n = 7)\). C, Cumulative frequency distributions of the interevent intervals of mEPSCs recorded in control cells (closed circles) and PSD-95-expressing cells (open circles).

A New Function For PSD-95 via the Stargazin Family of Molecules

PSD-95 over-expression

Both PSD-95 & Stargazin are localized at synaptic puncta

Removal of the c-terminal of stargazin (stargazinΔC) blocks its binding to PSD-95.

Enlarged AMPAR currents at synapses

stargazinΔC localization is more diffuse

Stargazin over-expression

stargazinΔC reduces AMPAR synaptic current

But stargazinΔC over-expression still increases extra-synaptic AMPAR current

No effect on synaptic currents but significant increases in extra-synaptic AMPAR current.

What do these results imply about the function of PSD-95?

That PSD-95 over-expression produces a significant increase in AMPAR but not NMDAR currents at the synapse.

What do these results imply about the function of stargazin?

That stargazin facilitates AMPAR expression on the surface of neurons.

What does the truncated stargazinΔC result imply about the function of the stargazin C terminus?

That the C-terminus is not necessary for stargazin to increase the surface expression of AMPARs but that it is necessary for clustering AMPARs at the synapse.

The C-terminus of Stargazin is a PDZ binding domain. Therefore what do these experiments suggest about the interaction of PSD-95 and Stargazin?

That PSD-95 binds Stargazin through its PDZ-domains and localizes it and the associated AMPARs to the synapse.
Ifenprodil blocks current through NR2B-rich NMDARs. Therefore, it can be used to determine how much of the NMDAR current is carried by NR2B-rich NMDARs.

1. The proportion of current carried by NR2B-rich receptors decreases with age.
2. The decrease in the NR2B-rich receptor contribution is much faster at the synaptic center than in the extrasynaptic domain.

Townsend et al, 2003
Extrasynaptic NMDAR receptors participate in evoked currents but not in most miniature synaptic currents.

Glutamate transporters remove glutamate from the synaptic cleft. Therefore, glutamate concentration decreases rapidly with distance.

Townsend et al, 2003
Hippocampal slice cultures comparing mEPSCs in neurons overexpressing PSD-95 and neighboring uninfected neurons

The frequency and amplitude of AMPAR responses increase